DETERMINATION OF HEAVY METALS IN VEGETABLE SAMPLES FROM A FARMLAND IN ISIHOR, OVIA NORTH EAST LGA, EDO STATE.

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CERTIFICATION

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DEDICATION

This research work is dedicated to God almighty for his infinity love and my beloved parents for their endless encouragement and care throughout my studies in this great citadel of learning University of Benin.

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ABSTRACT

This study aimed to determine the levels of heavy metals in pumpkin, bitter, and water leaves to evaluate their potential health risks and nutritional quality. Samples of the three types of leaves were collected from different locations in Evidence ISIOHOR OVIA NORTH EAST LGA, Benin City, Edo state and subjected to heavy metal analysis using established analytical methods. The concentrations of heavy metals, including iron (Fe), copper (Cu), manganese (Mn), lead (Pb), cadmium (Cd), and zinc (Zn), were determined and compared to international standards and guidelines.

Results revealed varying levels of heavy metals in the leaves, with differences observed among the three types. Pumpkin leaves exhibited elevated levels of iron, manganese, and zinc, while bitter leaves showed higher concentrations of copper. Water leaves displayed relatively lower levels of heavy metals compared to the other two types. However, all three leaf types had concentrations of heavy metals below regulatory limits, except for lead in water leaves, which exceeded the permissible level.

These findings highlight the importance of monitoring heavy metal levels in leafy greens, as they can serve as indicators of environmental contamination and potential health risks. While the concentrations of heavy metals in the studied leaves were generally within safe limits, the presence of elevated lead levels in water leaves suggests the need for further investigation and mitigation measures to ensure food safety.

In conclusion, this study provides valuable insights into the heavy metal content of pumpkin, bitter, and water leaves, contributing to efforts aimed at promoting food safety and nutritional quality. Continued monitoring and research in this area are essential to safeguarding public health and enhancing the sustainability of food production systems.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

A balanced diet must include plenty of vegetables. They offer a range of nutrients, such as fibre, vitamins, and minerals. They can aid in weight loss because they are low in calories. The majority of dietary standards advise consuming two to three servings of veggies daily. One cup of raw leafy greens or half a cup of other vegetables is often the suggested serving size.

Because plants have various parts, there are differences in the "DEFINITION" of a vegetable. All around the world, stems, leaves, blooms, fruts, and seeds are eaten as food. More precisely, according to (Dictionary.com (2015), it can be described as any plant, a portion of which is consumed for food.

PUMPKIN LEAVES: Also referred to as "kabocha" or "bitter melon" leaves, and known locally as "Ugu," these leafy greens are a common food in several regions of the world, including Asia and Africa. They taste slightly bitter, but they are also nutrient-dense and full of iron, vitamin C, and A. They can be sautéed, boiled, or stir-fried, among other cooking methods. Pumpkin leaves are utilised as a herbal cure for a variety of ailments in different cultures. Additionally, they have few calories—just 20 per 100 grammes.

Vitamin C found in pumpkin leaves aids in scar formation and wound healing. It also supports the preservation of strong teeth, skin, and bones. Additionally, it aids in the battle against reduces cholesterol, stops infection, etc. A number of minerals, including potassium, calcium, folic acid, magnesium, phosphorus, thiamine, niacin, copper, manganese, and proteins, are present in pumpkin leaves.

1.1.1 Background of Study

The presence of heavy metals in leafy greens have caused many problems to the human health hence this study work. To determine the amounts of heavy metal contamination in three frequently consumed leafy vegetables, this research and study work is to analyse the heavy metal content of pumpkin leaves (Telfairia occidentalis), bitter leaves (Vernonia amygdalina) and water leaves (Talinum triangulare).

1.1.2 Statement of Problem

Over the past few decades, there has been a rise in concern for health issues worldwide. Although the majority of these diseases are difficult to diagnose and may be acquired in childhood before becoming apparent in maturity, one cannot completely ignore the negative effects of modern technology advancement on the environment, even though it has greatly improved many people's quality of life. The creation of hazardous waste and other pollutants that are harmful to human health has increased as a result of industrialization and urbanization. Agricultural revolution that resulted in commercial farming using chemical pesticides, herbicides, fertilizers, and other agricultural chemicals. Over time, these heavy substances have a direct impact on human health and the ecosystem as a whole. The existence of heavy metals is one of the problems.

The toxicity of heavy metals has been demonstrated to be one of the main risks to health, carrying a number of health hazards. Naturally occurring metallic elements with high atomic weights and densities at least five times higher than water's are known as heavy metals. These metals have atomic numbers greater than 20 and metallic characteristics. Plants with roots, such as water leaves, bitter leaves, and pumpkins, can take heavy metals from the soil. These metals can originate from a variety of sources, including mining, industrial processes, agricultural practices (such as the use of tainted pesticides or fertilisers), and natural geological processes. If heavy metals are present in the irrigation water used to cultivate these plants, the metals may be absorbed by the roots and build up in the leaves. Rivers, streams, and groundwater contaminated by mining operations, industrial discharges, or inappropriate disposal of home or industrial waste are examples of contaminated water sources. Air pollution is another way that heavy metals can end up on plant leaves. Heavy metals can be released into the atmosphere by automobile exhaust, industrial pollutants, and other sources. Once there, they can land on plant surfaces and enter the plant's leaf tissues, where they can be absorbed. You Heavy metals can also indirectly enter the human system through the eating of meat or dairy products from animals that eat these polluted plants. As a result, as heavy metals ascend the food chain, they have the ability to bioaccumulate and biomagnify, possibly reaching greater amounts in animal tissues that people eat.

All things considered, the fact that bitter, water, and pumpkin leaves contain heavy metals emphasises how crucial it is to control environmental pollution in order to reduce the health concerns that could result from heavy metal exposure.

1.1.3 Justification of Study

Before now, studies have shown that there are quite a measure of heavy metals present in vegetables which gets into the human system through Consumption. This research is to assess the levels of heavy metals in pumpkin leaves, Bitter leaves and Water leaves.

1.1.4 Scope of Work

This study covers a farm land in EVIDENCE, ISIOHOR, OVIA NORTH EAST LGA, BENIN CITY, EDO STATE.

From here, the pumpkin, bitter and Water leaves will be assessed for the presence of heavy metals.

1.1.5 Aim and Objectives

The aim of this study is to determine the presence of heavy metals concentrations of pumpkin, bitter and water leaves from a farm land in ISIOHOR, Benin City, OVIA NORTH EAST LGA, EDO STATE

The objective of this study is to;

- quantify and examine the amount of heavy metals present in pumpkin, bitter and water leaves.

- Compare the levels of heavy metals with the permissible limits by the different health regulatory bodies like World health Organization (WHO).

The aim of this study is to determine the presence of heavy metals concentrations of pumpkin, bitter and water leaves from a farm land in ISIOHOR, Benin City, OVIA NORTH EAST LGA, EDO STATE

1.2 LITERATURE REVIEW

1.2.1 Pumpkin Leaf

Pumpkin leaves are derived from the squash plant, a type of gourd. Like other leafy greens like bitter or water leaves, pumpkin leaves can be cooked and consumed. They can be used in many different recipes, such as soups, stews, curries, and stir-fries, and have a moderate flavour. In various regions of the world, including Asia, Africa, and Latin America, pumpkin leaves are in vogue. Pumpkin leaves have several health advantages, such as enhancing immunity, decreasing inflammation, and facilitating better digestion. T. Occidentalis is a native of southern Nigeria and a member of the Cucurbitaceae family (Akoroda, 1990).

The scientific genus Cucurbita, which also includes melons, cucumbers, squash, and zucchini, is where pumpkin leaves are classified.
Pumpkin leaves specifically come from the Cucurbita pepo species.
The family Cucurbitaceae, which has over 900 distinct species of flowering plants, includes the g enus Cucurbita.
They are mostly used in soups and herbal treatments, and they are grown throughout West Africa, but especially in Southeast Nigeria (Nwanna *et al*., 2008).

Around the world, pumpkin leaves are a common ingredient in many different cuisines. Pumpkin leaves are frequently utilised in Kenyan, Ghanaian, and Nigerian cuisine throughout Africa.

Garlic, other spices, and palm oil are frequently used when cooking them. Pumpkin leaves are frequently used in Thai and Vietnamese cooking in Asia, where they are stir-fried or sautéed with garlic, chillis, and fish sauce. Pumpkin leaves are frequently utilised in Mexican and Peruvian cuisine throughout Latin America.

1.2.2 Nutritional Content, Cultivation, Structure and Storage

Antioxidants, which are abundant in pumpkin leaves, have been demonstrated to guard against cell damage. Additionally, they include substances that have been demonstrated to have hepatoprotective qualities, which means they may aid in shielding the liver from harm. Pumpkin leaves can also aid in infection prevention because they possess antibacterial qualities (Nwanna *et al* .,2008)

The primary ingredients of a Nigerian soup called egwusi are the young branches and leaves of the female plant. The huge, dark red seed (up to 5cm) can be eaten whole, pounded into a powder for a soup, or put into a porridge that is fermented. It is high in fat and protein. Although it can be permitted to spread out flat in a field, T. oreganalis is normally planted vertically on trestle-like constructions (Okoli and Mgbeogu, 1983). It is propagated by seed, which is kept safe from injury by being housed in a harder shell or another covering. It may endure years of drought and continue to exist in the root. This creeping plant thrives when bamboo sticks are used as stakes.

The fluted gourd fruit is fairly large; according to one study, its length ranges from 16 to 105 centimetres (6.3 to 41.3 cm), with an average diameter of 9 cm (Okoli and Mgbeogu, 1983).According to the same study, larger gourds can have up to 196 seeds per fruit, with each fruit typically measuring between 3 and 4.9 cm. in length (Okoli and Mgbeogu, 1983). In both the pistillate and staminate varieties, T. occidentalis flowers grow in sets of five with creamy - white and dark red petals contrasting with the light green colour of the fruit when young, and yellow when ripe (Okoli and Mgbeogu,1983). Dioecious flowering is most common in the fluted gound with few documented cases of monoecious flowering.

Although the Seeds of T-occidentalis store well, precautions must be taken when storing any portion of the plant, particularly if the gourd is to be stored whole. This is not a typical storage method, as the fluted gourd pod (the fruit itself) is highly perishable and can only be stored up to 4 weeks (Emebiri and Nwufo, 1990), If the gourd is left intact and proper storage and shipping are not practiced, pod rot can manifest, even from small lesions and can cause serious damage to the entire fruit, rendering it unusable (Emebiri and Nwufo,1990).

1.2.3 Bitter Leaf

The plant known as bitter leaf (Vernonia amygdalina) can grow to be a small tree or shrub that reaches a height of 23 feet. A common plant that grows practically everywhere, particularly in Africa's tropical regions. It's a versatile plant with a lot of possible applications. Because of its many medicinal benefits, which include treating stomachaches, skin infections, diabetes, sleeplessness, toothaches, acne, pneumonia, stoke, arthritis, exhaustion, coughing, and bleeding, it is considered a magnificent gift from God to humanity. Additionally, the plant's anti-bacterial, anti-inflammatory, anti-helminthic, and anti-parasitic qualities have been demonstrated. Bitter leaf is good for toning the body's essential organs, particularly the kidney and liver, and it detoxifies the entire system. Bitter leaf is one of the leafy vegetables in terms of nutrition ,that because to its great richness in proteins, vitamins, and mineral components including iron, phosphorus, calcium, potassium, zinc, copper, folic acid, and ascorbic acid, have been utilised to treat the symptoms of micronutrient deficiency. Bitter leaf is also said to have applications in the production of soap, decorations, beer, and stamina restoration in addition to helping to sequester carbon and slow down environmental deterioration. Because of this plant species' abundance of nutrients and myriad medical uses, the authors advise pregnant women, nursing moms, and other population groups to try it,.

Of the over 1,000 species of shrubs in the genus Vernonia, the most widely cultivated species is Vernonia amygdalina (Del.), sometimes known as bitter leaf (Muanya, 2013).It is a member of th e Astaraceae family.It is grown vegetatively in most of the West African countries, such as Niger ia, Cameroon, Gabon, and the Congo Democratic Republic, by cutting stems at an angle of 450 d egrees Celsius.
William Vernon, an English botanist, is the name of the plant.Another name for it is ironweed. Gardens are a common habitat for V. amygdalina (Schiffers, 2000).

Unlike other plants that are endemic to a certain place, it may adapt to a variety of conditions. It

is grown in fields and savannah zones across several nations (Ibrahim *et al*., 2010).

Despite being most commonly utilised in cuisine its therapeutic qualities have also been traditionally employed (Swee *et al*., 2010). As its name suggests, bitter leaf has a bitter flavour, yet it cooks up surprisingly well (Abosi and Raseroka, 2003). Bitter leaf is known by the following names: Ewuro (Yoruba), Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo), Chusa-doki Shiwaka (Hausa), Onugbo in Igbo-Eastern Nigeria, and Orugbo among the Itsekiri and Urhobo tribes in Nigeria.

This little plant grows in every place. During cultivation, V. amygdalina stems of varying lengths are chopped in the afternoon, soothed, and tied into bundles. To prevent desiccation, the bundles are stored over night, either standing in a basin of water or covered with a jute bag. These days, they are planted on loamy soil in the morning, at a depth of 2-5cm deep. Botanically, bitter leaf is a shrub or small tree that grows in tropical Africa. It attains the height of 2.5m with petiolate leaf of about 6mm in diameter that is elliptic in shape (Ijeh and Ejike, 2011).

1.2.4 Health Benefits of Bitter Leaf

The plant is used to cure diabetes, tuberculosis, diarrhoea, hepatitis B and C, jaundice, and cancer. Chemical investigations have showed considerable promise in the production of dietary supplements based on bitter leaves for these conditions (Muanya, 2013). By controlling a variety of cytokines and chemokines, polyherbal formulations containing bitter leaf as one of its active constituents boost immunity (Muanya, 2013). The herb helps mend the pancreas in addition to lowering blood sugar levels to a necessary degree. Diabetes can be healed if 10 handfuls of fresh leaves are squeezed into 10 litres of water and then consumed twice daily for a month.

A nutritious diet that includes bitter leaf may lower your chance of developing chronic illnesses like breast cancer.

1.2.5 Nutritional Benefits of Bitter Leaf

Bitter leaves are consumed as a vegetable. It is a widely used vegetable and herb in Southern and Eastern Nigerian cuisine, particularly in soups. Cooking the leaves and young shoots makes them into veggies that are high in protein, vitamins, and minerals. One green vegetable that has been used to address the issue of hunger and micronutrient deficiencies is bitter leaf, which is widely grown in tropical Africa (Ejoh *et al*., 2005).

The leaves are reasonably priced and abundant in many nutrients, including vitamin C and β-carotene, which are vital for human well-being. Additionally, it has high concentrations of minerals like potassium, phosphorus, calcium, and iron (Oshodi *et al*., 1992; Musa *et al*., 2011).

1.2.6 Water Leaf

The vegetable waterleaf is referred to by several names. It goes by as cariru, Surinam Purslane, Florida spinach, and Ceylon spinach. There is disagreement even about its scientific nomenclature. It makes use of both Talinum triangulare and Talinum fruticosum. Whatever name it goes by, it's tangy, crunchy, and healthy. It is said that waterleaf can reach a height of five feet and produces simple pink flowers. It is a great source of molybdenum, zinc, and iron, while waterleaf is native to the Americans and the Caribbeans, it has been cultivated in many places across the world.

1.2.7 Health Benefits

Waterleaf has a high nutritional value. But it also contains a lot of oxalate. Foods such as spinach, rhubarb, beets, sweet potatoes, and waterleaf naturally contain oxalate. Oxalate can cause kidney stones in people who have kidney problems. Blanching or heating can eliminate up to 50% of the soluble (dissolves in water) oxalate.

BONE HEALTH

Waterleaf is a great source of phosphorus and calcium, two minerals that are necessary for strong bones. In reality, studies have indicated that consuming calcium in the absence of phosphorus has very little effect on bone strength. The two components seem to function in tandem. They are particularly helpful for women over 60 who already have osteoporosis.

EYE HEALTH

Keeping your vitamin A levels adequate is crucial for maintaining healthy eyes. Studies show that vitamin A helps prevent cataracts, lessen the risk of retinal degeneration, and enhance eyesight in dim light. Vitamin A is abundant in waterleaf.

1.2.8 Nutritional Benefits of Water Leaf

Waterleaf is a rich source of the essential nutrients Calcium, Phosphorus, Iron, and Vitamin C. It is also a good source of Vitamin A, Thiamine, Riboflavin and Niacin.

The main concern when it comes to eating too much waterleaf stems from its oxalate content. Too much oxalate can be unhealthy for individuals with kidney disease. As such, those individuals should refrain from consuming raw waterleaf. Cooked waterleaf has approximately 50 percent less soluble oxalate than its raw counterpart. Otherwise, waterleaf is a healthy food that is low in both calories and sugar. Generally, it’s a good idea to try to eat three to four servings of vegetables per day.

Some popular waterleaf recipes include:

Gbure Elegusi, a Yoruba dish from Nigeria, Edikang Ikong soup, an Efik dish from Nigeria, roasted waterleaf with yams, plantains, and/or potatoes, stewed waterleaf with chicken, tomatoes, and onion

1.3 Heavy Metals

Elements having an atomic number greater than 20 and a density greater than $5g/cm^3$ are classified as heavy metals. The earth's crust contains naturally occurring elements known as heavy metals. Heavy metals include things like arsenic, cadmium, chromium, lead, and mercury. Both humans and animals may be poisonous to heavy metals, which can build up in the body over time. Depending on the type of metal and the extent of exposure, heavy metal exposure can result in a variety of health issues. Plants require heavy metals including Cu, Fe, Mn, Co, Zn, and Ni to grow and thrive. Plants require these micronutrients because they are needed to create cofactors, which are small molecules that help enzymes and proteins function properly. In other words, without micronutrients, plants wouldn't be able to grow and develop properly. Plants do not require Hg, Cr ,As or Pb for any of their metabolic functions, not even in trace concentrations (sami- stha sen Raychaudhurl 2021),hence they are referred to as non-essential heavy metals.

1.3.1 Iron

Iron is a heavy metal that is essential for which are small molecules that help enzymes and proteins function put it another way, plants couldn't grow and develop correctly without micronutrients. Since Hg, Cr, As, and Pb are not needed by plants for any of their metabolic processes—not even at trace levels—they are referred to as non-essential heavy metals (sami- stha sen Raychaudhurl 2021). similar to the heart and liver. Although iron poisoning is uncommon. It can occur when a person inadvertently or on purpose consumes a significant amount of iron. Iron poisoning can cause vomiting, diarrhoea, and abdominal pain.

Soil, water, and food are the main environmental sources of iron. Some foods, such as leafy green vegetables and red meat, have large amount of iron.

1.3.2 Copper

Heavy metals like copper are commonly utilised in industry to make cookware, electrical cables, plumbing pipes, and other items. Certain birth control tablets and IUDs are copper-containing devices. To stop algae from growing, copper sulphate is occasionally added to drinking water and swimming pools. Several industries, including the manufacture of paper and pulp, the refinement of petroleum, the preservation of wood, and agriculture, can contribute to copper contamination. Copper use in food packaging can potentially lead to contamination. Furthermore, foods derived from natural sources such as mussels, liver, mushrooms, almonds, and chocolate may also contain copper contaminants. (Kabata and Pendias, 1992).

Excessively high levels of copper in the body can cause a condition called Wilson's disease, which is a neurological disorder that results in copper deposition in the brain and kidney failure (Banum, 1982). In some instances, exposure to copper has resulted in jaundice and enlarged liver. It is suspected to be responsible for one form of metal fume fever (Wagoner Et soffioti,1976).

1.3.4 Manganese

A trace mineral that is vital to human health is manganese (Mn). Numerous foods, both plant and animal-based, contain it, such as leafy greens, whole grains, beans, nuts, and shellfish. The immune system, metabolism, and bone health all benefit from manganese. In addition, it contributes to the synthesis of energy and aids in blood sugar regulation. Extended exposure to lower Mn concentrations may increase the frequency of parkinsonian symptoms. Many enzymes in the body, including those that produce energy and protect the body from oxidative damage, require manganese as a cofactor. It contributes to brain and nerve function and is involved in the synthesis of connective tissue and bones. Moreover, manganese is necessary for the synthesis of hormones production and helps to regulate blood sugar levels. Manganese deficiency is rare, but can cause problems like reduced growth, infertility, and increased susceptibility to infections.(Roberto *et al*.2015)

1.3.5 Lead

Lead are a naturally occurring element present in the earth crust. Even at low concentrations, this heavy metal can be detrimental to human health. Lead can harm the brain and nervous system since it is a strong neurotoxin. In addition, it may result in issues with the blood, kidneys, and other organs. Young children are more susceptible to lead's negative effects. Natural processes such as rock weathering and volcanic activity can release lead into the environment. However, human activities such as the extraction and processing of lead-containing ores, the creation and consumption of lead-based products, and the disposal of trash containing lead are the most frequent sources of lead pollution. Following the release of lead into the environment can be taken up by plants, animals, and humans. Lead poisoning, or lead toxicity, is a term used to describe the harmful effects of lead exposure. It can cause a wide range of health problems, from developmental delays and learning difficulties in children, to high blood pressure and kidney problems in adults. It can even be fatal at very high levels. The effects of lead poisoning can be permanent, but the good news is that there are treatments that can help reduce the toxic effects of lead.

1.3.6 Cadmium

The earth crust contains the heavy element cadmium. Additionally, earth, air, and water all contain it. One hazardous element that can lead to major health issues is cadmium. It may cause harm to the liver, kidneys, and lungs. The chance of developing cancer can also rise with prolonged exposure to cadmium. Children should be extra careful around cadmium because of their increased sensitivity to its effects. Cadmium can damage plants and animals as well as pollute the environment, which raises environmental issues. There are multiple ways to come into contact with cadmium. One is through the atmosphere, when industrial sources leak cadmium into the atmosphere. Additionally, tainted food and drinking water can allow it to enter the body. Those who reside or work in industries that use or produce cadmium, such as mining and smelting, are also at risk of exposure. Smoking is another way that people can become exposed to cadmium, and this can raise blood and urine concentrations. The presence of cadmium in contaminated water can disrupt the body's necessary processes, which could lead to short- or long-term disorders (Jiang *et al*., 2015; Richter *et al*., 2017; Cao *et al*., 2018). The International Agency for Research on Cancer (IARC) has class.

1.3.7 Zinc

Zinc is a micronutrient that's important for human health, but it can also be a heavy metal if it's found in high concentrations. Like other heavy metals, exposure to high levels of zinc can be dangerous. Zinc can enter the body through the air, water, and food. Breathing in zinc dust is a common way to be exposed. Working in industries that use zinc, like smelting, can also lead to exposure. High levels of zinc in the body can cause nausea, vomiting, and diarrhea. It can also damage the liver and kidneys. 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).The surge in anthropogenic activities has exacerbated the problem of heavy metal pollution in the world, which, due to their indefinite persistence in the environment are non-degradable pollutants and can be detrimental for living beings at high levels (Witkowska *et al*., 2021). Zinc is one of the abundant transition elements and is also reported to be an essential trace element for all living organisms (Roohani *et al*., 2013; Jarosz *et al*., 2017; Lee, 2018). It is needed for the function of some organs and systems, as well as for growth, development, and tissue repair (Murgia *et al*, 2006; Zalewski, 2006).

1.3.8 Determination of Heavy Metals

Typically, spectroscopic and colorimetric methods are used to quantify the heavy metal ions. The type of metal ion and the accuracy of the chosen method dictate which one should be utilised. All metals can be studied using spectroscopic methods, however transition elements can be studied using colorimetric methodology in conjunction with UV-visible spectroscopy since these elements can generate coloured ions in solution. Spectroscopic methods are typically employed to quantify the heavy metals. Atomic absorption spectroscopy (AAS) and UV-visible spectroscopy are the two spectroscopic methods.

1.3.9 Atomic Absorption Spectrometry (AAS)

The atomic absorption spectrometry is a type of spectroscopy that uses the absorption of light to measure the concentration of an element in a sample. Essentially, a light source is passed through a sample, and then through a monochromator, which separates the light into its component wavelengths. Then, the light that's not absorbed by the sample is measured by a detector. The amount of light absorbed by the sample is proportional to the concentration of the element being measured.

Using this spectroscopic method, metal ions in solution are inhaled into an air-acetylene flame, where they atomize into the gaseous phase when they absorb enough energy. The concentration of the ions atomized from the solution determines the radiation strength absorbed. In the gaseous phase, the atoms are excited when incident monochromatic light is delivered through them from a source lamp (often a hollow cathode lamp). A sample injection port, an atomizing chamber, a hollow cathode lamp, a monochromator, a detector, and electronics/readout are the features that define the spectrometer.

The monochromator selects light of a certain wavelength that is passed through the gaseous atoms and this light is measured from the detector. The test solution is injected into the atomizing chamber by suction and is aspirated to flame to produce the gaseous atoms. This energy is transformed by the electronic component into absorbance, which is shown on the readout screen (Gary, 2014).

1.4 Samples Digestion

An orange-brown coloration was seen after adding 1 gram of each of the samples to a 10 millilitre combination of nitric acid.

This mixture was heated continuously for 1-2 hours until the brown vapours turned transparent. After that, it was lowered to room temperature and filtered through Whatmann No. 1 filter paper to get rid of any remaining particles.

The filtrate was ultimately put into sample bottles and brought to the atomic absorption spectrometer for measurement after being diluted in a 100 ml volumetric flask with distilled water.

THEORY OF AAS

A light source, usually a Hallow cathode lamp (HCL) or a continuous source, evaporates and excites the target element's atoms when a sample is placed in a flame or a graphite furnace. At particular wavelengths that match the element of interest's absorption lines, the light source releases radiation. The atoms in the atomized sample absorb radiation at wavelengths unique to the element under study as it travels through the sample. The absorption directly relates to the element's concentration in the sample. Using a calibration curve or standard reference material, the concentration of the element can be found by measuring the reduction in light intensity brought on by absorption. As an analytical method, AAS has various benefits. as an analytical technique. It has high sensitivity, allowing for the detection of trace amounts of elements in a sample. It also provides good selectivity , as the absorption lines are specific to each element, minimizing interference from other substances present in the sample. AAS is relatively simple to operate and provides rapid results, making it commonly used method in analytical laboratories.

Beer's law

Beer's law states that the rate of light absorption is proportional to the concentration of the solution.

Lambert's Law

This law states that the absorbance of light and path length are directly proportional

Beer Lambert's Law

This law states that for a given material sample path length and concentration of the sample are directly proportional to the absorbance of the light.

The Beer-Lambert Law is expressed as :

A=£Lc

Where

A=the amount of light absorbed for a particular wavelength by the sample.

£= Is the molar extinction coefficient

L= the distance covered by light through the solution

c=the concentration of absorbing species

A=Log10(lo/l)

Where

A is the absorbance

lo is the intensity of the transmitted light

A=-LogT

Where

T is the transmittance

A=-LogT=£Lc.

CHAPTER TWO

2.0 MATERIALS AND METHODOLOGY

2.1 MATERIALS

Pumpkin leaves sample

Bitter leaves sample

Water leaves sample

Funnel
Sieve (0.5mm)

Reagents bottles

Spatula
Sample bottle

Digestion tubes

Kjeldahl's Flask

Conical flask

Measuring cylinder

Safety equipments (gloves, lab coat, goggles)

Standard solution of heavy metals

Volumetric flask

Atomic Absorption spectrometer

Nitric acid (HNO3)

Deionized Distilled water.

BITTER LEAVES: Also known as Vernonia amygdalina in botany, this tropical plant is native to several regions of Africa.

Botanical Description: Bitter leaf is a member of the Asteraceae family and is a perennial plant. Its leaves are elliptic and have a distinct bitter flavour.

Geographic Distribution: It is indigenous to tropical Africa, with Nigeria, Ghana, and Cameroon being among the countries where it is most common. Other regions of the continent are also home to the plant.

In traditional African medicine, bitter leaf is prized for its therapeutic qualities. It is thought to possess several health advantages, such as anti-inflammatory, anti-diabetic, and anti-malarial qualities.

Nutritional Content: Iron, calcium, potassium, vitamin C, and other vitamins and minerals abound in the plant. These add to its nutritious content.

WATER LEAVES: A leafy vegetable used in Nigerian cooking, water leaves are also referred to botanically as Talinum triangulare.

Botanical Information: Water leaf, or Talinum triangulare, is a member of the Portulacaceae family. The herb is succulent and has triangular-shaped leaves.

A common vegetable in Nigerian cooking is water leaf. It frequently adds a distinct flavour and nutritious value to soups, stews, and other classic recipes.

Nutritional Value: Antioxidants, vitamins, and minerals abound in water leaf. It is a nutrient-dense supplement to the diet since it includes minerals including calcium, iron, vitamin C, and vitamin A.

2.2 METHOD

2.2.1 PRE SAMPLING TREATMENTS

To be sure there was no contamination, all equipments and containers used to collect samples were carefully washed with distilled water. The samples collected were some leafy vegetables

Pumpkin leaves (Telfairia occidentalis)

Bitter leaves (Vernonia amygdalina)

Water leaves (Talinum triangulare)

2.2.2 SAMPLE COLLECTION AND PREPARATION

Each of the samples was gotten from a farm land in Evidence ISIOHOR OVIA NORTH EAST local government area, Benin City, Edo State.

The leaves were carefully removed from their stalks and thoroughly and carefully washed with deionised distilled water to remove any form of contaminants, thereafter it was sun-dried for 3-7 days to remove all residual moisture.

The dried samples were grounded finely with mortar and pestle and sieved through a 0.5mm mesh sieve to obtain uniform particle size.

2.2.3 DIGESTION

Analytical chemistry uses the process of digestion to disassemble a sample into its constituent parts. Digestion is employed in heavy metal analysis to separate the plant material into its constituent for independent examination. Although there are many alternative digesting techniques.

There are two types of digestion

-wet digestion (wet ashing)

-Dry digestion (dry ashing)

Wet digestion:

Strong acid is used in the wet digesting process to break down the plant material. The plant material is heated to a high temperature, causing the acid to react with it and disintegrate it into its constituent parts. After that, the constituent components are divided and examined using methods atomic absorption spectroscopy. The plant material is entirely broken down during the wet digesting process, making it a harmful procedure. This is in contrast to other techniques that can analyse a material without damaging it, including X-ray fluorescence.

Dry Digestion:

The plant material is heated to a high temperature and then totally burned in the dry digesting process, commonly referred to as the ashing method. After that, the leftover ash is dissolved in acid and examined using the same methods as the wet digestion process. The dry digesting method has the advantage of being less expensive and faster than the wet digestion approach. It may, however, be less accurate because it is more challenging to guarantee that every last bit of plant material gets burned.

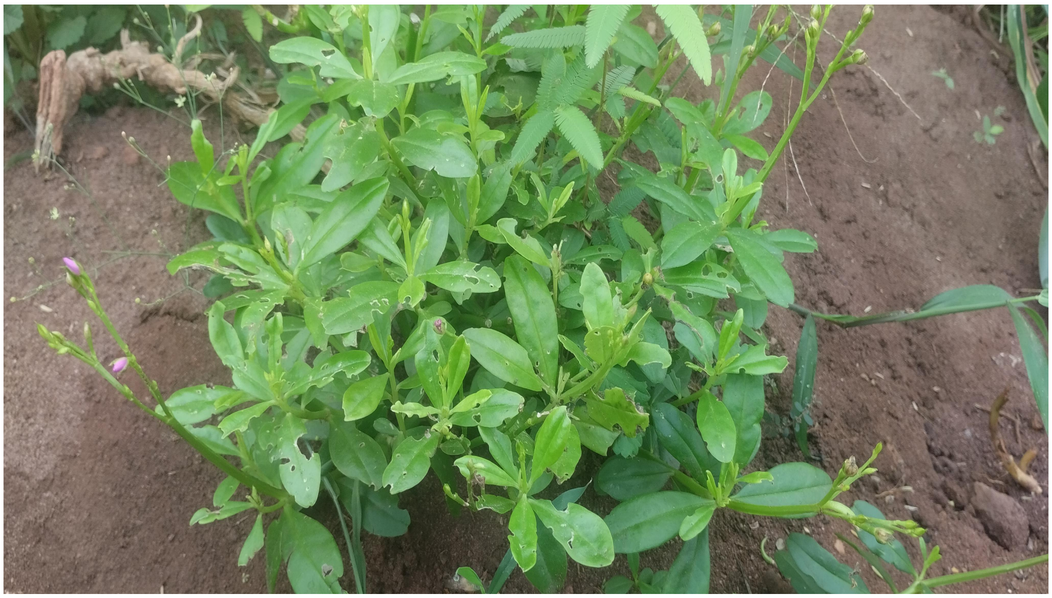

Plate2. 1: Water Leaf

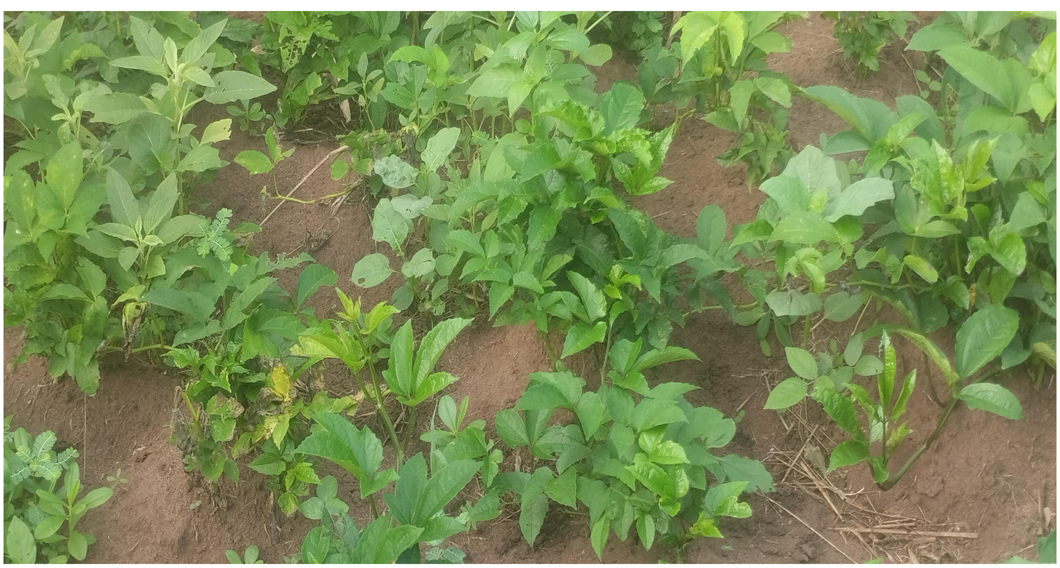

Plate 2.2: Pumpkin Leaf

Plate 2.3: Bitter Leaf

Plate 2.4: Dry Bitter Leaf

Plate 2.5: Dry Water Leaf

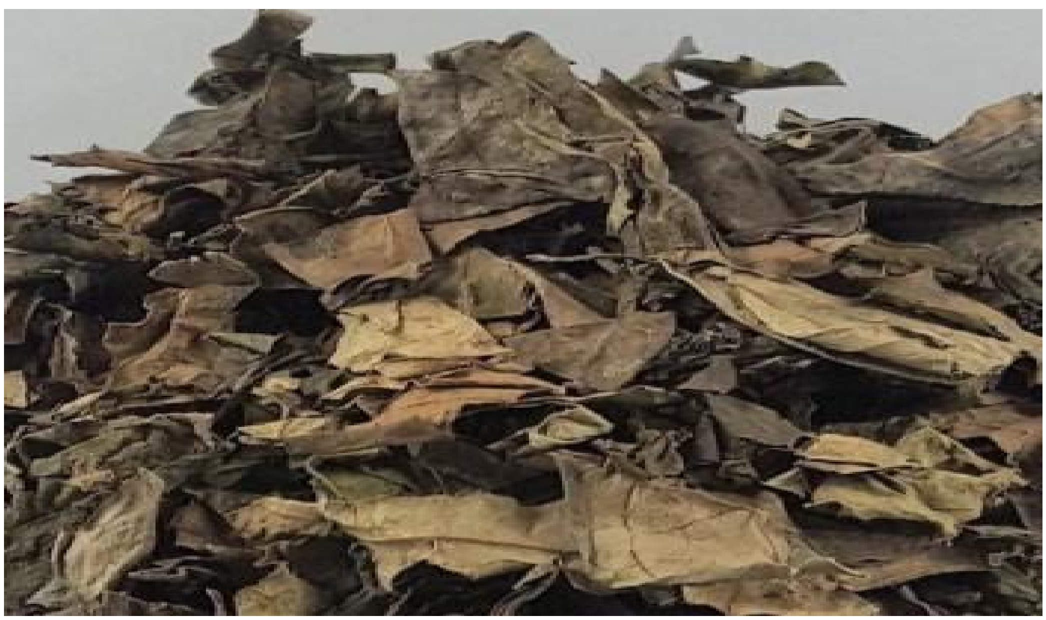

Plate 2.6: Dry Pumpkin Leaf

Plate 2.7: Ground samples

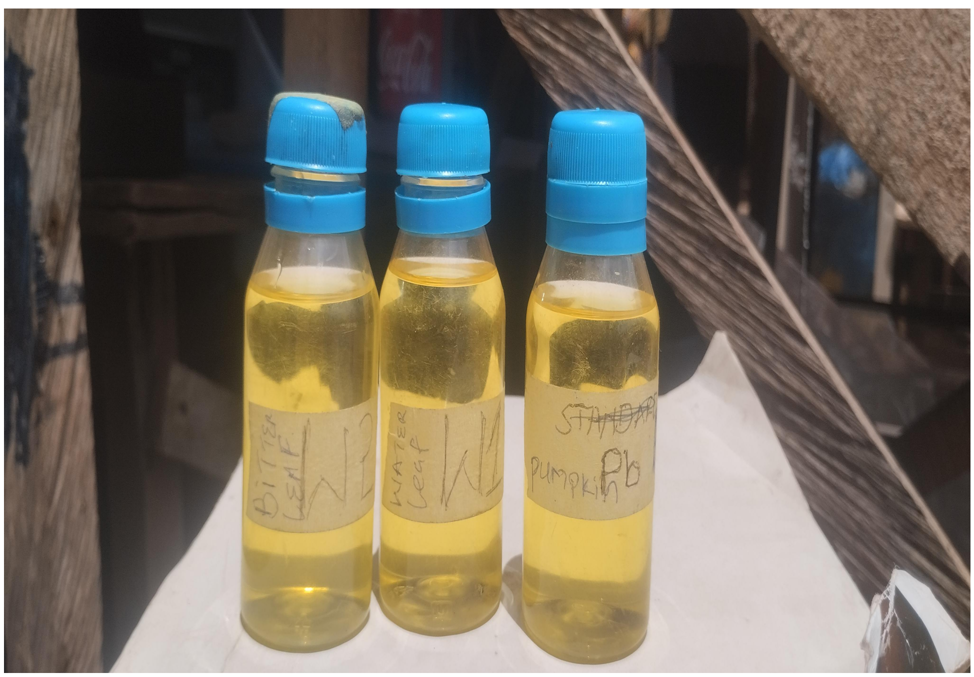

Plate 2.8: Digested Sample

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

Because of their toxicity, soil partitioning, and plant accumulation, heavy metals are regarded as the most significant components of pollution originating from the terrestrial environment. Because heavy metal has a detrimental influence on human health as well as the nutritional values of agricultural products. As a result, in order to meet the standards established by the World Health Organisation and other international regulators, there must be sufficient control over the concentration of heavy metals in food.

Here are the results of the experiment

Table 3.1: Heavy Metal Analysis in each Sample

Heavy metals	Pumpkin leaf sample A mg/kg	Pumpkin leaf sample B	Pumpkin leaf sample C	Bitter leaf sample A	Bitter leaf sample B	Bitter leaf sample C	Water leaf sample A	Water leaf sample B	Water leaf sample C
Fe	5.400	5.200	7.100	4.100	4.300	5.400	3.000	3.200	4.000
Cu	0.040	0.020	0.080	0.030	0.010	0.100	0.030	0.030	0.030
Mn	1.890	1.800	3.500	1.380	1.300	2.400	4.370	4.150	5.890
Pd	0.010	0.010	0.040	ND	0.020	0.090	0.010	0.010	0.750
Cd	0.010	0.030	0.060	0.010	0.030	0.050	0.010	0.030	0.040
Zn	1.800	1.500	2.300	1.100	1.000	1.600	1.000	1.000	1.200

Table .3. 2: Mean Table

Heavy metals	Pumpkin leaf sample	Bitter leaf sample	Water leaf sample
Fe	5.900	4.600	3.400
Cu	0.050	0.470	0.120
Mn	2.300	5.080	4.800
Pd	0.020	0.040	0.260
Cd	0.030	0.030	0.030
Zn	1.870	1.230	1.070

Generally speaking, a "mean table" is a table that shows the means (average values) of several variables for various groups or circumstances. Usually, while talking about a mean table, the main emphasis is on analysing the patterns and trends that the means in the table disclose. It is clear from the mean table comparing the iron content of water, bitter, and pumpkin leaves that the mean iron concentration of pumpkin leaves is the greatest of the three leaf kinds. Compared to bitter and water leaves, this finding show that pumpkin leaves are a strong source of iron.

Differences in these plants' physiological traits and nutritional makeup may be the cause of this discrepancy in iron concentration. Compared to bitter and wet leaves, pumpkin leaves may be more able to absorb and collect iron from the soil or water. Furthermore, the way the soil is composed, how it is farmed, and the surrounding environment can all affect how much iron is absorbed by plants.

In summary, the mean table that shows that pumpkin leaves have a greater iron content than bitter or water leaves emphasises the potential nutritional value of pumpkin as a dietary iron source. To improve dietary recommendations and support optimum health, more research might look into the variables influencing these variations and the leafy greens' overall nutritional profiles.

Table 3.3: Mean levels of Heavy Metals (mg/kg) in Vegetable Samples and their Standard Deviation

Heavy metals	Pumpkin leaf sample	Bitter leaf sample	Water leaf sample
Fe	5.900±0.850	4.600±0.570	3.400±0.430
Cu	0.050±0.025	0.470±0.040	0.120±0.013
Mn	2.300±0.780	5.080±0.580	4.800±0.770
Pd	0.020±0.014	0.040±0.040	0.260±0.350
Cd	0.030±0.020	0.030±0.020	0.030±0.010
Zn	1.870±0.330	1.230±0.260	1.070±0.090

It is noteworthy that among the three leaf kinds, pumpkin leaves had the lowest mean value and standard deviation for lead concentration in the mean and standard deviation table comparing the lead level of water, bitter, and pumpkin leaves. According to this research, lead levels in pumpkin leaves are often lower than in water and bitter leaves.

There are a number of possible explanations for the reduced lead content in pumpkin leaves, such as variations in plant physiology, lead uptake processes, and environmental exposure. For instance, compared to water and bitter leaves .pumpkin leaves may have a lesser propensity for absorbing and storing lead from the soil.

Furthermore, soil makeup, agricultural techniques, and pollution levels in the environment can all affect how much lead is absorbed by plants.

The potential consequences for human health and environmental safety could arise from the reduced mean and standard deviation values of lead found in pumpkin leaves. The brain system, kidneys, and cardiovascular system are among the human health issues that lead can cause. Lead is a hazardous heavy metal. As a result, eating foods like pumpkin leaves that have a reduced lead level may help lessen the chance of exposure to lead and the health problems that come with it.

In conclusion, the mean and standard deviation table emphasises the possible health benefits of ingesting pumpkin as a source of leafy greens with lower lead contamination. It shows that pumpkin leaves have a lower lead concentration than both water and bitter leaves. In order to improve public health and inform dietary recommendations, more research could examine the underlying causes of these variations as well as assess the leafy greens' overall nutritional profiles and safety.

Table 3.4: Permissible Guideline for Heavy Metals in Vegetables

Heavy metals	Values in mg/kg
Fe	0.540
Cu	4.000
Mn	0.310
Pd	0.100
Cd	0.200
Zn	60

Sources: FAO/WHO (1999),
FAO/WHO(JECFA2005)

Table 3. 5: WHO Comparison Table

Heavy metals	Pumpkin leaf sample	Bitter leaf sample	Water leaf sample	WHO STANDARD
Fe	5.900	4.600	3.400	0.540
Cu	0.050	0.470	0.120	4.000
Mn	2.300	5.080	4.800	0.310
Pd	0.020	0.040	0.260	0.100
Cd	0.030	0.030	0.030	0.200
Zn	1.870	1.230	1.070	60

According to these results, all three varieties of leaves are very rich sources of iron, offering concentrations that are far greater than the advised level. These plants' high iron content may have significant effects on human nutrition and health. An important micronutrient for several physiological functions, including as energy metabolism, oxygen transport, and immunological function, is iron. Eating foods high in iron, such bitter and wet leaves, pumpkin, can help a lot with dietary iron requirements and iron deficiency-related health problems.

Even while having a high iron content is advantageous, it's important to think about the possible health risks associated with consuming too much iron. Overconsumption of iron can result in iron overload, which can have detrimental consequences on health including oxidative stress and gastrointestinal irritation. As a result, people who have certain medical issues or dietary limitations might need to watch how much iron-rich food, like these leaves, they consume. In conclusion, the remarkably high iron content of water, bitter, and pumpkin leaves in comparison to the WHO criteria highlights their nutritional significance as abundant dietary iron sources. But while adding these leaves to their diet, people should be aware of their total iron intake as well as dietary balance and personal health considerations. Subsequent investigations may delve into tactics to maximise the dietary advantages of these veggies high in iron while reducing the possible hazards linked to consuming an excessive amount of iron.

According to these results, the copper concentrations in all three varieties of leaves are considerably lower than the advised level. An important micronutrient, copper is involved in energy metabolism, antioxidant defence, and connective tissue development, among other physiological activities. While too much copper might be harmful to one's health, it is still necessary for good health. These leaves may not be important sources of dietary copper because of their low copper concentration. Even while low copper consumption is uncommon in diets that are well-balanced, people who primarily rely on these veggies for their nutrients may not be getting enough copper in their diets. A lack of copper can cause anaemia, weakened immune system, and anomalies in the bones, among other health issues.

In conclusion, it appears that the water, bitter, and pumpkin leaves may not be significant sources of dietary copper due to their lower copper content when compared to the WHO norm. Even if they have additional nutritional advantages, people should make sure their diet is balanced and full of a range of foods high in important micronutrients like copper. Subsequent investigations may investigate methods for augmenting the copper concentration of these veggies or pinpointing substitute dietary sources of copper for persons with particular dietary requirements.

These results imply that all three varieties of leaves are very rich sources of manganese, offering amounts that are significantly greater than the advised level. An important micronutrient, manganese is involved in the creation of bones, the metabolism of carbohydrates, and the protection against free radicals. Eating manganese-rich foods, such as bitter and wet leaves, pumpkin, can help to meet manganese requirements in the diet and promote general health. However, while manganese is necessary for health, excessive manganese intake can lead to toxicity and adverse health effects. Chronic manganese exposure has been linked to neurological disorders such as Parkinson's disease and cognitive impairment. Therefore, individuals should be mindful of their overall manganese intake and consider dietary balance and individual health factors when incorporating these leaves into their diet.

In conclusion, the manganese concentration of water, bitter, and pumpkin leaves is remarkably higher than the WHO criterion, highlighting their nutritional significance as abundant dietary manganese sources. Even though these leaves have many health advantages, people should eat them in moderation as part of a balanced diet to reduce their risk of manganese poisoning. In order to maximise the nutritional advantages of these manganese-rich plants while maintaining acceptable manganese intake levels, more research may be conducted.

From the data above, the lead concentrations in these varieties of leaves are far lower than the advised level except for that of water leaf. The brain system, kidneys, and cardiovascular system are among the human health issues that lead can cause. Lead is a hazardous heavy metal. Thus, eating foods like water leaves, bitter, and pumpkin that have a lower lead concentration may help minimise the chance of lead exposure and the health problems that go along with it. These leaves are comparatively safe options in terms of lead contamination because of their low lead level. However, while assessing the general safety and nutritional worth of these leafy greens, it's important to take into account aspects other than just lead content. To guarantee the safety and quality of food items, thorough risk assessments should take a variety of pollutants and nutritional aspects into account.

According to the data from the table above lead content in water leaf is higher when compared to the WHO STANDARD.

This could be as a result of various factors, including environmental contamination, industrial activities, agricultural practices, or water pollution.

When lead is found in amounts higher than allowed by law or when it endangers the environment or public health, it is regarded as a contamination. Although lead is a naturally occurring element, human activity has greatly raised the amount of lead in the environment, causing widespread contamination. In order to safeguard public health and the environment, it is critical to address the sources of lead pollution, as evidenced by the elevated lead levels found in water leaves. Implementing strategies to lower industrial emissions, enhancing waste management procedures, keeping an eye on and cleaning up polluted areas, and supporting sustainable farming methods to reduce lead exposure in food production are some of the steps that may be taken to mitigate lead contamination. To further guarantee food safety and safeguard public health, food products must be routinely tested for lead contamination.

In conclusion, the fact that bitter leaves and pumpkin had lower lead contents than the WHO threshold implies that these vegetables might be less hazardous in terms of lead contamination. However, in order to reduce the chance of exposure to lead from other sources, people should keep up excellent agricultural and food processing standards. In order to further lower the lead levels in these veggies and enhance food safety generally, more research may be conducted.

These results imply that the cadmium amounts in all three varieties of leaves are considerably lower than the advised level. One dangerous heavy metal that is known to have a negative impact on human health is cadmium, especially on the kidneys and bones. Consequently, eating foods lower in cadmium, such pumpkin, bitter, and water leaves, which have levels below the WHO threshold, may help lessen the risk of cadmium exposure and the health risks that go along with it. These leaves are comparatively safe options in terms of cadmium contamination because of their low cadmium level. However, while assessing the overall safety and nutritional value of these leafy greens, it's important to take into account aspects other than just the concentration of cadmium. To guarantee the safety and quality of food items, thorough risk assessments should take a variety of pollutants and nutritional aspects into account.

According to these results, the zinc concentrations in all three varieties of leaves are considerably lower than the advised level. An important micronutrient, zinc is involved in many physiological processes, such as DNA synthesis, wound healing, and immunological function. Thus, eating foods low in zinc, such bitter and wet leaves, pumpkin, may raise your risk of zinc insufficiency and related health problems. These foods also have zinc levels below the WHO recommended limit

These leaves might not be significant sources of dietary zinc because of their low zinc concentration. People who consume large amounts of these veggies as their main food sources may not be getting enough zinc. Consequently, it's critical that people eat a balanced diet full of a range of foods high in important micronutrients, such as zinc.

Finally, the low zinc concentration of water, bitter, and pumpkin leaves relative to the WHO criterion implies that these vegetables could not be important dietary zinc sources. Even if they have additional nutritional advantages, people should make sure their diets are varied and include foods high in zinc in order to meet their nutritional demands. Future studies might look into ways to increase the zinc content of these veggies or find different dietary sources of zinc for people with particular

3.6CONCLUSION

This study evaluated the possible effects of heavy metal levels on food safety and nutritional quality by analysing the amounts of these metals in water, bitter, and pumpkin leaves. The results of the research showed that the three different types of leaves had different amounts of heavy metals, including iron, copper, manganese, lead, cadmium, and zinc.

Compared to bitter and water leaves, pumpkin leaves showed higher concentrations of iron, manganese, and zinc, indicating that they may be good providers of these vital elements. Bitter leaves have a higher copper content, which suggests that they are important for delivering this mineral. Although water leaves had a lower overall heavy metal content than the other two varieties, they did show elevated levels of lead, which calls for more research and mitigating actions to guarantee the safety of food. With the exception of lead in water leaves, where concentrations of the heavy metal surpassed legal limits, most of the examined leaves' heavy metal concentrations were within safe bounds. These results highlight how crucial it is to keep an eye on the amounts of heavy metals in leafy greens in order to protect the general public's health and advance food safety.

To sum up, this study adds important knowledge about heavy metal contamination in water, bitter, and pumpkin leaves. It also emphasises the need for ongoing research and management techniques to guarantee the safety and nutritional value of leafy greens in food production systems.

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