ASSESSMENT OF HEAVY METALS IN LEAFY VEGETABLES PURCHASED FROM USELU MARKET USING ATOMIC ABSORPTION SPECTROSCOPY

BY

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CERTIFICATION

This is to certify that this research project was carried out and put together by UDO GOODNEWS INNOCENT, with Matriculation Number PSC2008012, a student of the Department of Chemistry, Faculty of Physical Science, University of Benin, Benin City, Edo State under supervision and guidance in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc. Honors) Degree in Pure Chemistry.

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DEDICATION

This project is specifically dedicated to God Almighty for his love, mercy, guidance and protection over my life and also to my parents Late Mr. Peter Udoh and Mrs. Uwem Peter for their unending love, counsels, prayers and support that have kept me through my stay in the University of Benin.

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TABLE OF CONTENTS

ABSTRACT

This study is to assess heavy metal content in three leafy vegetables. Telfairia occidentalis (Pumpkin leaf), Amaranth green (Green leaf) and Celosia argentea (Shoko leaf). Samples were collected from Uselu market, Egor local government area of Benin city in Edo State, Nigeria in the month of February. The samples were prepared using Atomic Absorption Spectroscopy (AAS) method. The level of five different heavy metals namely Lead (Pb), Cadmium (Cd), Zinc (Zn), Chromium (Cr) and Iron (Fe) were analyzed using Atomic Absorption Spectroscopy (AAS). Result obtained from the analysis shows that Pumpkin leaf contained a mean value of 2.00mg/kg Fe, 0.01mg/kg Cr, 2.11mg/kg Zn, while Cd and Pb were below detection limit (BDL). Shoko leaf had a mean value of 3.85mg/kg Fe, 0.04mg/kg Cr, 1.52mg/kg Zn, 0.01mg/kg Cd and Pb was below detection limit (BDL). Green leaf had a mean data of 7.70mg/kg Fe, 0.03mg/kg Cr, 1.64mg/kg Zn while Cd and Pb were below detection limit (BDL). Among the three samples, the mean value of all the heavy metals are below standard regulatory limit of 425.50mg/kg Fe, 0.03mg/kg Cr, 99.40mg/kg Zn, 0.20mg/kg Cd and 0.30mg/kg Pb except for Cr in Shoko leaf which mean value is slightly above that of standard regulatory limit. The samples are safe for human and animal consumption, but attention should be paid to Shoko leaf to avoid the level of Cr to be higher than it is by being mindful of the site for cultivation of the crop.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE

1.1 INTRODUCTION

Taxonomically classed as a member of the Angiospermae family, leafy vegetables are plant leaves that are eaten as a vegetable and may come with fragile petioles and shoots. They are also known as leafy greens, pot herbs, vegetable greens, or just greens (DuPont and Bennett, 2010). Edible plant leaves are known as green leafy vegetables, dark green leafy vegetables, leafy greens, or greens. Certain leafy greens can be eaten raw, while others might need to be cooked (Rodríguez et al, 2006). As the name suggests, these veggies are easily recognized due to their edible leaves and green color (Torkashvand and Sokhansanj, 2015). Eating leafy green vegetables could help to prevent cognitive deterioration as people age (Martha et al, 2017).

A vegetable can be more precisely described as "any plant, part of which is used for food," with "the edible part of such a plant" serving as a supplementary definition. A more accurate description would be any portion of the plant that is eaten for food that is not a fruit or seed, such as mature fruits that are included in a main course. (Stasz and Paull, 2009). Leafy vegetables are a great option for managing weight because of their low-calorie content, they are also great source of nutrients since they are low in fat, high in dietary fiber, and rich in folate, ascorbic acid, vitamin k, magnesium, and K (Aune et al, 2017). They are also rich in phytochemicals, including flavonoids and β-carotene.

A simple method to ensure your body gets the vitamins and minerals it needs for optimal wellness is to include leafy greens in your diet. They include significant amounts of iron, calcium, magnesium, potassium, vitamins. Vitamin A, promote the health of your skin and eyes (Eun et al, 2020). Vegetable and fruit-rich diets have been shown to lower blood pressure (Appel et al., 1997), lower the risk of heart disease and stroke (Hu, 2003), prevent some cancers (Boeing et al., 2012), lower the risk of digestive and eye issues (Rodriguez and Remesar, 2002), and improve blood sugar levels, which may help control appetite (Kahn et al, 2006).

Romaine lettuce, butter lettuce, red leaf lettuce, escarole, endive, spring mix, spinach, cabbage, kale, arugula, and chard are examples of leafy green goods. But in this case, our attention will be on Shoko, a Yoruba term for Celosia argentea, spinach, and pumpkin. Efo Shoko is a variety of Celosia that is consumed in soups, stews, and side dishes in portions of Nigeria, Benin, Congo, and Cameroon as well as in Indonesia and India. It is sometimes referred to as Lagos Spinach or Nigerian Spinach (Olasupo et al, 2019). According to (Gruben and Denton, 2004), it is classified as Celosia Argentea taxonomically. This native plant has leaves that are rich in folic acid and beta-carotene (Olasupo et al, 2019). Often referred to as the plumed cockscomb or silver cock's comb, Celosia argentea is an herbaceous plant of tropical origin in the Amaranthaceae family from India and Nepal (Lattier and Black 1995). The plant is known for its very bright colors.

Celosia needs full sun, six hours of sunshine every day, and soil that is either slightly acidic or slightly alkaline. It can withstand extreme heat, but freezing kills it. Depending on the variety, this sun-loving annual can reach heights of 6 to 36 inches and widths of up to 24 inches. The National Gardening Association. This vegetable has a lot of vitamins, minerals, and antioxidants. In the body, it is similarly high in calcium, iron, and zinc (Olasupo et al, 2019). One of the most popular dark green leafy vegetables is fluted pumpkin, or Ugwu in Igbo dialect. However, many of us are not aware of its health benefits. This plant is indigenous to West Africa, and it is primarily cultivated and consumed in the southern of Nigeria (Agishi, 2021).

For an adequate harvest, ugu leaves mature three to four weeks after germination. They continue to mature as long as regular harvesting is conducted. To harvest ugu, carefully cut the mature stem with a knife to prevent injuring the mode. With proper management, harvesting can continue for up to six months. April and May are the best months to grow fluted pumpkins because of the few amounts of rain that falls during this time. Planting comes after the soil has been dressed. The seeds should be spaced two to three feet apart. It is recommended to complete this by late evening or very early in the morning (Guardian.ng, 2018; Obasi, 2020).

English Name: Fluted Pumpkin Leaves. Local Name: Ugu (Igbo) Botanical Name: Telfairia Occidentalis.

Prepare pumpkin leaves the same way you would spinach or Swiss chard. Pumpkin leaves are typically used in African soup recipes. They are delicious in crunchy fresh stir-fries in Zambia, and they can be diced or shredded and added to soups, sauces, stews, and various sishebos (Unilever, 2024). Pumpkin leaves are used in Nigeria to make native, vegetable, okra, egusi, and ogbono soups. According to (Demandafrica, 2019), uga leaves are also used to prepare tomato stews, sauces, porridge yam, and porridge beans. Crucial minerals, vitamins, and antioxidants are found in pumpkin leaves, including iron Fe, potassium K, sodium Na, phosphorus P, calcium Ca, and magnesium Mg (Kayode et al., 2011).

They possess sufficient levels of vitamins A, B, and C. In addition, the leaves offer several skin benefits in addition to their vitamin A benefits for vision. Moreover, the presence of vitamin C aids in the formation of scar tissue and the healing of wounds (Pulse.ng, 2023). These vitamins are essential for your body's development and healthy cell structure (Shubham Pandey, 2024). In Nigeria, the term "amaranth green" is commonly referred to as "green leaf". In Yoruba, amaranth green is also referred to as Tete. The Greek word amarantos, which meaning unfading, is where the word "amaranth" originates. The idea that the plant is eternal led to the naming of the species. The seeds of the amaranth plant are used to propagate. Amaranth plant grows best in any average well-drained acidic soil with good air circulation. Thrives in temperatures between 70- and 85-degrees Fahrenheit; needs at least six hours of direct sunlight (Angela, 2023).

Young leaves of Amaranthus dubius can be consumed either fresh or cooked. It is typically prepared in Uganda using peanut sauce, onions, and tomatoes (Jenna, 2023). In Nigeria, amaranth greens are typically consumed as an ingredient in soups and served alongside a food high in carbohydrates, such as garri or mashed yam (Funke, 2016). You can use amaranth leaves as a food coloring. Pink and red are the primary colors that are utilized (Ganesan and Kumaresan, 2015). Currently known as Red Dye No. 2, amaranth food coloring was outlawed in the US in 1976 (FDA's Regulatory Process). A cup of amaranth leaves can provide 97% of your daily need for vitamin A, an antioxidant, among other nutrients. Additionally, they are abundant in antioxidant flavonoid polyphenols like beta-carotene, zeaxanthin and lutein which provide a protective layer against oxidative stress caused by free radicals. Vitamin A is also needed for healthy skin and proper vision (Nikita, 2024).

Because amaranth greens are abundant in iron, vitamins A, B6, and folate, they also aid in the prevention of anemia. Increases in hemoglobin and red blood cell count are also facilitated by the high iron and folic acid levels (Gaga, 2017). In trace amounts, heavy metals are unquestionably vital components for both humans and plants (Oladebeye, 2017). According to (Järup, 2003), heavy metals are defined as those metals that have a specific density of greater than 5 g/cm3 and have a negative impact on both the environment and living things. They have lengthy biological half-lives and are not biodegradable (Heidarieh et al., 2013). These metals are zinc (Zn), manganese (Mn), cobalt (Co), arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), nickel (Ni), and mercury (Hg).

1.1.1 Background of Study

The body may absorb heavy metals in several ways. They are contact with the skin, ingestion, and inhalation. However, the specific emphasis of this study is on heavy metals that are ingested through leafy vegetables. Leafy Vegetables with may encounter heavy metals through soil, water, or air pollution.

Higher concentrations of heavy metal contamination are seen in leafy vegetables, which poses a health risk to humans. Human health may be seriously endangered by these metals, especially at levels over the extremely low limits required by the body (Gupta et al, 2008). Therefore, measures must be taken to slow down the rate at which these metals are consuming and damaging the health of humans and animals.

1.1.2 Statement of Problems

These heavy metals can also be obtained from garbage, such as liquid, solid, or hazardous waste deposited in the soil. Hazardous or radioactive waste can be among contaminants (Onibokun and Kumuyi, 1996; UNDP, 2006). Excessive levels of micronutrients or heavy metals in soil can have harmful effects on plants and even constitute a health risk to humans (Murugesan et al, 2008). Vegetables grown on waste dump sites abandoned for an extended period of time, however studies have shown that these plants can absorb large amounts of heavy metals from contaminated and polluted soils (Cobb et al, 2000; Benson and Ebong, 2005).

The world is aware of the health risks that accompany exposure to heavy metals, and reports of ingesting them through food are common (Zhuang et al, 2009). These metals can have an impact on the body's essential organs, including the blood, bones, kidneys, and liver. Continuous exposure to cadmium can cause nephrotoxicity, immunological disorders, and bone toxicity in addition to having a negative impact on the liver and lungs (Patrick, 2003 and Klaassen et al, 1999).

Long-term lead exposure can hinder children's intellectual development and eventually lead to nephropathy and cardiovascular problems (Ekong et al, 2006, Goyer, 1993, and Navas-Acien et al, 2007). Elevated Cu concentration in the body can cause liver damage and gastrointestinal problems, while higher Zn exposure can negatively impact the immune system and lower HDL levels in the body (Gaetke et al, 2003). The toxicity resulting from exposure to heavy metals can be either acute or chronic, depending on the duration of exposure (Dorne, 2011 and Jarup, 2003).

1.1.3 Justification

One of the most significant global health concerns is the presence of heavy metal pollution in the environment, even at low concentrations, and the long-term cumulative health impacts which follow from it (Oladebeye, 2017). These metal contamination of edible leafy plants causes serious health problems that may be fatal or expensive to treat. When consumed in quantities large enough to result in health problems for both humans and animals, vegetables can absorb heavy metals and collect them in both their edible and inedible sections (Bahemuka and Mubofu, 1991; Alam et al, 2003).

Hunger and malnutrition are caused in both humans and animals when heavy metals contaminate soil, which makes up only 7.5% of the land on Earth that is used for agriculture. And it is subsequently carried by erosion, leaching into rivers, streams, and other water bodies, affecting them and possibly causing them to become unhealthy for human consumption or to die. One of the main sources of heavy metal contamination in soil and crops is toxic waste and waste disposal sites. This is the rationale for the design of this study, which aims to ascertain the heavy metal contents in leafy vegetables from particular vegetable plantations and Nigerian market locations.

1.1.4 Scope of Work

These leaves: Pumpkin, Shokor and Green leaves were gotten from Uselu market for analysis of the concentration of five heavy metals. This research therefore covers Uselu market, Egor local government area if Benin city, Edo State.

1.1.5 Aim

The aim of this work, is to determine and measure the concentration of five heavy metals which includes Lead (Pb), Cadmium (Cd), Zinc (Zn), Chromium (Cr) and Iron(Fe) in pumpkin, Shokor and green leaves sold at Uselu market.

1.1.6 Objectives

1. To determine the presence of heavy metals in selected vegetables.

2. To compare the concentration of heavy metals in these selected vegetables with the permissible limit set by regulatory bodies.

3. To determine if these vegetables are safe for human consumption.

4. To make data available for government use through publication.

1.2 LITERATURE REVIEW

1.2.1 Heavy Metals

Globally, the problem of heavy metal pollution in leafy greens poses a serious threat to food safety and public health. Heavy metals are one of the main pollutants of leafy greens (Mapanda et al, 2005). Heavy metals can be classified as essential or non-essential. The different levels of heavy metals that are necessary for living things are called essential metals. The following are some examples of essential heavy metals: zinc (Zn), magnesium (Mg), manganese (Mn), copper (Cu), chromium (Cr), iron (Fe), molybdenum (Mo), nickel (Ni), selenium (Se), and cobalt (Co). Even at low concentrations, non-essential heavy metals can have a detrimental effect on the health of living things. Mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) are a few examples.

The body's many biological and biochemical processes may get disrupted if hazardous levels of heavy metals are consumed over an extended period of time. (Oladebeye, 2017). Vegetables, particularly leafy vegetables, can become contaminated with heavy metals through a variety of means. Some of these techniques include soil pollution, air pollution, water pollution, and agricultural inputs.

1.2.1.1 Soil Pollution

A chemical or material that is out of place, present in a soil at a higher concentration than usual, and negatively affects any non-targeted organism is referred to as soil pollution (Rodríguez-Eugenio et al, 2018). There are two categories of heavy metal pollution in soil: naturally occurring and man-made. The former results from natural processes that are part of the formation of soil, such as elemental dissolution via mineral weathering and background sources (Tapia-Gatica et al, 2022). Wastewater irrigation, pesticides, fertilizers, animal dung, leaded paint, sewage sludge, petroleum distillate spills, coal combustion residue, and trash disposal are examples of intentional or anthropogenic contamination (Masindi et al, 2018).

In addition to being a fantastic source of nutrients and contaminants, soil is crucial for both socio-ecological sustainability and human health. The influx of heavy metals from processes like industrialization, urbanization, and agriculture leads to an increase in soil pollution. Heavy metals, in contrast to organic contaminants, are non-biodegradable and can persist in the soil for over 150 years. Human health is seriously impacted by the ongoing rise in heavy metal concentration in the soil brought on by improper farming practices. The content of heavy metals in the soil is frequently increased by the long-term use of wastewater for agricultural irrigation (Ayda et al, 2023). The crops grown there are contaminated by the heavy metals in the soil, especially green vegetables.

1.2.1.2 Water Pollution

For socioeconomic progress to continue, water is essential. Human activities including overusing water resources, traveling, and releasing industrial waste into bodies of water without permission are the main causes of water pollution. Untreated domestic and industrial wastewater can seriously contaminate water supplies and have a long-term impact on aquatic ecosystems. The harmful water streams include organic solvents, nitrates, synthetic colors, and pigments. (Lone et al, 2017). Discharged into adjacent rivers are untreated industrial effluent and municipal wastewater from cities, which degrade the aquatic ecosystem. (Chowdhury et al, 2015). Heavy metals that enter rivers, oceans, or aquatic environments will be regarded as important pollutants due to their high toxicity, persistence, and bioaccumulation qualities. (Anita et al, 2023).

1.2.1.3 Air Pollution

Many heavy metals are naturally occurring environmental elements, but because of indiscriminate anthropogenic activity driven by the desire for contemporary comforts and improved living standards, their biochemical balance and geochemical processes have been changed. Consequently, the urban populace has encountered an extensive array of detrimental health consequences, such as cancer and harm to vital organs, in addition to pulmonary and cardiovascular inflammation and acute respiratory diseases. (Ritusmita et al, 2023). Heavy metal emissions into the atmosphere are attributed to industrial businesses' activities.

1.2.2 HEALTH EFFECTS IN SELECTED HEAVY METALS

From the perspective of human health, each of the heavy metal imparts different effects and symptoms (Lesmana et al, 2009).

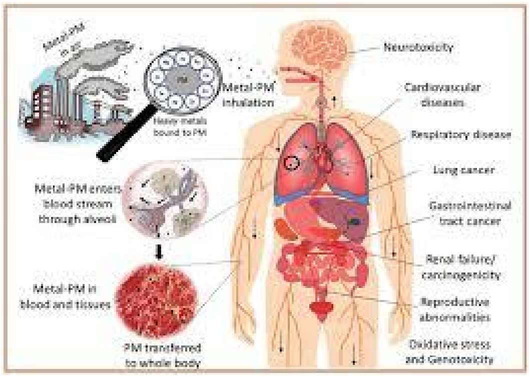


Fig. 1.1 Diagram showing how heavy metals affect the human internal organs.

1.2.2.1 Iron (Fe)

Although iron is necessary for human health and plant growth, excessive concentrations of the mineral can be hazardous. Anthropogenic activities such industrial emissions, mining, and agricultural practices involving the use of soil amendments and fertilizers containing iron are the main causes of iron contamination in leafy vegetables (Alloway, 2013). Soil iron levels are also influenced by natural processes, such as the weathering of rocks and sediments that are rich in iron (Bolan et al, 2010). In leafy vegetables, excessive iron absorption can interfere with a number of physiological functions, including as photosynthesis, food uptake, and enzyme activity (Liu et al, 2003). Chlorosis, leaf necrosis, stunted growth, and decreased yield are common signs of iron poisoning (Kabata-Pendias and Mukherjee, 2007). Eating green plants tainted with high iron concentrations may be harmful to human health.

Oxidative stress, liver damage, and gastrointestinal problems can result from long-term exposure to iron toxicity (Xiong et al, 2016). Furthermore, iron may increase the toxicity of other heavy metals by interacting with other metals in the body (Ratcliffe et al, 2019). The overabundance of iron in plants can be mitigated by methods like phytoremediation, which uses plants to absorb, immobilize, or decompose pollutants from the soil (Salt et al, 1998). The safety and quality of leafy vegetables are seriously threatened by excessive iron, which also has an adverse effect on human health and plant health.

1.2.2.2 Chromium (Cr)

In small quantities, chromium is also a necessary heavy metal for humans and plants, but in higher concentrations, it is toxic. Because of their large surface area and quick transpiration rates, leafy crops are especially susceptible to chromium contamination. Chromium is found in soil naturally, mostly as chromite ore, which can weather and release chromium into the surrounding environment (Bai et al, 2017). Anthropogenically, chromium is released into the environment by mining, industrial waste disposal, and mining processes (Alloway, 2013). Through a variety of pathways, including as active transport and passive diffusion, leafy crops absorb chromium through their roots (Shanker et al, 2005).

The oxidation state of the chromium in the soil, its pH, and the amount of organic matter all affects the amount chromium plants can absorb (Dube et al, 2003). Chromium is absorbed and then builds up in plant tissues, especially in the edible parts like leaves. Chronic exposure to high-concentration green vegetables or an excessive consumption of them can result in chromium poisoning, which can cause gastrointestinal issues, respiratory issues, and cancerous implications (O'Brien et al, 2003). Furthermore, over time, chromium buildup in the body can result in long-term health issues (O'Brien et al, 2003). Humans can convert methemoglobin to hemoglobin by being exposed to increased concentrations of chromium compounds (Koutras et al, 1965).

1.2.2.3 Zinc (Zn)

Another vital heavy metal that is required in trace amounts by both plants and animals is zinc. For both humans and animals, zinc is a vital nutrient that is required for the proper operation of numerous metallo-enzymes. Alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase, superoxide dismutase, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymerase are some of these enzymes. Acute oral zinc exposure might result in symptoms such pancreatitis, vascular shock, dyspeptic nausea, vomiting, diarrhea, and tachycardia (Salgueiro et al, 2000).

1.2.2.4 Lead (Pb)

Unlike other metals like zinc, copper, and manganese, which have biological purposes, lead is a very toxic heavy metal that disrupts several physiological processes in plants (Monisha et al, 2014). Lead toxicity, commonly referred to as lead poisoning, can be acute or persistent. Acute exposure might result in vertigo, hallucinations, arthritis, lethargy, weariness, headache, hypertension, stomach discomfort, and renal failure. Acute exposure mostly happens in the workplace and in some lead-using manufacturing industries. According to (Martin and Griswold 2009), prolonged exposure to lead can induce mental retardation, birth defects, psychosis, autism, allergies, dyslexia, weight loss, hyperactivity, paralysis, muscle weakness, brain damage, kidney damage, and even death.

Lead causes hemoglobin deficiencies, mental issues, liver and kidney problems, and a lower fertility rate in living things (Samuel et al, 2022; Swaran and Shruti, 2017;). Due to dust adhesion and transfer into the tissues, plants cultivated in lead-contaminated soils develop low levels of lead in the edible parts of the plant (Finster et al., 2004). Lead and its compounds have accumulated in the air, water, and soil as a result of human activities such mining, manufacturing, and burning fossil fuels (Monisha et al., 2014). Anyone who has encounter lead can become poisoned.

People who work with metals, drink water from pipes made of older metals (lead), take more medicine or supplements than is recommended, live in an area with high levels of air or water pollution, eat a lot of metal-containing foods, or use non-edible metal products (paint) are most likely to be affected by this. Due to their developing bodies and increased sensitivity to the negative effects of heavy metals, children are more susceptible to heavy metal poisoning (Cleveland clinic, 2022). The primary causes of the disease are lead ions from household and environmental sources, yet the risk of lead toxicity can be decreased with appropriate preventive measures (Brochin et al, 2008).

1.2.2.5 Cadmium (Cd)

Heavy metal cadmium is extremely poisonous and detrimental to most organ systems. Humans are widely contaminated by it; the main causes include cigarette smoke, welding, and contaminated food and drink (Bernhoft, 2013). Cadmium exposure can happen when someone consumes tainted food, water, soil, or dust, or when they breathe in tobacco smoke or particulate matter from the surrounding air (ATSDR, 2012). The International Agency for Research on Cancer has categorized cadmium and its compounds as Group 1 carcinogenic for humans (Henson and Chedrese, 2004). Natural processes including volcanic eruptions, weathering, and river transport release cadmium into the environment, as do some human activities like mining, smelting, smoking tobacco, and burning municipal garbage and manufacture of fertilizers. (Monisha et al, 2014).

Even though most developed nations have seen a noticeable decrease in cadmium emissions, workers and residents in the contaminated areas still worry about it. Acute and long-term intoxications are both possible with cadmium (Chakraborty et al., 2013). Numerous organs have been shown to be poisonous to cadmium, as will be covered later. Through oxidative stress (Matović et al., 2012), epigenetic modifications in DNA expression (Wang et al., 2012), and the blockage or upregulation of transport pathways (Thévenod et al., 2010), cadmium causes tissue damage, especially in the kidney tubule's proximal S1 segment (Vesey, 2010). Additionally, phosphate fertilizers, detergents, and refined petroleum products all contain cadmium as an impurity. Furthermore, the geochemical mobility of Cd has been enhanced by acid rain and the ensuing acidification of soils and surface waters. when a result, surface-water concentrations of Cd tend to rise when the pH of lake waterfalls (Campbell, 2006). The Jintsu River Valley, close to Fuchu, Japan, is home to the most remarkable and well-known case of cadmium poisoning, which was caused by dietary consumption of the metal. The illness known as itai itai, which translates to "ouch, ouch," affected the victims. The combination of painful osteomalacia (bone disease) and kidney malfunction is the cause of the symptoms. Irrigated rice tainted by an upstream mine that produced Pb, Zn, and Cd was identified as the cause of cadmium toxicity in the Jintsu River Valley (Oladebeye, 2017).

Highly poisonous and unnecessary, cadmium is known to negatively impact cellular enzymatic systems, cause oxidative stress, and cause nutritional deficiencies in plants (Irfan et al., 2013). Educating consumers about the value of eating a variety of foods and making sure they are getting the right amount of micronutrients (zinc, iron, and calcium) are two additional tactics that can be used at the consumption point to reduce the rate of absorption and body retention of cadmium (Heather et al, 2020). One may also think of mitigating at other points in the food supply chain. These include restricting the amount of stabilizers containing cadmium in plastics and cadmium-based ceramic glazes, as well as doing away with galvanized equipment used in postharvest processing (ATSDR, 2012).

1.2.3 PUMPKIN LEAF

Pumpkin leaf taxonomically classified as Telfairia occidentalis is a tropical vine grown in West Africa as a leaf and for its edible seeds. It is commonly called fluted pumpkin, ugu (in Igbo language), okwukwo - wiri (in Ikwerre language) and ikong- ubong (in Efik and Ibibio languages), "Akwukwor ri" (in Etche language). Telfairia occidentalis is a member of the family Cucurbitaceae and its indigenous to Southern Nigeria (Akoroda, 1990).

1.2.3.1 Scientific Classification Of Pumpkin

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Telfairia
Species	T. occidentalis

1.2.3.2 Structure

The fluted pumpkin fruit is quite large; one study documented a range of 16→105 centimeters (6.3→41.3 in) in length, and an average of 9 cm in diameter (Okoli et al., 1983). The same study found the seed count in larger gourds to reach upwards of 196 per fruit, typically measuring between 3.4 and 4.9 cm in length (Okoli et al., 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 color of the fruit when young, and yellow when ripe (Okoli et al., 1983). Dioecious flowering is most common in the fluted gourd, with very few documented cases of monoecious flowering.

Fig. 1.2 Pumpkin leaf

1.2.3.3 Nutritional Content

Considered an ↑oil seed↓, the fluted pumpkin is high in oil (30%) (Akoroda, 1990). Shoots of T. occidentalis contain high levels of potassium and iron, while seeds are composed of 27% crude proteins and 53% fats (Aiyelaagbe and Kintomo, 2002) The leaves contain a high amount of antioxidants and hepatoprotective and antimicrobial properties (Nwanna et al, 2008).

The young shoots and leaves of the female plant are the main ingredients of a Nigerian soup, ofe egwusi. The large (up to 5 cm), dark-red seed is rich in fat and protein and can be eaten whole, ground into powder for a kind of soup, or made into a fermented porridge.

1.2.3.4 Cultivation

T. occidentalis is typically grown vertically on trestle-like structures; however, it can be allowed to spread flat on a field (Okoli et al, 1983). A beneficial outcome of growing the gourd flat is the suppression of weeds, especially when intercropped with a tall, upright plant such as maize. The growing period begins in April or May when seeds are planted; (Emebiri and Nwufo, 1990) the first leaves and shoots can be harvested after a month and can be collected every 2–4 weeks thereafter (Okoli et al, 1983). Seeds are planted directly in the soil, typically in groups of three to increase output in a case of a failed germination (Akoroda, 1990). Fruit is typically harvested between October and December (Emebiri and Nwufo, 1990). The seeds are subsequently collected and dried; a portion of them are consumed, while the remainder are stored for the following planting season. Although dependent upon soil type, the fluted gourd is able to ratoon and subsequently produce many flushes of fruit over long periods (Aiyelaagbe and Kintomo, 2002). It is able to ratoon with the highest degree of success in well-drained soils (Akoroda, 1990). It is propagated using the seeds. Its seed is housed in another greater covering or hard shell which protects it from harm. It survives drought and can retain its life in the root even after many years. It is a creeping plant and grows well if staked with bamboo sticks.

1.2.3.5 Pest And Pathogens

A major concern of buyers and sellers of fluted gourd is pod rot. Infection occurs most frequently during transport, although it can also arise before the plant is harvested, starting as a small lesion that creates an avenue for pathogens to penetrate the fruit (Emebiri and Nwufo, 1990). It is most common for the affected area to appear brown in colour, indicating contamination by R. stolonifer or Erwina. However, the infected area may also appear black (indicating the presence of Aspergillis niger) or grey (B. theobromae) (Emebiri and Nwufo, 1990). Other symptoms associated with the presence of these pathogens include softening of the pod tissue accompanied by a pungent odour, or watery fluid in the fruit (Emebiri and Nwufo, 1990). Pod rot can be reduced by avoiding damage to the fruit during harvest and transport (Emebiri and Nwufo, 1990).

1.2.3.6 Uses

The edible seeds can be boiled and eaten whole, or fermented and added to ogili (Badifu, 1993). The fluted gourd has been traditionally used by indigenous tribes as a blood tonic, likely due to its high protein content (Akoroda, 1990). Flour produced from the seeds can be used for high-protein breads (Giami, 2003). Furthermore, the shoots and leaves can be consumed as vegetables, as Nigerians use it to make soup, stew, egg sauce and vegetable sauce (Akoroda, 1990). When T. occidentalis is prepared for herbal medicine, it is used to treat sudden attack of convulsion, malaria, and anaemia; it also plays a vital and protective role in cardiovascular diseases.

1.2.4 SHOKO LEAF

Shoko leaf taxonomically classified as Celosia argentea, commonly known as the plumed cockscomb or silver cock's comb is an herbaceous plant of tropical origin in the Amaranthaceae family from India and Nepal. The plant is known for its very bright colors. In India and China, it is known as a troublesome weed (Grant, 1954).

1.2.4.1 Scientific Classification Of Shoko Leaf

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Order	Caryophyllales
Family	Amaranthaceae
Genus	Celosia
Species	C. argentea

1.2.4.2 Structure

Celosia argentea is a tender annual that is often grown in gardens, it can also grow perennially (Extension Gardener, 2023). It blooms in mid-spring to summer. The plant exhibits dodecaploidy. The flowers are tiny and hermaphrodite, they are packed in narrow, pyramidal, plume-like heads 4→10 inches (10→25 cm) long with vivid colors including shades of orange, red, purple, yellow and cream (Plant Finder, 2023). It is propagated by black seeds. The seeds come in capsules (Plant Finder, 2023); they are extremely small, up to 43,000 seeds per ounce (Dave's Garden, 2013).

1.2.4.3 Cultivation

As these plants are of tropical origin, they grow best in full sunlight and should be placed in a well-drained area. Full sunlight means they should get at least 8 hours of direct sunlight. For healthy growth plant them in the area where they get early morning sunlight and afternoon shade. In the afternoon the sunlight is mostly harsh especially in hot summer. Afternoon shade will save the plant from excessive heat (Shiny Plant, 2020). The flowerheads can last up to 8 weeks, and further growth can be promoted by removing dead flowers (Dave's Garden, 2013).

1.2.4.4 Uses

It is used in Africa to help control growth of the parasitic Striga plant. It can also be used in soaps (AVRDC, 2013). The leaves and flowers are edible and are grown for such use particularly in west Africa and Southeast Asia (Grubben and Denton, 2004). Celosia argentea var. argentea or "Lagos spinach" is one of the main boiled greens in West Africa, where it is known as soko yòkòtò (Yoruba) or farar áláyyafó (Hausa) (ECHO, 2010; Hanelt et al, 2001).

1.2.5 GREEN LEAF

Green leaf also known as Amaranthus is a cosmopolitan group of more than 50 species which make up the genus of annual or short-lived perennial plants collectively known as amaranths. Some of the more well-known names include "prostrate pigweed" and "love lies bleeding" (Kassia 2017; Plant family, 2015) Some amaranth species are cultivated as leaf vegetables, pseudocereals, and ornamental plants(Bensch et al, (2003). Catkin-like cymes of densely packed flowers grow in summer or fall (Kindersley, 2008).

1.2.5.1 Scientific Classification

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Order	Caryophyllales
Family	Amaranthaceae
Subfamily	Amaranthoideae
Genus	Amaranthus

1.2.5.2 Description

Amaranth grain contains phytochemicals that are not defined as nutrients and may be antinutrient factors, such as polyphenols, saponins, tannins, and oxalates. These compounds are reduced in content and antinutrient effect by cooking (Amaranth Institute, 1992; Hotz, 2007)

1.2.5.3 Uses

Amaranth species are cultivated and consumed as a leaf vegetable in many parts of the world. It is used for the preparation of soup in most southern part of Nigeria.

1.2.6 DETERMINATION OF HEAVY METALS

The heavy metal ions are usually quantified using colorimetric and spectroscopic techniques. The choice of the method to be used is determined by its accuracy and the metal ion type. Spectroscopic methods are suitable for all metals while colorimetric technique is suitable for only metals within the visible region. There are two spectroscopic techniques: Absorption Atomic Spectroscopy (AAS) and UV-Visible Spectroscopy.

1.2.6.1 Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectroscopy (AAS) is an analytical technique used for quantitative and qualitative determination of concentration of metal ions or atoms in a sample by free atoms in the gaseous state. Atomic absorption spectroscopy is based on absorption of light by free metallic ions.

In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution, or directly in solid samples via electrothermal vaporization, and other research like pharmacology etc.

Atomic Absorption Spectroscopy follows the principle of the Beer-Lambart law. It is a linear relationship between the absorbance and the concentration, molar absorption coefficient and optical path length of light passing through a solution.

The law states that the intensity of the transmitted radiation passing through a solution decreases exponentially with increasing in concentration (c) and thickness (b) of the medium.

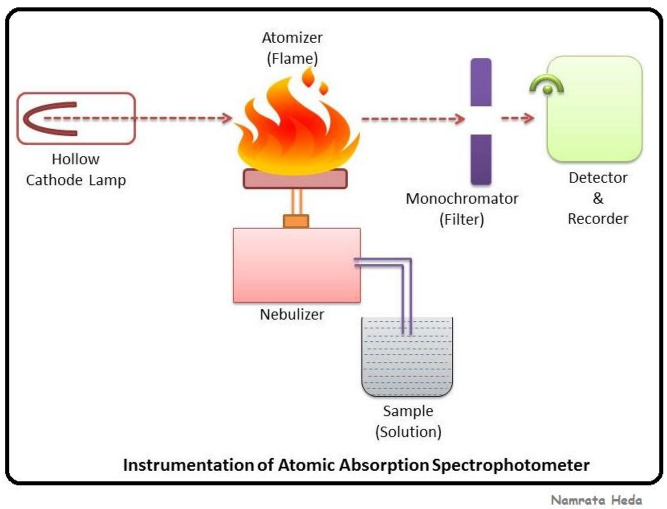
$A = \varepsilon c l$

Where: * A is the absorbance measured.

* ε is the molar absorptivity (constant specific to the element and wavelength).

* c is the concentration of the analyte.

* l is the path length of the light through the sample.



The AAS technique follow several steps for the analysis of a sample:

Step 1: Sample Preparation:

The sample is typically dissolved in a suitable solvent (acids, water) to create a liquid solution. This ensures the analytes are present as free atoms, ready for absorption.

For solid samples like ores or minerals, additional steps like grinding and digestion may be required to break down the matrix and liberate the analytes.

Step 2: Atomization:

The prepared solution is nebulized into a fine mist and introduced into a high-temperature flame (air-acetylene or nitrous oxide-acetylene mix). The intense heat in the flame excites the electrons in the analyte atoms, promoting them to higher energy levels.

Step 3: Absorption:

Simultaneously, a hollow cathode lamp containing the same element as the analyte emits a specific wavelength of light that corresponds to the energy difference between the excited and ground state of the analyte atoms.

As the emitted light passes through the atomized sample, some photons are absorbed by the excited analyte atoms, causing them to return to their ground state. This absorption decreases the intensity of the light at the specific wavelength.

Step 4: Measurement and Analysis:

The light intensity before and after passing through the sample is measured by a detector.

The difference in intensity is directly proportional to the concentration of the analyte in the sample, following the Beer-Lambert law.

Step 5: Calibration and Quantification:

To determine the actual concentration of the analyte, the instrument is calibrated using standard solutions containing known concentrations of the element.

By comparing the measured absorbance of the sample to the calibration curve, the concentration of the analyte in the original sample can be calculated.

Feedback Mechanism:

The measured absorbance directly provides feedback on the concentration of the analyte in the sample. This feedback loop allows the AAS to analyze various samples efficiently and determine their elemental composition with high accuracy.

In summary, AAS utilizes the unique absorption properties of elements to accurately quantify their concentration in samples. By preparing the sample, atomizing the analytes, measuring their absorption of specific light, and applying the Beer-Lambert law, this technique helps us understand the elemental makeup of diverse materials across various scientific and industrial fields.

While atomic absorption spectroscopy is the technique used for the measurement of heavy metals, the measurement of the heavy metals is known as atomic absorption spectrometry.

Atomic Absorption Spectrometry is a technique used for the measuring of quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. The atoms absorb ultraviolet or visible light and make transitions to higher energy level.

1.2.7 DIGESTION

This is the process in which volatile materials in a sample are combusted. These volatile materials include organic materials such as carbohydrates, fats and oils, protein etc. After digestion, the inorganic materials remaining are usually the trace metal elements.

Two major methods can be used carry out digestion procedure and they are:

- Dry ashing
- Wet ashing

1.2.7.1 Dry Ashing

Dry ashing is the process of oxidizing an organic sample with oxygen or air at high temperature leaving the inorganic component for analysis. The simplest method for decomposing of organic sample is to heat the sample over a flame in an open dish or crucible until all carbonaceous material have been oxidized to carbon dioxide. The technique is used for sample which has carbon content.

1.2.7.2 Wet Ashing

This is a method used to dissolve metals into solution in the presence of organic molecules prior to elemental analysis. All acid procedures make use of oxidizing agents to break down the organic matters.

This technique involves irradiating the sample in strong acids in a closed vessel and raising the temperature creating a combination of acids which increases the speed of the thermal decomposition as well as the solubility of the metals in the solution which makes it possible for elemental analysis. For this study, triacid digestion was used.

CHAPTER TWO

2.0 MATERIALS AND METHOD(S)

2.1 MATERIALS

Beakers

Standard flask

Sample bottles

Volumetric flask

Blender

Measuring cylinder

Conical flask

Whatman filter paper

Glass rod

Spatula

Funnel

Digestive flask

Gloves

Burner

Sand bath

Reagents

Nitric acid

Perchloric acid

Sulphuric acid

2.2 METHOD

2.2.1 PRE-SAMPLING TREATMENTS

All analysis were carried out following standard analytical methods. All reagents were of analytical grade. All glass wares thoroughly washed and dried prior usage for experiment.

2.2.2 SAMPLES

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

Pumpkin leaf (Telfairia occidentalis)

Green leaf (Amaranth green)

Shoko leaf (Celosia argentea)

2.2.3 SAMPLE COLLECTION AND PREPARATION

The three samples each were obtained from Uselu market, Egor local government are, Benin City, Edo State.

The leaves were carefully separated from their stalks and further washed thoroughly with water to remove any surface contaminants thereafter it was sundried for a week to remove all moisture content. The dried samples were grounded finely with blender.

2.2.4 SAMPLE DIGESTION

2g of each grounded vegetable sample was digested in a 10ml mixture of three acids which includes, Nitric acid (HNO_3), Sulphuric acid (H_2SO_4) AND Perchloric acid ($HCLO_4$) in the ratio of 5:1:1. An orange, brown coloration was observed. A blank matrix was also prepared using only the mixture of the three acids. The mixtures were left over night for about 12 hours. The mixtures were heated for about 45 minutes to 1 hour continually until transparent fumes were observed which indicated that the samples were completely digested. Thereafter the mixture was allowed to cool and then it was filtered using Whatman No. 1 filter paper to remove any form of residual undigested particles. The filtrate was poured into the volumetric flask and made up to 100ml mark using distilled water. It was finally transferred into the sample bottles for analysis by the atomic absorption spectrometer (AAS).

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

This work is designed to evaluate the levels of heavy metals in leafy vegetables that are collected in Uselu market. Details are shown in the tables below:

Table 3.1: Heavy metals concentration value in leafy vegetable sample after analysis.

Heavy Metals	Pumpkin Leaf (Mg/kg)	Shoko Leaf (Mg/kg)	Green Leaf (Mg/kg)	Blank
LEAD	BDL	BDL	BDL	BDL
CADMIUM	BDL	0.01	BDL	BDL
ZINC	2.17	1.58	1.63	0.12
CHROMIUM	0.02	0.04	0.04	0.02
IRON	2.50	4.10	8.20	0.40

BDL →Below Detection Limit

Table 3.2: Second concentration value

Heavy Metals	Pumpkin Leaf (Mg/kg)	Shoko Leaf (Mg/kg)	Green Leaf (Mg/kg)	Blank
LEAD	BDL	BDL	BDL	BDL
CADMIUM	BDL	0.01	BDL	BDL
ZINC	2.19	1.60	1.80	0.03
CHROMIUM	0.02	0.06	0.04	0.01
IRON	2.30	4.40	8.00	0.40

Table 3.3: Mean value of leafy vegetable samples

Heavy Metals	Pumpkin Leaf (Mg/kg)	Shoko Leaf (Mg/kg)	Green Leaf (Mg/kg)
LEAD	BDL	BDL	BDL
CADMUIM	BDL	0.01	BDL
ZINC	2.11	1.52	1.64
CHROMIUM	0.01	0.04	0.03
IRON	2.00	3.85	7.70

Table 3.4: Comparison of the mean value of the samples with standard regulatory limit.

Heavy Metals	Pumpkin Leaf (Mg/kg)	Shoko Leaf (Mg/kg)	Green Leaf (Mg/kg)	FAO/WHO permissible limit (Mg/kg)
LEAD	BDL	BDL	BDL	0.30
CADMUIM	BDL	0.01	BDL	0.20
ZINC	2.11	1.52	1.64	99.40
CHROMIUM	0.01	0.04	0.03	0.03
IRON	2.00	3.85	7.70	425.50

3.1.1 DISCUSSION

The analysis results of heavy metal concentrations in leafy vegetables collected (alongside their mean values) from the Uselu market in Benin-city, Edo state, according to Tables 3.1, 3.2, and 3.3 provide valuable insights into the safety of these sampled food products. The absence of lead in all samples, indicated by concentrations below detection limit, is a comforting finding. This is because lead is a highly toxic heavy metal that can cause severe health issues, particularly neurological problems (Jarup, 2023), and its absence reflects effective agricultural practices or minimal environmental contamination in the growing areas, ultimately safeguarding consumer health. This finding is consistent with the study of the heavy metal in the sample.

Moreover, Cadmium, which is another toxic heavy metal, was detected in only one sample, the Shoko Leaf, at a very low mean concentration of 0.01 Mg/kg. There is need for a continuous monitoring of this heavy metal. The potential health risks associated with cadmium accumulation in the body, particularly kidney damage (Yan & Allen, 2021).

Also, Zinc was found to be present in all samples of this study at varying concentrations. While zinc is necessary for various physiological functions, excessive intake can lead to adverse health effects (Plum, *et al., 2010)*. The mean concentrations of zinc were 2.11 Mg/kg for Pumpkin Leaf, 1.52 Mg/kg for Shoko Leaf, and 1.64 Mg/kg for Green Leaf.

The concentration of Chromium in all three samples was also measured. Although at relatively low concentrations ranging from 0.02 to 0.04 Mg/kg, Chromium was detected in all the samples. The mean concentrations of chromium in this study were 0.01 Mg/kg for Pumpkin Leaf, 0.04 Mg/kg for Shoko Leaf, and 0.03 Mg/kg for Green Leaf.

Furthermore, Iron was detected in all samples, with the highest concentration observed in the Green Leaf sample. Iron is necessary for various physiological functions, including oxygen transport in the body. The mean concentrations of iron were 2.00 Mg/kg for Pumpkin Leaf, 3.85 Mg/kg for Shoko Leaf, and 7.70 Mg/kg for Green Leaf.

Table 3.4 on the other hand, presents a comparison analysis of the mean values of the samples and the standard regulatory limit. For lead (Pb), cadmium (Cd), and chromium (Cr), all samples showed concentrations below detection limit (BDL) except for Shoko Leaf which contained a mean concentration of 0.04Mg/kg. This indicates compliance with the FAO/WHO permissible limits of 0.30 Mg/kg for lead, 0.20 Mg/kg for cadmium, and 0.03 Mg/kg for chromium in the

Pumpkin Leaf and Green leaf, but non-compliance in the Shoko leaf. Therefore, the result is comforting as it suggests that the leafy vegetables sampled (except for Shoko leaf) do not pose significant health risks associated with these heavy metals.

However, for zinc (Zn) and iron (Fe), the mean concentrations in all samples were well below the FAO/WHO permissible limits of 99.40 Mg/kg for zinc and 425.50 Mg/kg for iron. The mean concentrations of zinc ranged from 1.52 to 2.11 Mg/kg, while iron concentrations ranged from 2.00 to 7.70 Mg/kg. These results indicate that the levels of zinc and iron in the leafy vegetables are significantly lower than the established regulatory limits, which makes the vegetable healthy for human consumption.

CONCLUSION

Human beings and animals can come in contact with heavy metals through several means which are inhalation from polluted air, dermal contact from surfaces with heavy metals and ingestion from food, drugs or other substances taken. This study focuses contact with heavy metals through ingestion of leafy vegetables contaminated by heavy metals. Not all heavy metals are toxic, some like iron, zinc, chromium etc are essential to the body for certain biochemical and physiology activities in trace amount because elevated level of heavy metals are very toxic to the body and health which can lead to various diseases.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). (2012). Toxicological profile for cadmium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Agishi, E. C., Adesope, O. M., & Tijani-Eniola, H. (2021). An appraisal of the traditional and contemporary uses of fluted pumpkin (Telfairia occidentalis) in Nigeria. Journal of Medicinal.

Aiyelaagbe, I.O.O, and A.A Kintomo. "Nitrogen Response of Fluted Pumpkin (Telfairia occidentalis Hook. F) Grown Sole or Intercropped with Banana." Nutrient Cycling in Agroecosystems 64 (2002): 231-35.

Akoroda, M. O. "Ethnobotany of Telfairia occidentalis (Curcurbitacae) among Igbos of Nigeria." Economic Botany (1990): 29-39. JSTOR 4255209.

Alam, M.G., Snow, E.T., Tanaka, A. (2003). Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. Sci Total Environ 308: 83-96.

Amaranthaceae | plant family". Encyclopædia Britannica. Retrieved 2015-06-02.

Amarteifio, G., & Mosase, K. R. (2019). Nutritional quality of leafy vegetables: Evaluation of mineral composition. International Journal of Food Science, 2019, 1-8.

Anyasor, G. N., Adejumo, T. O., Oyelade, O. J., et al. (2018). Dietary Carotenoids and Their Role in Combating Vitamin A Deficiency: A Review of the Literature. Cogent Food & Agriculture, 4(1), 1476641.

Appel, L. J., Moore, T. J., Obarzanek, E., et al. (1997). A clinical trial of the effects of dietary patterns on blood pressure. New England Journal of Medicine, 336(16), 1117-1124.

Arun K Shanker, Carlos Cervantes, Herminia Loza-Tavera, S Avudainayagam. Chromium toxicity in plants. Environment international 31 (5), 739-753, 2005.

Aune, D., Giovannucci, E., Boffetta, P., Fadnes, L. T., Keum, N., Norat, T., ... & Tonstad, S. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. International Journal of Epidemiology. 2017.

Badifu, Gabriel I.O. "Food Potentials of Some Unconventional Oilseeds Grown in Nigeria - a Brief Review." Plant Foods for Human Nutrition 43.3 (1993): 211-24.

Bahemuka, T-E. and Mubofu E.B. (1991). Heavy metals in edible green vegetables grown along the sites of the Sinza and Msimbazi Rivers in Dar es Salaam,Tanzania. Food Chemistry 66: 63-66.

Bai Yanjie, Jia-Ping Chen, Guo-Chong Chen, Xiao-Ping Wang, Liqiang Qin. Dietary fiber and metabolic syndrome: a meta-analysis and review of related mechanisms. Nutrients 10 (1), 24, 2017.

Bensch; et al. (2003). "Interference of redroot pigweed (Amaranthus retroflexus), Palmer amaranth (A. palmeri), and common waterhemp (A. rudis) in soybean". Weed Science. 51: 37–43.

Bernhoft A Robin. Cadmium toxicity and treatment. The Scientific World Journal 2013 (1), 394652, 2013.

Bertelsen, M. (2003). Vegetables: Definition and classification. Encyclopedia of Food Sciences and Nutrition (Second Edition), 6132-6136.

Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., ... & Watzl, B. (2012). Critical review: vegetables and fruit in the prevention of chronic diseases. European Journal of Nutrition, 51(6), 637-663.

Brian J Alloway. Sources of heavy metals and metalloids in soils. Heavy metals in soils: trace metals and metalloids in soils and their bioavailability, 11-50, 2013.

Brochin Robert, Siena Leone, Dylan Phillips, Nicholas Shepard, Diane Zisa, Allan Angerio. The cellular effect of lead poisoning and its clinical picture. GUJHS 5 (2), 1-8, 2008.

Celosia argentea (Plumosa Group) 'New Look'. Plant Finder. Missouri Botanical Garden. n.d. Retrieved 4 January 2023.

Celosia argentea. North Carolina Extension Gardener Plant Toolbox. North Carolina State University. n.d. Retrieved 4 January 2023.

Celosia. AVRDC. Retrieved 13 December 2013.

Chakraborty Debargha, Subhajit Bhar, Jayjit Majumdar, SC Santra. Heavy metal pollution and phytoremediation potential of Avicennia officinalis L. in the southern coast of the Hoogly estuarine system. International Journal of Environmental Sciences 3 (6), 2291-2303, 2013.

Cleveland clinic, 2022.

Cobb George P, Kristin Sands, Melissa Waters, Bobby G Wixson, Elaine Dorward-King, Accumulation of heavy metals by vegetables grown in mine wastes. Environmental Toxicology and Chemistry: An International Journal 19 (3), 600-607, 2000.

Cockscomb. Dave's Garden. Retrieved 13 December 2013.

Dorne, J.L.C.M.; Kass, G.E.N.; Bordajandi, L.R.; Amzal, B.; Bertelsen, U.; Castoldi, A.F.; Heppner, C.; Eskola, M.; Fabiansson, S.; Ferrari, P.; et al. Human risk assessment of heavy metals: Principles and applications. Met. Ions Life Sci. 2011, 8, 27–60.

Dube BK, Kamlesh Tewari, J Chatterjee, C Chatterjee. Excess chromium alters uptake and translocation of certain nutrients in citrullus. Chemosphere 53 (9), 1147-1153, 2003.

DuPont, M. S., & Bennett, R. N. Polyphenols from leaf vegetables. Phytochemistry Reviews. 2010.

ECHO Archived May 10, 2010, at the Wayback Machine.

Ekong EB, BG Jaar, VM Weaver Lead-related nephrotoxicity: a review of the epidemiologic evidence. Kidney international 70 (12), 2074-2084, 2006.

Emebiri L. and Nwufo, M., "Pod Rots of Fluted Pumpkin (Telfairia Occidentalis Hook. F.) in mo State, Nigeria." International Biodeterioration (26)1. (1990): 63-68.

Eun Jong-Bang, Ahmed Maruf,Protiva Rani Das &Seung-Hee Nam. A review of encapsulation of carotenoids using spray drying and freeze drying. 3547-3572, 2019.

Finster E Mary, Kimberly A Gray, Helen J Binns. Lead levels of edibles grown in contaminated residential soils: a field survey. Science of the Total Environment 320 (2-3), 245-257, 2004.

Gaetke Lisa M, Ching Kuang Chow; Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology 189 (1-2), 147-163, 2003.

Gaga Mina, Sergey E Borisov, Keertan Dheda, Martin Enwerem, Rodolfo Romero Leyet, Lia D'Ambrosio, Rosella Centis, Giovanni Sotgiu, Simon Tiberi, Jan-Willem Alffenaar, Andrey Maryandyshev, Evgeny Belilovski, Shashank Ganatra, Alena Skrahina, Onno Akkerman, Alena Aleksa, Rohit Amale, Janina Artsukevich, Judith Bruchfeld, Jose A Caminero, Isabel Carpena Martinez, Luigi Codecasa, Margareth Dalcolmo, Justin Denholm, Paul Douglas, Raquel Duarte, Aliasgar Esmail, Mohammed Fadul, Alexey Filippov, Lina Davies Forsman. Effectiveness and safety of bedaquiline-containing regimens in the treatment of MDR-and XDR-TB: a multicentre study. European Respiratory Journal 49 (5), 2017.

Giami, Sunday Y. "Effect of Germination on Bread-Making Properties of Wheat-Fluted Pumpkin (Telfairia occidentalis) Seed Four Blends." Plant Foods for Human Nutrition 58 (2003): 1-9.

Goyer Robert A, Lead toxicity: current concerns. Environmental health perspectives 100, 177-187, 1993.

Grubben, G. J. H., & Denton, O. A. (2004). Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands.

Gupta, U.C., Subhas, C., Gupta, M.D. (2008). Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. Commun Soil Sci Plant Anal 29: 1791-1807.

Ha, E. K., Ju, Y. S., Jeong, H. S., et al. (2020). Dietary intakes of vitamin E, vitamin C, and β-carotene and risk of Alzheimer's disease: a meta-analysis. Alzheimer's & Dementia: The Journal of the Alzheimer's Association, 16(11), 1519-1529.

Hanelt et al., Mansfeld's encyclopedia of agricultural and horticultural crops, 2001 Google Books

Heavy Metal Contamination in Leafy Vegetables Grown in Jazan Region of Saudi Arabia: Assessment of Possible Human Health Hazards Asim Najmi, Mohammed Albratty , , Abdul Jabbar Al-Rajab, Hassan A. Alhazmi, Sadique A. Javed, Waquar Ahsan, Zia ur Rehman, Rym Hassani and Saad S. Alqahtani, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharm Int. J. Environ. Res. Public Health 2023, 20, 2984 Int. J. Environ. Res. Public Health 2023, 20, 2984.

Heavy Metals in the Vegetables Collected from Production Sites Article in Health Promotion Perspectives. December 2013. PubMed

Heidarieh Marzieh, Mohammad Ghannadi Maragheh, Mehrdad Azizi Shamami, Mehdi Behgar, Farhood Ziaei, Zahra Akbari, Evaluate of heavy metal concentration in shrimp (Penaeus semisulcatus) and crab (Portunus pelagicus) with INAA method, SpringerPlus 2, 1-5, 2013.

Henson C Michael, P Jorge Chedrese. Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Experimental biology and medicine 229 (5), 383-392, 2004.

Hotz C, Gibson RS (2007). "Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets". J Nutr. 137 (4): 1097⇥100.

How to grow colorful celosia: The complete guide. Shiny Plant. 2020-12-19.

Hu Han-Hwa Gunnel E Carlsson, Anders Möller, Christian Blomstrand, Takashi Ueda, Katsufumi Mizushige, Kazushi Yukiiri, Tsutomu Takahashi, Masakazu Kohno, Terry BJ

Kuo, Chang-Ming Chern, Cheryl CH Yang, Hung-Yi Hsu, Wen-Jang Wong, Wen-Yung Sheng, European stroke initiative recommendations for stroke management, Cerebrovascular Diseases 16 (4), 311-337, 2003.

Irfan Mohd, Shamsul Hayat, Aqil Ahmad, Mohammed Nasser Alyemeni. Soil cadmium enrichment: Allocation and plant physiological manifestations. Saudi journal of biological sciences 20 (1), 1-10, 2013.

Jarup, L. (2003). Hazards of heavy metal contamination. *British medical bulletin*, *68*(1), 167-182.

Jenkins, D. J., et al. (2002). Glycemic index of foods: a physiological basis for carbohydrate exchange. The American Journal of Clinical Nutrition, 76(1), 5-56.

Joshua L Ratcliffe, David I Campbell, Beverley R Clarkson, Aaron M Wall, Louis A Schipper. Water table fluctuations control CO_2 exchange in wet and dry bogs through different mechanisms. Science of the Total Environment 655, 1037-1046, 2019.

Kabata-Pendias, A. and Mukherjee, A.B., 2007. Trace Elements from Soil to Human. Springer, Berlin, 550.

Kahn Steven E, Rebecca L Hull, Kristina M Utzschneider Mechanisms linking obesity to insulin resistance and type 2 diabetes, Nature 444 (7121), 840-846, 2006.

Kayodé AP Polycarpe, MJ Rob Nout, Anita R Linnemann, Joseph D Hounhouigan, Emmerich Berghofer, Susanne Siebenhandl-Ehn. Uncommonly high levels of 3-deoxyanthocyanidins and antioxidant capacity in the leaf sheaths of dye sorghum. Journal of agricultural and food chemistry 59 (4), 1178-1184, 2011.

Kiple, K. F., & Ornelas, K. C. (Eds.). (2000). The Cambridge World History of Food. Cambridge University Press.

Klaassen Curtis D, Jie Liu, Supratim Choudhuri, Metallothionein: an intracellular protein to protect against cadmium toxicity. Annual review of pharmacology and toxicology 39 (1), 267-294, 1999.

Koutras GA, AS Schneider, M Hattori, WN Valentine. Studies on chromated erythrocytes. Mechanisms of chromate inhibition of glutathione reductase.British journal of haematology 11 (3), 360-369, 1965.

Lattier, J. D., & Black, R. J. (1995). Cockscomb (Celosia argentea var. cristata) Cultivar Evaluations in Mississippi. HortScience, 30(2), 353-354.

Legacy: The Official Newsletter of Amaranth Institute" (PDF). Amaranth Institute. 1992. pp. 6–9. Archived from the original (PDF) on 2012-10-12. Retrieved 2011-09-19.

Lesmana Sisca O., Novie Febriana, Felycia E Soetaredjo, Jaka Sunarso, Suryadi Ismadji. Biochemical Engineering Journal 44 (1), 19-41, 2009.

Liu, P., et al. Analysis of heavy metals sources for vegetable soils from Standing Province China. Agriculture Science China, 10(1), 109-119.

Lone, S.A., Jeelani, G. and Deshpande, R.D. (2017). Evaluating the sensitivity of glacier to climate by using stable water isotopes and remote sensing. Environ. Earth Sci., 76: 598.

Mapanda, F., Mangwayana, E.N., Nyamangara, J. and Giller, K.E. (2005). The effects of long-term irrigation using water on heavy metal contents of soils under vegetables. Agriculture, Ecosystem and Environment, 107:151-156.

Mapanda, F., Mangwayana, E.N., Nyamangara, J., Giller, K-E. (2005). Impacts of sewage irrigation on heavy metals distribution and contamination. Environ Intem 31: 05-812.

Martha Clare Morris, Yamin Wang, Lisa L. Barnes, David A. Bennett, and Christy C. Tangney. Nutrients and bioactives in green leafy vegetables and cognitive decline: Prospective study. Neurology. 2017.

Martin Sabine, Wendy Griswold. Human health effects of heavy metals. Environmental Science and Technology briefs for citizens 15 (5), 1-6, 2009.

Masindi, V. and Muedi, K.L. (2018). "Environmental contamination by heavy metals". Heavy metals In Tech.

Matović Vesna, Aleksandra Buha, Zorica Bulat, Danijela Đukić-Ćosić, Milica Miljković, Jasmina Ivanišević, Jelena Kotur-Stevuljević. Route-dependent effects of cadmium/cadmium and magnesium acute treatment on parameters of oxidative stress in rat liver. Food and chemical toxicology 50 (3-4), 552-557, 2012.

Murugesan AG, S Maheswari, G Bagirath, Biosorption of cadmium by live and immobilized cells of Spirulina platensis. INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH (IJER) 2 (3), 307-312, 2008.

National Gardening Association. (n.d.). Celosia. Retrieved from https://garden.org/pl

Navas-Acien Ana, Eliseo Guallar, Ellen K Silbergeld, Stephen J Rothenberg; Lead exposure and cardiovascular disease—a systematic review. Environmental health perspectives 115 (3), 472-482, 2007.

Nwanna, Esther Emem, et al. "Antioxidant and Hepatoprotective Properties of Telfairia occidentalis Leaf (Fluted Pumpkin)." Thesis and Dissertations (Biochemistry) (2008) Antioxidant and Hepatoprotective Properties Of Telfairia Occidentalis Leaf (Fluted Pumpkin)". Archived from the original on 2013-12-06. Retrieved 2013-12-06.

Nwufo, M. I. "Effects of Water Stress on the Post-harvest Quality of Two Leafy Vegetables, (Telfairia occidentalis) and - Storage Pterocarpus Soyauxii during Storage." J Sci Food Agric 64 (1994): 265-69.

Obasi, M. E., Chikezie, P. C., Nwankwo, C., & Obasi, I. A. (2020). Effect of Different Planting Spacing on Growth and Yield of Fluted Pumpkin (Telfairia occidentalis Hook F.) in Afikpo, Southeast Nigeria. American Journal of Agriculture and Forestry, 8(5), 139-143.

Okoli, Bosa E., and C. M. Mgbeogu. "Fluted Pumpkin, Telfairia occidentalis: West African Vegetable Crop." School of Biological Sciences, University of Port-Harcourt 37.2 (1983): 145-49.

Oladebeye, A. O. (2017) Assessment Of Heavy Metals In Nigerian Vegetables And Soils In Owo And Edo Axes Using X-Ray Fluorescence (XRF) Technique.

Olasupo, A. O., Owoyale, J. A., & Asafa, S. A. (2019). Nutritional and Phytochemical Analysis of Celosia argentea (Linn) Leaf. International Journal of Biochemistry Research & Review, 29(2), 1-8.

Onibokun, A.G. and Kumuyi, A.J. (1996). Urban poverty in Nigeria: towards sustainable strategies for its alleviation. Centre for African Settlement Studies and Development, Ibadan, Nigeria. CASSAD Monograph Series. Plants for Economic Development, 5(1), 43.

Patrick Lyn, Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity. Alternative medicine review 8 (2), 2003.

Plum, L. M., Rink, L., & Haase, H. (2010). The essential toxin: impact of zinc on human health. *International journal of environmental research and public health*, *7*(4), 1342-1365.

Rastrelli, L., Manna, F., & Falcone, C. (2017). Flavonoids: A Review of Biological Activities. The Italian Journal of Biochemistry, 66(4), 483-499.

Reynolds, A., Mann, J., Cummings, J., Winter, N., Mete, E., & Te Morenga, L. (2019). Dietary Fiber and Health Outcomes: An Umbrella Review of Systematic Reviews and Meta-Analyses. The American Journal of Clinical Nutrition, 109(4), 1359-1371.

RHS A→Z encyclopedia of garden plants. Dorling Kindersley. 2008. p. 1136. ISBN 978-1405332965.

Rodríguez, H. G., Ramírez, R., & García, R. Green leafy vegetables. The Complete Book on Spices & Condiments. 2006.

Rodriguez-Carmona, M., & Remesar, X. (2002). Obesity, adipo-vascular axis, and vascular risk. Current Opinion in Clinical Nutrition and Metabolic Care, 5(4), 407-412.

Rodríguez-Eugenio Natalia , Michael McLaughlin, Daniel Pennock (2018). Soil pollution: a hidden reality.

Salgueiro MJ, M Zubillaga, A Lysionek, G Cremaschi, CG Goldman, R Caro, T De Paoli, A Hager, R Weill, J Boccio. Zinc status and immune system relationship: a review. Biological trace element research 76, 193-205, 2000.

Salt E David , RD Smith, Ilya Raskin. Phytoremediation. Annual review of plant biology 49 (1), 643-668, 1998.

Samuel Collin M., Senthil Kumar Venkatraman, Naveensubramaniam Vijayakumar, V Kanimozhi, S Muhammad Arbaaz, RG Sibiya Stacey, Jogannagari Anusha, Rajan Choudhary, Vladislav Lvov, Gabriel Ibrahin Tovar, Fedor Senatov, Sivasankar Koppala, Sasikumar Swamiappan. Bioaccumulation of lead (Pb) and its effects on human: A review. Journal of Hazardous Materials Advances 7, 100094, 2022.

Shubham Pandey, Julaiba Tahsina Mazumder, Ravindra Kumar Jha, Homoatomic flatlands beyond graphene: A new avenue for gas sensors, Coordination Chemistry Reviews 507, 215747, 2024.

St. Clair, Kassia. The Secret Lives of Color. 2017. Penguin Group. p. 130. ISBN 978-0143131144.

Stasz, T. E., & Paull, R. E. (2009). Vegetables: An Overview. In The Produce Contamination Problem: Causes and Solutions (pp. 1-12). Academic Press.Vegetables:

Swaran JS Flora, Shruti Agrawal. Arsenic, cadmium, and lead. Reproductive and developmental toxicology, 537-566, 2017.

Tapia-Gatica Jaime, Iván Selles, Manuel A. Bravo, Catherine Tessini, Wilson Barros-Parada, Alexey Novoselov, Alexander Neaman. Global issues in setting legal limits on soil metal contamination: A case study of Chile. Chemosphere; Volume 290, March 2022, 133404.

Thévenod Frank, Prabir K Chakraborty, Wing-Kee Lee, Malte Molitor, Natascha A Wolff. Cadmium induces Wnt signaling to upregulate proliferation and survival genes in sub-confluent kidney proximal tubule cells. Molecular cancer 9, 1-16, 2010.

Torkashvand, A. M., & Sokhansanj, S. A review on the role of green leafy vegetables in human health. International Journal of Nutrition and Food Sciences. 2015.

Toxicity, mechanism and health effects of some heavy metals Monisha JAISHANKAR, Tenzin TSETEN, Naresh ANBALAGAN, Blessy B. MATHEW, Krishnamurthy N. BEEREGOWDA. Department of Biotechnology, Sapthagiri College of Engineering, Bangalore-57, Karnataka, India. Interdiscip Toxicol. 2014; Vol. 7(2): 60→72.

UNDP and CivilSociety Organizations, UNITEDNATIONSDEVELOPMENTPROGRAMME UNDP, 2006.

Vesey A David. Transport pathways for cadmium in the intestine and kidney proximal tubule: focus on the interaction with essential metals. Toxicology letters 198 (1), 13-19, 2010.

Wang Xiangtao, Yifei Guo, Li Yang, Meihua Han, Jing Zhao, Xiaoliang Cheng. Nanomaterials as sorbents to remove heavy metal ions in wastewater treatment. J. Environ. Anal. Toxicol 2 (7), 154-158, 2012.

Xiong TianTian, Camille Dumat, Antoine Pierart, Muhammad Shahid, Yuan Kang, Ning Li, Georges Bertoni, Christophe Laplanche. Environmental geochemistry and health 38, 1283-1301, 2016.

Yan, L. J., & Allen, D. C. (2021). Cadmium-induced kidney injury: Oxidative damage as a unifying mechanism. *Biomolecules*, *11*(11), 1575.

Zhuang Ping, Murray B McBride, Hanping Xia, Ningyu Li, Zhian Li, Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Science of the total environment 407 (5), 1551-1561, 2009.