THE USE OF *Mangifera indica* and *Psidium guajava* TO ASSESS THE LEVELS OF AIRBORNE TRACE METALS IN UGBOWO CAMPUS, UNIVERSITY OF BENIN

BY

NDUOMA EMMANUEL OMEDE

PSC2105229

SUBMITTED TO THE DEPARTMENT OF CHEMISTRY

PHYSICAL SCIENCE

UNIVERSITY OF BENIN

BENIN CITY, EDO STATE

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) IN PURE CHEMISTRY

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by NDUOMA EMMANUEL OMEDE, PSC2105229, in the Department of Chemistry, Faculty of Physical Science, University of Benin, in partial fulfillment of the requirements of the award of Bachelor of Science (B.Sc.) degree in Pure Chemistry.

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DEDICATION

This report is dedicated to God Almighty, the Wellspring of life and wisdom. I also extend my heartfelt dedication to my parents, siblings, and cherished ones, whose unwavering presence have been a beacon of support, especially during challenging times.

ACKNOWLEDGEMENT

My deepest gratitude goes to God Almighty for His love and grace throughout my B.Sc. program in this great institution and for His favour during the project endeavour. I also wish to express my heartfelt thanks to PROF. E.E. UKPEBOR, my project supervisor, whose invaluable support and intellectual input greatly contributed to the accomplishment of this study. His meticulous corrections and supervision made this research a success, and is deeply appreciated. I want to use this opportunity to also acknowledge the Head of Department, Prof. E.E. IRABOR, Prof. (Mrs) Ukpebor, Dr. Dibie, Dr. (Mrs) Emeribe, and all other members of staff, both academic and non-academic, for their relentless support and assistance. I am also grateful to the laboratory technicians, Mr. Amayo, Mr. Lucky and Mr. Clinton, for their resolute guidance. I wish to also acknowledge the contributions of my ever-supportive project partners: Favour Iyare, Favour Igbinosa, Praise Igumah, and Michael Udofa, for their keen support in the success of this project work. To all my friends and colleagues: Mirabelle, Godswill, Destiny, Success, Chidinma, Marvelous, Chiemelie, Divine, Pamela, Joseph, Emmanuel, Favour and Eseosa, I am truly grateful for your advice and encouragement. My most profound appreciation goes to my parents, Mr. Robert and Mrs. Anne OMEDE, and my siblings, Kenechukwu, Osokam and Kelikume. Their unwavering and steadfast support, financially and emotionally, have been indispensable; not only in this project work, but throughout my entire degree programme and life journey.

ABSTRACT

The importance of tree plant species as biological components in the ecosystem is significant. These plants are used as shade, beautification, and even food. Urban locations are also bound to face numerous human activities that cause the introduction of many pollutants, including trace (heavy) metals into the environment. This research work is designed to determine the level of airborne trace metals (Cr, Fe, Ni, and Pb) present in the environment using *Psidium guajava* and *Mangifera indica*. The bark and leaf samples of these trees were collected, digested and analyzed using the Atomic Absorption Spectrometer (AAS). The bark samples of *Mangifera indica* gave a mean concentration of Cr – 0.24mg/kg, Fe – 15.85mg/kg, Ni – 0.11mg/kg, and Pb – 0.04mg/kg while the leaf samples gave a mean concentration of Cr – 0.28mg/kg, Fe – 15.70mg/kg, Ni – 0.09mg/kg, and Pb - 0.03mg/kg. Also, the data obtained from *Psidium guajava* bark samples gave a mean concentration of Cr – 0.19mg/kg, Fe – 2.25mg/kg, Ni – 0.03mg/kg, and Pb – 0.02mg/kg while the leaf samples resulted in a mean concentration of Cr – 0.15mg/kg, Fe – 2.70mg/kg, Ni – 0.06mg/kg, and Pb – 0.04mg/kg. The leaf and bark samples of *Mangifera indica* accumulated the highest level of trace metals, indicating that they are good biomonitors of airborne trace metals in Ugbowo Campus. The level of chromium (Cr) is above the permissible limit in all the plant species suggesting the contamination of the study area. The levels of other trace metals reported in this study are below the permissible limit. The findings of this work therefore shows that the study area is unsafe in Cr levels but safe and clean in the levels of other airborne heavy metals (Fe, Ni, and Pb).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Contamination of the environment by hazardous heavy metals is a serious worldwide problem that compromises the natural purity of air, water, and soil (Lubal, 2024). Heavy metal contamination is recognized as a significant contributor to severe environmental issues and poses risks to human health, such as reduced microbial activity in soil, along with losses in fertility and crop yields (Dogan et al., 2014). These metals are recognized as environmental contaminants due to their harmful nature, persistence in the environment and possible accumulation in the human body during bioaccumulation (Mitra et al., 2022). According to Csuros and Csuros (2002), a heavy metal is defined as a metal with a density greater than $5 g/cm^3$ (i.e. specific gravity greater than 5) and has atomic number greater than 20 (Hazrat et al., 2019). They include Cr, Ni, Cu, Zn, Cd, Pb, Fe, Hg, Mn and As. These metals are often referred to as "silent killers", when they are dense by more than $5 g/cm^3$.

Natural sources of heavy metals in the environment may be through volcanic activity, weathering of metal bearing rocks, erosion and other geologic processes, or anthropogenically, through urban runoff, agriculture, and industrial processes (Cakaj et al., 2024). Most human exposure to these substances stems from anthropogenic activities, which in turn introduce heavy metals into the ecosystem. Whether these hazardous materials will be toxic will depend on the exposure available to the living organisms in terms of quantity and duration of time (Abd Elnabi et al., 2023). Heavy metals exist in nature and are vital for various life processes; however, they become dangerous once their levels rise beyond normal concentrations. Some of the most common heavy metals that pollute the environment are Mercury, Cadmium, Arsenic, Chromium, Nickel, Copper and Lead (Mitra et al., 2022).

Fig 1.1 Diagrammatic details of heavy metals in the environment

Unlike organic pollutants, heavy metals cannot degrade naturally and tend to accumulate within living organisms. Consequently, increasing emphasis is placed on using plant components such as leaves, shoots, and bark for biomonitoring. Any organism, or part of it, that helps detect pollutants through their concentrations, visible symptoms, or structural alterations is known as a bioindicator or biomonitor. Generally, the concept of biomonitoring can be described as the systematized utilization of living things or their reactions to detect the status or variations of the setting (Badamasi, 2017).

Plant biomonitoring is becoming more widely used, particularly in Europe, as an effective substitute for older instrumental techniques in studying how natural and anthropogenic substances settle from the air onto land. Out of all the techniques of biomonitoring, the plants-based systems have earned a significant level of attention due to their effectiveness in detecting pollution of heavy materials. Trees act as natural purifiers of the environment, and their leaves and bark serve as valuable indicators for assessing environmental quality. The value of trees as bio monitors is especially acute since they are immobile and, therefore cannot flee, they offer an ongoing set of long-term measurements on the level of contamination in their tissues.

This research work hence examines the usefulness of using tree bark and leaves as biomonitors to measure the level of environmental quality using heavy metal detection and analysis.

1.1.1 BACKGROUND OF STUDY

Biomonitoring refers to the use of living organisms or their biological materials to obtain information about specific substances present in the biosphere. One major advantage of using biomonitors for environmental assessment is their low cost compared to direct pollution measurements, since they require no specialized equipment that must be installed or protected from damage. When biomonitors are distributed over a large region and are repeated frequently, then, they can be used over large areas to record and assess heavy metal accumulations. Also, they allow determining where the emissions are emitted and confirming the overland transportation of heavy metals (Dogan et al., 2007). Due to industrialization and urbanization, the issue of heavy metal pollution has become worse in recent times as a result of heavy metal deposition from different sources including vehicle emissions, combustion processes, aircraft exhaust and other anthropogenic sources. However, plant parts, especially bark and leaves, are now being utilized as very sensitive tools in the determination of environmental pollution (Parmar et al., 2016).

Plants are like living sponges which absorb pollutants through a number of pathways. In this project work, *Mangifera indica* (Mango) and *Psidium guajava* (Guava) leaves and barks were properly investigated to detect trace metal pollution in the environment. Tree plants act as perfect biomonitors of atmospheric air pollution. Earlier literature observed that the chlorophyll and protein contents of the leaves of the plant species are lower in trees in polluted environments than their equivalents in clean environment (Fowotade et al., 2025).

The use of tree bark for biomonitoring is beneficial due to its long-term pollutant retention capacity and widespread availability, all while leaving the tree unharmed. Its inherent porosity contributes significantly to its ability to collect and store analytes effectively. The air pollution caused by road traffic in urban areas is also captured due to long-term bioaccumulation in the trees bark (Swislowski et al., 2020).

Bark and leaves are more commonly used in biomonitoring than other parts of the tree structure like buds, flowers or needles because they can store a greater amount of analyte (heavy metals) (Matin et al., 2016).

The leaves and the bark provides a larger surface area for particulate matter. It was established that the amount of particulate matter which had settled on the leaves of the trees studied was about 148mg/cm^2 (Song et al., 2015).

This mode of biomonitoring gives accurate quantification of heavy metal concentration in plant tissues and accurate assessment of environmental pollution levels.

1.1.2 STATEMENT OF PROBLEM

Heavy metal pollution is increasingly recognized as a significant environmental issue due to its harmful effects in various regions worldwide. These inorganic pollutants are being released in our waters, soils and into the atmosphere owing to the booming agriculture and metal industries, poor disposal of waste, fertilizers and pesticides (Briffa et al., 2020). In our study area, car mobile traffic, combustion by school-related activities, and possibly emissions by a local transportation center or airplane are main sources of heavy metal deposition. Without biomonitoring, we are not aware of whether the levels are above the safe limits or what threat it poses when exposed to human health.

Previous research in Ibadan has indicated high concentration of heavy metals (Pb, Zn, Cu, etc.) in tree barks in high traffic and industrial regions when compared to control sites, indicating actual exposure risk (Ejidike and Onianwa, 2015).

Therefore, there is a lack of information regarding the concentrations of specific heavy metals in the environment, particularly in areas where nearby trees could serve as biomonitors.

1.1.3 JUSTIFICATION OF STUDY

Assessing heavy metal concentrations in tree bark and leaves is significant for various reasons, spanning public health, environmental protection, economic impact, and adherence to regulations. They include:

• **Public health protection:** The main significance of the determination of heavy metals in tree bark and leaves is the protection of human health. Conducting regular monitoring assists in detecting large amounts of contamination and therefore be able to intervene in time to save those living in that environment.

• **Environmental Health Assessment:** The analysis of heavy metals in the tree bark and leaf is used to indicate the quality of air and the environment. It is an indicator of how polluted the environment is, caused by agricultural practices, industrial activities and urban pollution. This data is crucial in evaluating the general health of ecosystems, and the areas that need remedial action.

1.1.4 SCOPE OF WORK

This research work covers the assessment of levels of Lead (Pb), Iron (Fe), Chromium (Cr) and Nickel (Ni) in tree bark and leaves of Mango (Mangifera indica) and Guava (Psidium guajava) respectively, around Ugbowo Campus, University of Benin, Benin City, Edo State, Nigeria.

1.1.5. AIM AND OBJECTIVES

AIM

The aim of this study is to determine the level of heavy metals present (specifically Pd, Fe, Cr and Ni) in the tree bark and leaves of Mango (Mangifera indica) and Guava (Psidium guajava) around Ugbowo Campus, University of Benin, so as to access the level of air pollution.

OBJECTIVES

To achieve the aforementioned aim, the following objectives were set to:

• Carefully procure samples of the tree parts (bark and leaves).

• Extract the heavy metals present using acid based wet digestion.

• To determine the levels of Pb, Fe, Cr, and Ni in digested samples using AAS.

• To compare these results with the permissible limits set by the WHO and other applicable regulatory organizations.

• Make recommendations to regulatory bodies and general public on the findings from this research.

1.2 LITERATURE REVIEW

1.2.1 BIOMONITORING

Biomonitoring refers to measuring and evaluating toxicants or their metabolites in tissues, secreta, excreta or a combination of both or any one of both in relation to an acceptable reference with a comparison of the exposure and health risk (Dhananjayan et al., 2022).

Biomonitoring involves the systematic observation of compounds or the detection of cellular or molecular changes in living organisms, with the aim of assessing potential risks and impacts of chemical exposure. The benefits of biomonitoring compared to environmental monitoring are diverse and it has been regarded as a useful tool in ecological and human health surveillance (Costa and Teixeira, 2014).

When a chemical is released into the environment, it typically disperses across various environmental matrices and is subsequently absorbed by both target and non-target organisms. Once inside the organism, the chemical undergoes absorption, distribution, metabolism, and excretion. These processes can result in the accumulation of the toxicant within the organism's biological systems, causing biochemical, cellular, and physiological changes that can be detected through biomonitoring studies. The purpose of a biomonitoring study is the health risk assessment, it is the assessment of the internal dose of the toxicant entering the body and estimating the burden of the toxicant within the body, as well as quantifying the total sum of adverse effect of the toxicant (Dhananjayan et al., 2022).

Consequently, biomonitoring processes have been said to have a role to play in terms of demonstrating the pollutants and trends in the real-time and retrospective method of monitoring environmental pollution through the use of the biomonitors (Chaudhary et al., 2022).

1.2.2 MANGO *(Mangifera indica)*

Mango *(Mangifera indica)* is among the most important fruits all over the world as it contains nutritional values and various phytochemicals and performs various functions. Some of the nutrients found in mango fruit include carbohydrates, fatty acids, vitamins, and minerals. The mango fruit has a huge economic value, as well as nutritional value across the globe. It has many crucial nutrients and bioactive compounds, which are responsible for its functional effects (Yahia et al., 2023).

Mango trees are evergreen, long-living, and grow rapidly. Their dark green, glossy, and pointed leaves persist year-round. In tropical climates, these trees can attain heights of 30–40 meters, though growth is slower in subtropical regions. There are hundreds of varieties of the mango fruit with their own distinctive taste, form, and size.The exocarp of mangoes is smooth, initially green in unripe fruits, and matures to colors ranging from golden yellow to crimson red, yellow, or orange-red according to the cultivar. The endocarp is a sizable ovoid-oblong cavity enclosing a single seed, while the mesocarp (pulp) is orange-yellow. Its taste is good and full bodied and it has a sweet, marginally tart taste. Mangoes are eaten fresh and used to make canned or frozen slices, which are popular globally. They are abundant in polyphenols, plant-based micronutrients with significant health effects. Different parts of the mango tree, such as the leaves and bark, have medicinal uses in traditional practices. The mango kernel is decoded, e.g., to treat diarrhea, hemorrhage, bleeding hemorrhoids because of its vermifuge and astringent properties, extracts of unripe fruit, bark and leaves are used due to its antibiotic effect and an aqueous stem bark extract of *Mangifera indica* is used in Cuba as a diarrhea remedy, fever remedy, gastritis remedy, and ulcer remedy (Lauricella et al., 2017). The porous structure of Mango bark and broad leaves makes it a good collector of airborne pollutants overtime; therefore, it can be used as a biomonitor of atmospheric air pollutants.

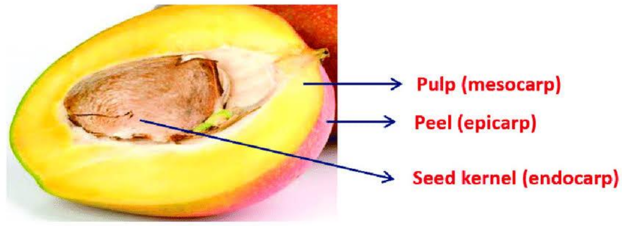

Fig. 1.2 Major parts of Mango fruit

Fig 1.3 Mango fruit and leaves

1.2.2.1 Scientific Classification of *Mangifera indica*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Sapindales

Family: Anacardiaceae

Genus: Mangifera

Species: Mangifera indica

1.2.3 GUAVA *(Psidium guajava)*

Guava *(Psidium guajava)* is a unique, long-established plant in the Myrtaceae family, rich in medicinal and nutritional benefits. It grows widely in tropical regions like India, Indonesia, Pakistan, Bangladesh, and South America and is a popular fruit. Different parts of the guava tree, including the leaves and bark, are traditionally used to treat stomachaches, diabetes, diarrhea, and other illnesses. Guava leaves are dark green, oval to elliptical, with an obtuse tip. The leaves, pulp, and seeds are used to treat respiratory and gastrointestinal problems and help increase platelet levels in dengue patients. Guava leaves also have antispasmodic, cough-relieving, anti-inflammatory, antidiarrheal, antihypertensive, anti-obesity, and antidiabetic effects. Animal studies show that guava leaf extracts can act as antitumor, anticancer, and cytotoxic agents. They are also rich in nutrients and bioactive compounds beneficial to health (Kumar et al., 2021).

Guava leaves are often considered non-conventional food products, as they are not typically consumed by people. Nevertheless, both guava fruit and leaves possess significant nutritional and medicinal properties and are recognized as valuable sources of active compounds. Almost all these bioactive compounds have been reported to have positive effects such as antimicrobial effects, anti-inflammatory effects, anti-carcinogenic effects, and cardio protective effects. Their antioxidant properties make them useful substances as food additives (Khanna et al., 2025).

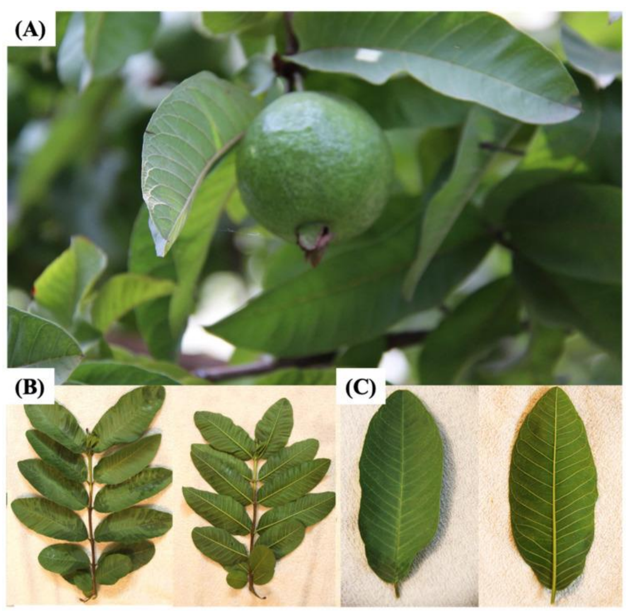

*Fig 1.4 (**A**) Guava fruit and leaves, (**B**) bunch of guava leaves with dorsal view on the left and ventral view on the right, (**C**) guava leaf with dorsal view on the left and ventral view on the right.*

Fig 1.5 Internal view of guava fruit

1.2.3.1 Scientific Classification of *Psidium guajava*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: Psidium

Specie: Psidium guajava

1.2.4 HEAVY METALS

The term “heavy metals” refers to metallic elements that possess high atomic masses and densities above 5 g/cm³. They are categorized heavy given the fact they have high atomic weight propensity to influence stability as compared to lighter metals. Many heavy metals listed in the periodic table are naturally present in the biosphere, including water, soils, and rocks, and are also released into the environment through anthropogenic activities, primarily from commercial and industrial sources. Though a few of these metals are important to biological functions in trace, most are highly toxic and capable of producing severe health effects even without being consumed in high amounts. Nonetheless, recent experimental studies reveal that some of them, such as Ni, Cu and Zn, are essential to human beings. Heavy metals cause a public health issue due to their persistence in the environment and accumulation within biological systems, particularly in the atmosphere (Jaishankar et al., 2014).

1.2.4.1 Classification of Heavy Metals

Heavy metals are classified into two: essential and non-essential metals based on their role in human health.

Essential Heavy Metals

These metals are highly required in the metabolism of the human body and body physiological processes, yet in little amount. They may lead to toxicity when consumed in large amounts particularly when contaminated in the environment or over-dressed in form of supplementation. Some examples include:

Iron (Fe): Among the elements in the Earth's crust, iron is the fourth most abundant. Production of hemoglobin and transportation of oxygen in blood needs iron, whose lack results in Anemia. Nevertheless, overload of iron may result into oxidative stress, resulting in liver and heart damage and the situation is especially hazardous in people with genetic disorders and hemochromatosis. Iron deficiency, Anemia, is a common nutritional deficiency worldwide, leading to fatigue, weakened immunity, and impaired cognitive function (WHO, 2015).

Zinc (Zn): Among the elements in the Earth's crust, zinc is the 24th most abundant. It exists in lead, copper, and iron ores as it is too reactive as an element to exist in its free form. It has primarily to do with the immune system, wound healing and production of DNA. Although deficiency affects the levels by weakening the immunity and growth, excessive levels may cause nausea and vomiting as well as reduction of immunity. Zinc is needed in the immune system, in healing the wounds and in the production of the proteins. It also helps in keeping the skin, hair, and nails healthy. Zinc deficiency may result in growth retarding, hypogonadism and depressed immune capability.

Copper: Copper is essential for the formation of several enzymes involved in energy production, iron metabolism, and neurotransmitter function. Shellfishes, whole grain, nuts, and seeds are some good sources of copper. Copper deficiency may cause anemia, musculoskeletal dysfunctions and heart complications.

Manganese: This metal is necessary for bone health, wound healing and metabolism. Manganese deficiency can cause impaired bone growth and reproductive issues.

Non-Essential Heavy Metals

Non-essential heavy metals are significant environmental pollutants with serious health implications. They are generally absorbed through contaminated food, water or air and are not easily excreted from the body. Unlike essential heavy metals, which are necessary in trace quantities for biological activities, non-essential heavy metals do not contribute any known benefit to biological systems. Some of them include:

Lead (Pb): Lead, a toxic metal naturally present in the Earth's crust, has become a major environmental pollutant due to its widespread use. Exposure occurs mainly via contaminated soil, water, or metal objects. Lead is a serious neurotoxin that can harm children's brain development, lower IQ, and affect kidney and reproductive health.

Mercury (Hg): Mercury is a hazardous heavy metal that occurs naturally and is found throughout the environment. Its major sources are environmental pollution from mining and industrial wastes. Mercury exposure can damage the nervous and renal systems, causing tremors, memory impairment, and mental disorders

Cadmium: Cadmium is a poisonous heavy metal found in industrial, environmental, and agricultural contexts. Its use in zinc mining byproducts, electroplating, batteries, pigments, and plastics has increased environmental pollution. Cadmium can accumulate in crops grown in contaminated soil and water and has been detected in alcoholic beverages like wine, beer, and spirits. Therefore, ingestion through food and drinks is the main route of exposure for people.

1.2.5 LEAD (Pb)

Fig 1.6 Lead metal

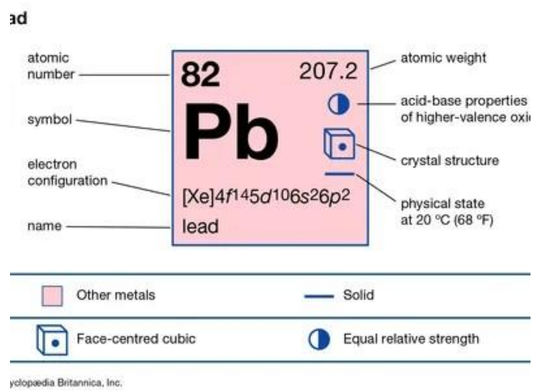

Fig 1.7 Lead in the periodic table

Lead is a naturally occurring toxic metal found in the Earth's crust. Its extensive use has contributed to environmental contamination, human exposure, and widespread health problems worldwide. Humans are primarily exposed to lead through contaminated soil, water, or metal-based containers and utensils. Lead is a potent neurotoxin that can cause poisoning in children, impair brain development, reduce IQ, and damage the kidneys and reproductive organs. Lead poisoning is a well-known disease arising from excessive intake of the metal and can damage the nervous, Skeletal, circulatory, enzymatic, endocrine, and immune systems of those exposed to it (Zhang et al., 2015). Lead enters the environment through mining, smelting, industrial production, recycling, and its use in different products. Most of the world’s lead is used in manufacturing lead-acid batteries for automobiles. Lead is used in many products, including pigments, paints, solder, stained glass, lead crystal glassware, ammunition, ceramic glazes, jewelry, toys, some traditional cosmetics, and some traditional medicine (WHO 2024). High levels of lead exposure can severely harm the brain and central nervous system, causing coma, convulsions, or death. Surviving children may suffer permanent cognitive and behavioral impairments. Even low-level exposure can affect multiple organs, particularly the developing brain, reducing IQ, attention span, and educational outcomes, and increasing behavioral problems. Lead can also cause anemia, high blood pressure, kidney damage, and reproductive harm. Once released into the environment, lead can be absorbed by plants, animals and humans. Lead poisoning, also known as lead toxicity, refers to the detrimental consequences of lead exposure. It can result in a wide array of health issues, ranging from developmental delays and learning disabilities in children to hypertension and kidney disorders in adults. In severe cases, lead poisoning can be fatal. Although the effects of lead poisoning may be irreversible, there are treatments available to mitigate its toxic impact.

1.2.6 CHROMIUM

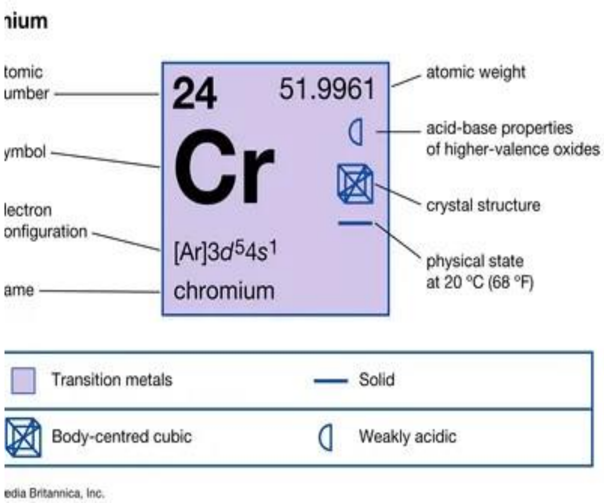

Fig 1.8 Chromium metal

Fig 1.9 Chromium in the periodic table

Chromium, a transition metal in Group VI of the periodic table, is a hard, crystalline steel-gray element with an atomic number of 24 and a density of 5.28 g/cm³ (Kumar et al., 2023). It is among the most abundant elements in the Earth's crust and ranks as the sixth most abundant transition metal. Chromium occurs naturally in ores containing other elements, such as ferric chromite ($FeCr_2O_4$), crocoite ($PbCrO_4$), and chrome ochre (Cr_2O_3). It is also recognized as a highly toxic metal when present in drinking water. Chromium naturally found in different oxidation states ranging from 2+, 3+ and 6+, with the trivalent Cr (III) and hexavalent Cr (VI) are the most stable forms in nature (Abbas et al., 2016).

Chromium primarily exists as trivalent (III), the stable form, or hexavalent (VI), a strong oxidant. Elemental chromium (0) is not naturally found. Trivalent chromium (III) is an essential trace metal needed for glucose tolerance factor formation and insulin metabolism. Commercial applications of chromium compounds include tanning, corrosion inhibition, plating, glassware-cleaning solutions, wood preservative, manufacture of safety matches, metal finishing, and the production of pigments (Barceloux and Barceloux, 1999).

Chromium is commonly applied in medical and dental implants and tools, as it provides a corrosion-resistant oxide layer on metal surfaces. In small amounts, it is used for medical purposes and contributes to lipid and protein metabolism in the human body. However, at sufficiently high concentrations particularly hexavalent chromium is toxic and carcinogenic (Achmad et al., 2017). Chromium (VI) is linked to a range of health problems, while chromium (III) is essential in trace amounts for normal metabolism and insulin action. Chromium (VI) is the more toxic form commonly found in industrial processes, whereas chromium (III) is the beneficial form naturally present in foods.

1.2.7 IRON

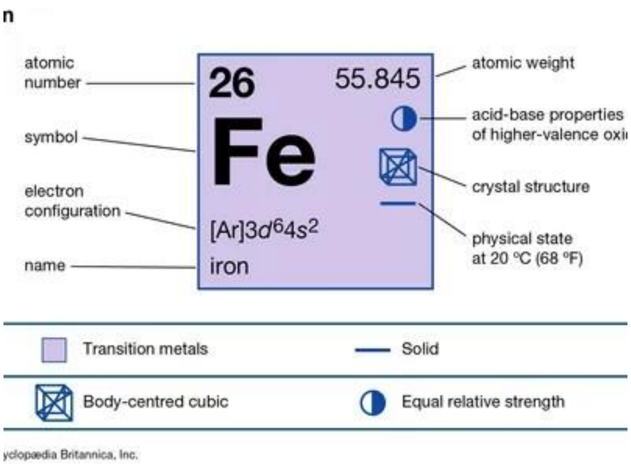

Fig 1.10 Iron in the periodic table *Fig 1.11 Iron metal*

Iron (Fe), derived from the Latin word ferrum, is a metallic element with a lustrous, grayish appearance. It belongs to Group 8 (VIIIb) of the periodic table, with an atomic number of 26 and an atomic weight of 55.847 g/mol. Iron has a melting point of 1538°C and a boiling point of 3000°C. Iron constitutes about 5% of the Earth's crust, making it the second most abundant metal after aluminum and the fourth most abundant element overall, following oxygen, silicon, and aluminum. It is the primary component of the Earth's core. In the human body, the average iron content is approximately 4.5 grams (0.004% of body weight). About 65% of this iron is found in hemoglobin, which carries oxygen from the lungs to tissues throughout the body. Roughly 1% is incorporated into enzymes that regulate intracellular oxidation, while the remaining iron is stored in organs such as the liver, spleen, and bone marrow for later conversion into hemoglobin. Red meat, egg yolk, carrots, fruit, whole wheat, and green vegetables contribute most of the 10–20 milligrams of iron required each day by the average adult (Britannica, 2025).

Iron is an essential heavy metal and a vital component of hemoglobin and myoglobin. It is also present in numerous human enzymes, playing a critical role in oxygen transport and cellular metabolism. High concentrations of iron are found in red blood cells and muscle tissue. Iron is included in many over-the-counter multivitamin and mineral supplements and is therapeutically administered at higher doses to prevent or treat iron deficiency anemia. When taken at recommended daily allowances or replacement doses, iron generally has minimal or no adverse effects on the liver. However, excessive intake, whether intentional or accidental, can cause serious toxicities, including acute liver damage (National Institute of Diabetes and Digestive and Kidney Diseases, 2018).

1.2.8 NICKEL

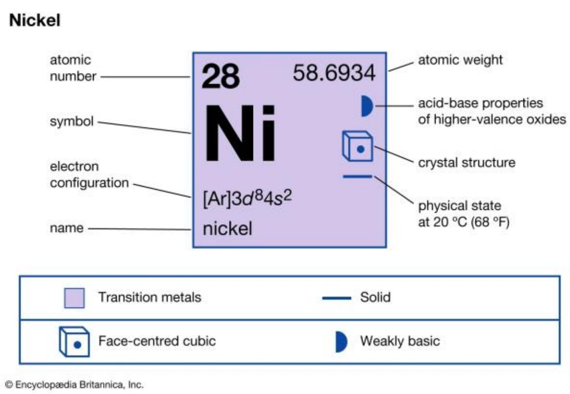

Fig 1.12 Nickel in the periodic table

Fig 1.13 Nickel metal

Nickel (Ni) is a naturally occurring metallic element found in the Earth's crust. Its unique physical and chemical properties make it highly valuable in the production of alloys used for jewelry, medical implants, stainless steel, Ni-Cd batteries, and nickel-plating applications. Nickel is also a widespread environmental contaminant and pollutant, exposing humans through various routes and causing multiple toxic effects. Of particular concern is its recognized carcinogenic potential. As a transition metal, nickel can exist in five oxidation states (−1, 0, +2, +3, and +4), with the +2 state being the most prevalent under normal conditions (Munoz and Costa, 2012).

Nickel is a hard, ductile, silvery-white transition metal. It is the 28th element in the periodic table, has an atomic weight of 58.6934g/mol and density of 8908 kg/m³. It has a high melting point at about 1455 °C and boiling point of 2913 °C. It belongs to group 10 of the periodic table along with iron, cobalt, palladium, platinum and five other elements.Nickel is the 24th most abundant element in the Earth's crust and ranks as the fifth most abundant element by weight, following iron, oxygen, magnesium, and silicon. In nature, it is commonly found combined with elements such as antimony, arsenic, and sulfur. Nickel has a wide range of applications, including inexpensive jewelry, keys, paper clips, clothing fasteners (such as zippers, snap buttons, and belt buckles), stainless steel household utensils, electrical equipment, armaments, coins, alloys, metallurgical and food processing industries, pigments, and catalysts (Genchi et al., 2020).

Nickel is required in minute amounts for enzyme function, but overexposure can lead to skin irritation, respiratory issues, and allergic reactions.

1.2.9 DIGESTION

Digestion is a technique employed in analytical chemistry to disintegrate a sample into its constituent parts. When it comes to analyzing heavy metals, digestion is utilized to break down plant material into its elemental components for separate analysis. In analytical chemistry, digestion process entails preparing samples by dissolving organic molecules in order to separate and examine metal trace elements, which are inorganic substances that remain after digestion. This procedure is necessary for precise analysis, especially when using processes such as AAS. This digestion can be carried out in two primary ways: Wet Digestion and Dry Ashing. However, the wet digestion process is commonly preferred for plant samples because it is more accurate and reduces contamination.

Wet Digestion

The wet digestion process utilizes potent acid to disintegrate the plant material. The acid is heated to an elevated temperature, leading to a reaction with the plant material and breaking it down into its constituent parts. These components are subsequently isolated and examined through processes like AAS or ICP-MS. The wet digestion technique completely breaks down the plant material and this adaptability is essential for efficiently digesting various samples and guaranteeing precise mineral content analysis.

Dry Ashing

The ashing method, also referred to as the dry digestion method, entails subjecting the plant material to high temperatures until it is fully incinerated. Subsequently, the resulting ash is dissolved in an acid and examined using the same procedures employed in the wet digestion method. The dry digestion method offers the advantage of being quicker and more cost-effective compared to the wet digestion method. Nevertheless, its accuracy may be compromised as it is more challenging to guarantee the complete incineration of all plant material.

Both techniques, each with unique benefits and drawbacks, are essential for getting samples ready for real analysis. The type of analysis needed, the sample's characteristics, and the particular requirements of the analytical technique being used all influences the choice of digestion method used.

1.2.10 ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Atomic Absorption Spectrophotometry (AAS) is an analytical technique employed to measure the concentration of metal atoms or ions in a sample. Metals constitute approximately 75% of the chemical elements found in the Earth. While metals are essential in many materials, they can also act as contaminants or toxic substances. Therefore, accurately determining metal content is crucial across various fields, including quality control, toxicology, and environmental monitoring.

Principle of Atomic Absorption Spectroscopy

The fundamental principle of Atomic Absorption Spectrophotometry (AAS) is that all atoms or ions absorb light at specific, characteristic wavelengths. For example, when a sample containing copper (Cu) and nickel (Ni) is exposed to light at copper’s specific wavelength, only the copper atoms or ions will absorb that light, while the nickel remains unaffected. The intensity of light absorbed at a specific wavelength is directly proportional to the concentration of the atoms or ions that absorb it. Within an atom, electrons occupy discrete energy levels. When the atom is exposed to its characteristic wavelength, it absorbs energy in the form of photons, causing electrons to transition from their ground state to higher, excited energy states.

The radiant energy absorbed by the electrons is directly related to the transition that occurs during this process. Furthermore, since the electronic structure of every element is unique, the radiation absorbed represents a unique property of each individual element and it can be measured.

Atomic Absorption Spectrophotometry (AAS) utilizes these basic principles to carry out practical, quantitative measurements of metal concentrations. A standard AAS instrument is composed of four primary components: the light source, the atomization system, the monochromator, and the detection system, as shown in the figure below:

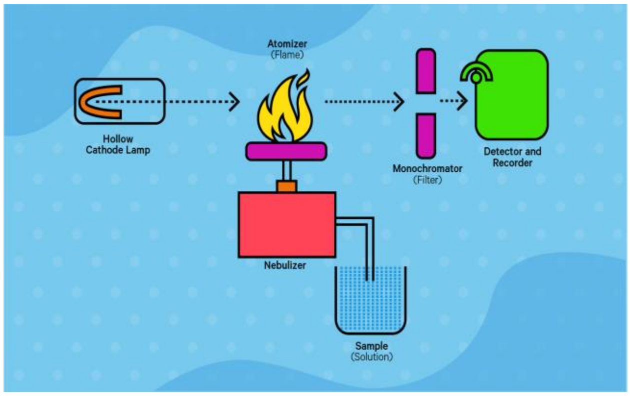

Fig 1.14 Schematic diagram of a typical atomic absorption spectrometer

In a typical experiment, the sample, either liquid or solid, is atomized in either a flame or a graphite furnace. The free atoms are then exposed to light, typically produced by a hollow-cathode lamp, and undergo electronic transitions from the ground state to excited electronic states. The light produced by the lamp is emitted from excited atoms of the same element that is to be determined, therefore the radiation energy corresponds directly to the wavelength absorbed by the atomized sample. A monochromator is placed between the sample and the detector to reduce background interference. From here, the detector measures the intensity of the beam of light and converts it to absorption data.

Although solid samples can be analyzed using AAS, this method is generally limited to the more costly graphite furnace technique, which allows the sample to be heated through controlled electrical energy rather than a direct flame. Additionally, AAS is typically used only for the analysis of metal atoms. This is because metals exhibit narrow, bright, and well-defined single emission and absorption lines, making them ideal for this technique.

Interpreting results in Atomic Absorption Spectrophotometry (AAS) is straightforward and follows Beer's law, which states that absorbance is directly proportional to the concentration of the analyte. As a result, the concentration of the analyte can be determined from the electrical signal generated by the detector, with higher absorbance corresponding to higher concentrations. Wavelengths typically used for the analysis of heavy metals using AAS include: Cadmium (Cd) at 228.8nm, Cobalt (Co) at 240.7nm, Chromium (Cr) at 357.9nm, Copper (Cu) at 324.8nm, Lead (Pb) at 217nm, Manganese (Mn) at 279.5nm, Nickel (Ni) at 232nm and Zinc (Zn) at 213.9nm (Wasim et al., 2019).

Beer's Law

Beer's law (sometimes called the Beer-Lambert law) states that the absorbance is proportional to the path length, b, through the sample and the concentration of the absorbing species, c:

$A \propto b \cdot c$

The proportionality constant is sometimes given the symbol a, giving Beer's law an alphabetic look:

$A = a \cdot b \cdot c$

The constant **a** is called the absorptivity. More formally, the proportionality constant is represented by ε and is called the extinction coefficient:

$A = \varepsilon \cdot b \cdot c$

If ε has molar units, it is called the molar extinction coefficient, or the molar absorptivity. The molar absorptivity varies with wavelength, and Beer's law is more accurately written as a function of λ:

$A(\lambda) = \varepsilon(\lambda) \cdot b \cdot c$

The direct relationship between absorbance and concentration illustrated by Beer's law often makes absorbance a more useful mode for spectra than transmittance (Ball, 2006).

ATOMIZING TECHNIQUES

They are of two main types:

• Flame Atomic Absorption Spectroscopy (FAAS)

• Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)

ADVANTAGES AND LIMITATIONS OF AAS

The advantages of AAS most certainly outweighs the limitations as listed in the table below:

ADVANTAGES	LIMITATIONS
Low cost per analysis	Cannot detect non-metals
Easy to operate	New equipment is quite expensive
High sensitivity (up to ppb detection)	More geared towards analysis of liquids
High accuracy	Sample is destroyed
Mostly free from inter-element interference	
Wide applications across many industries	

APPLICATIONS OF AAS

AAS is used in a wide range of chemistry applications, including environmental, food, pharmaceuticals, and industrial analysis.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Reagents/Chemical used

64.5% Nitric acid (HNO_3): Used for sample digestion due to its strong oxidizing properties.

Perchloric acid ($HClO_4$): Enhances the digestion process and aids in breaking down organic matter.

Distilled water: Used for sample dilution and preparation to ensure contamination-free analysis.

2.1.2 Instruments/Apparatus used

Instruments:

Electronic analytical balance

Laboratory grinding mill

Sand bath: Hot plate and pot filled with sand

Atomic Absorption Spectrometer

Apparatus:

300ml digestion flask

100ml volumetric flask

Funnels

Spatula

Measuring cylinder

Whatman filter paper

Wash bottle

Sample bottles

2.2 METHODS

2.2.1 STUDY AREA

The study was conducted within the environs of Ugbowo Campus, University of Benin, Benin City, Edo State. Specifically, the sampling site was located in front of the Department of Chemistry, University of Benin. The area contains a diverse array of tree species, making it suitable for environmental monitoring. This location was selected due to its representation of campus traffic, which includes a wide range of motorized vehicles such as trailers, tankers, tippers, trucks, and cars. Natural environmental factors, such as wind and local climatic conditions, also influence the area. Only trees older than 10 years and situated within 4 meters of the highway were chosen for the study (Ukpebor et al., 2010). Based on these criteria, Mangifera indica (Mango) and Psidium guajava (Guava) trees were selected because of their abundance and year-round availability. The coordinates of the study site are 6°23′57.858″ N, 5°36′53.971″ E.

Fig 2.1 Map of Study Area: Ugbowo Campus, University of Benin

2.2.2 SAMPLE COLLECTION AND STORAGE

The samples of the leaves were gathered in a systematic way, in the outer canopy, at 2 meters to 4 meters in height, closest to the highway. The collection was achieved through the use of extendable scissors and polyethylene bags to avoid cross-contamination. Mature leaves of current-season growth were selected only and not the juvenile growing foliage or senescence yellowing foliage likely to show a different metal content profile. About 10g of fresh leaf material was cut off each from each tree, and leaves of various branches were sampled to provide a representativeness of the whole canopy (Guleryuz et al., 2020).

Bark samples from the trees were carefully collected using a clean stainless-steel penknife. Sampling was performed at a convenient height of approximately 1.5 to 2.0 meters above the ground (Ukpebor et al., 2010). This standardized height minimizes ground-level contamination effects while ensuring consistent exposure to atmospheric pollutants. pollutants. Multiple bark samples were collected from each tree to account for spatial variability around the trunk circumference. The collected samples were left unwashed, securely sealed, and immediately stored in polyethylene bags to preserve their integrity.

All sampling equipment were thoroughly sterilized with detergent solution and rinsed with deionized water before use and between sampling locations to prevent cross-contamination. The collected samples were immediately placed in pre-labeled polyethylene bags and sealed to prevent moisture loss and contamination during transport. Subsequently, all samples were transported to the laboratory for further processing and analysis.

2.2.3 SAMPLE TREATMENT

The treatment process began with thorough washing of samples to remove surface contaminants, dust, and atmospheric particles that could interfere with the determination of accumulated heavy metals in plant tissues.

Washing Procedure: Bark and leaf samples were initially subjected to gentle brushing using soft-bristled brushes to remove loose debris and surface particles. The samples were first washed thoroughly with clean tap water to remove dirt and debris. They were then rinsed three times with distilled water to eliminate any remaining foreign particles and surface contaminants adhering to the bark.

Fig 2.2 Washed bark and leaf samples of Mangifera indica (left) and Psidium guajava (right)

Drying and Grinding: Following washing, samples were dried to constant weight in an oven maintained at 105°C ± 3°C for 3 hours. This temperature was selected to ensure complete moisture removal while preventing thermal degradation of organic compounds and loss of volatile metals. Dried samples were allowed to cool in a desiccator before further processing.

A laboratory grinder, thoroughly cleaned, rinsed with distilled water, and dried, was used to grind the samples into a fine powder. The powdered samples were then stored in pre-labeled plastic containers and properly sealed to prevent contamination. The grinder was washed after each use to avoid cross-contamination between samples. Grinding the samples increases their surface area, which enhances the efficiency of heavy metal detection

Fig 2.3 Ground bark and leaf samples

<u>Digestion Procedure:</u> All apparatus used were thoroughly washed with distilled water before the digestion process. A widely used acid-based wet digestion method was used for the plant samples (Palma et al., 2015; Standard Methods Online, 2025), with slight modifications. 2g each of the bark and leaves samples was accurately measured using an electronic analytical balance into a 300ml digestion flask. 30ml of concentrated (64.5%) nitric acid (HNO_3) was then added slowly to each sample, and the mixture was allowed to stand for about 15 minutes to enable cold digestion.

The samples were then heated gently in a sand bath for about 45 minutes until violent foaming subsided and dense orange fumes evolved, indicating breakdown of organic matter. 10ml of concentrated perchloric acid ($HClO_4$) was then added gently to each flask while maintaining gentle heating. The acid mixture of 30ml HNO3 and 10ml HClO4 in a 3:1 ratio was used to facilitate oxidation and breakdown of organic matter. Both concentrated acids were not added at the same time to the samples in order to prevent explosion.

Heating was continued until the solutions became clear and colorless, signifying complete digestion. The digested solutions were allowed to cool to room temperature and then filtered using Whatman No.1 filter paper into clean 100ml volumetric flasks to remove any residual undigested particles. The filtrates in the 100ml volumetric flasks were then diluted with distilled water to the 100ml mark to ensure uniformity and make the solutions instrument-friendly. The diluted solutions were transferred into labelled sample bottles and stored at 4°C until analysis.

Heavy metal concentrations, including Lead (Pb), Iron (Fe), Chromium (Cr) and Nickel (Ni) were determined using an Atomic Absorption Spectrophotometer (AAS). This laboratory work was conducted in duplicates with the presence of blank solutions.

All digestion procedures were carried out in a fume cupboard with appropriate safety equipment including nose masks, hand gloves, and laboratory coats.

Fig 2.4 Digestion process of samples

2.2.4 SAMPLE ANALYSIS

The concentrations of selected heavy metals (Lead [Pb], Iron [Fe], Chromium [Cr] and Nickel [Ni]) were determined using Atomic Absorption Spectrophotometry (AAS) following standard operating procedures. The AAS was calibrated using standard metal solutions of known concentrations, and blank. Each sample was analyzed in duplicates to ensure accuracy, and the mean concentrations were calculated.

Quality Assurance and Control

To ensure reliability and validity of results, the following quality control measures were observed:

- All glass wares were soaked overnight and properly rinsed with distilled water before use.
- Reagent blanks were prepared and analyzed to correct for background contamination.
- Duplicate analyses and calibration checks were done at regular intervals.

Data Interpretation and Analysis

The concentrations of heavy metals are expressed in mg/kg. Data were subjected to descriptive statistics (mean) and compared against World Health Organization (WHO) permissible limits for heavy metals in plants. All analyses were performed in duplicate, and results are expressed as mean concentration in mg/kg

Mean = (A+ B) / 2

where **A** represent **Result 1** and **B** represents **Result 2**

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 RESULTS AND DISCUSSION

The four specified heavy metals: Chromium (Cr), Iron (Fe), Nickel (Ni) and Lead (Pb) are all detected in the plant parts. Duplicate readings and recording was carried out for each heavy metal determination via AAS. The mean of the values were evaluated and thus tabulated as shown in Tables 3.1 to 3.4. The evaluated values of the mean concentration are approximated to two places of decimal for easy interpretation and discussion. This is also done for uniformity in data reporting. The mean concentration of the instrumentally measured values of the heavy metal types that constitutes environmental pollution in Ugbowo Campus, University of Benin, is hereby presented in Tables 3.1 to 3.4

Table 3.1 Concentration of heavy metals (Cr, Fe, Ni, Pb) in the bark and leaf samples of Mangifera indica

	METAL CONCENTRATION (mg/kg)			
PLANT PART	Cr	Fe	Ni	Pb
Bark	0.24	15.85	0.11	0.04
Leaf	0.28	15.70	0.09	0.03
Allowable limit by FAO/WHO (Okedeyi et al., 2013)	0.02	20.00	1.63	0.43

<u>For plant bark sample:</u> As unveiled in Table 3.1, all the heavy metals sampled for were identified. The highest value is found in Iron (Fe) at 15.85mg/kg and the least value found in Lead (Pb) at 0.04mg/kg. When compared to the maximum allowable limit for edible plants, there is a breach in the value of Chromium (Cr) at 0.24mg/kg and is above the regulatory limit (0.02mg/kg) set by FAO/WHO (Okedeyi et al., 2013). Every other heavy metal detected complies with the allowable limit.

For plant leaf sample: As seen in Table 3.1 above, the highest value of heavy metals is also found in Iron (Fe) at 15.70 mg/kg and the least value is seen in Lead (Pb) at 0.03 mg/kg. The concentration of Chromium in this leaf samples is above the allowable limit and therefore violates the regulatory limit set by FAO/WHO. The rest heavy metals detected complies to that of the allowable limit set by FAO/WHO.

Table 3.2 Concentration of heavy metals (Cr, Fe, Ni, Pb) in the bark and leaf samples of Psidium guajava

	METAL CONCENTRATION (mg/kg)			
PLANT PART	Cr	Fe	Ni	Pb
Bark	0.19	2.25	0.03	0.02
Leaf	0.15	2.70	0.06	0.04
Allowable limit by FAO/WHO (Okedeyi et al., 2013)	0.02	20.00	1.63	0.43

For plant bark sample: The table above shows that there is a breach in the permissible limit of Chromium heavy metals. The levels of Fe, Ni and Pb, all complies to the allowable limit set by FAO/WHO (Okedeyi et al., 2013).

For leaf sample: The highest concentration of heavy metals is found in Iron (Fe) at 2.70 mg/kg, while the lowest in Lead (Pb) at 0.04mg/kg. When compared with the allowable limit of

Chromium (Cr) set by FAO/WHO, there is a breach in the concentration of Chromium (0.15mg/kg).

Table 3.3 A comparison between levels of heavy metals (mg/kg) in the bark samples

Heavy metals	Mangifera indica bark	Psidium guajava bark
Cr	0.24	0.19
Fe	15.85	2.25
Ni	0.11	0.03
Pb	0.04	0.02

Based on specific tree bark analysis, Mangifera indica bark accumulated 15.85 mg/kg Fe as the highest and 0.04 mg/kg Pb has the least heavy metals. The biomonitoring potential of Mangifera indica in respect to Cr and Ni is 0.24mg/kg and 0.11mg/kg respectively. Considering Psidium guajava, the range of bioaccumulated heavy metals levels is 2.25mg/kg - 0.02mg/kg. This comparison shows that the bark of Mangifera indica is a good biomonitor of Chromium (0.24mg/kg), Iron (15.85mg/kg), Nickel (0.11mg/kg), and Lead (0.04mg/kg). This result therefore shows that the nature of the tree bark has a significant influence on the concentration of the metals. Tree having rough bark (Mangifera indica) gave higher concentration values than that with relatively smoother bark (Psidium guajava).

Table 3.4 A comparison between levels of heavy metals (mg/kg) in the leaf samples

Heavy metals	Mangifera indica leaf	Psidium guajava leaf
Cr	0.28	0.15
Fe	15.70	2.70
Ni	0.09	0.06
Pb	0.04	0.04

The range of heavy metals divulged by the leaves of Mangifera indica is 15.70mg/kg - 0.03 mg/kg. The leaves of Psidium guajava revealed the highest biomonitoring potential towards Fe (2.70 mg/kg) and the least at Pb (0.04 mg/kg). The levels of Ni and Pb are almost the same value. Comparatively, as seen in Table 3.4 above, the concentration of heavy metals found in Mangifera indica leaves is higher than those in Psidium guajava leaves. This result therefore shows that the broader the leaves (Mangifera indica), the higher the concentration of heavy metals.

CONCLUSION

The results of this experiment indicates that Mango (Mangifera indica) and Guava (Psidium guajava) have the capacity to absorb trace levels of heavy metals in the environment they are located in, which confirms their future use as an effective biomonitor in environmental pollution. All samples were found to have a higher concentration of Chromium (Cr) than the WHO/FAO allowable limit of 0.02mg/kg, which suggests the possibility of anthropogenic sources that include vehicular emissions, industrial discharges or atmospheric deposition. Both plant species had lower than the permitted concentration of iron (Fe), indicating that there was no significant iron contamination in the study area. The levels of nickel (Ni) and lead (Pb) were also found to be within the permissible range, which further indicated that little contamination of these two types of metals was present.

In comparison, Mangifera indica showed greater concentrations of chromium and nickel as compared to Psidium guajava indicating that mango tree might be more efficient in metal uptake or be more exposed by their canopy cover and leaf structures. The accumulation of metallic levels was generally more in the bark than the leaf and this is an indication of the direct exposure of the bark to atmospheric particulates and deposition.

In general, the results show that Mangifera indica and Psidium guajava can be considered appropriate biomonitor to evaluate the quality of environment and monitor the tendencies of heavy metal pollution.

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