CONCENTRATIONS OF NICKEL, CADMIUM, MERCURY AND OXIDATIVE STRESS STATUS OF TOMATOES FROM USELU AND IKPOBA HILL MARKET IN BENIN CITY

BY

MICHELLE ESEOGHENE SALUBI

LSC1806609

DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

APRIL, 2024

CONCENTRATIONS OF NICKEL, CADMIUM, MERCURY AND OXIDATIVE STRESS STATUS OF TOMATOES FROM USELU AND IKPOBA HILL MARKET IN BENIN CITY

BY

MICHELLE ESEOGHENE SALUBI

LSC1806609

AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

APRIL, 2024.

CERTIFICATION

This is to certify that this research titled "**NICKEL, CADMIUM, MERCURY AND OXIDATIVE STRESS STATUS OF TOMATOES FROM USELU AND IKPOBA HILL MARKET IN BENIN CITY"** was carried out by **"MICHELLE ESEOGHENE SALUBI"** with matriculation number **"LSC1806609"** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

_____ _____

Dr. (Mrs) G. E. OGBEIDE **Date**

(Project Supervisor)

_____ _____

Prof A. A. ENUNEKU **Date**

(Head of Department)

DECLARATION

I "**MICHELLE ESEOGHENE SALUBI**" declare that "**NICKEL, CADMIUM, MERCURY AND OXIDATIVE STRESS STATUS OF TOMATOES FROM USELU AND IKPOBA HILL MARKET IN BENIN CITY**" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

…….. …….

MICHELLE ESEOGHENE SALUBI **Date**

DEDICATION

I express my sincere gratitude to God, The source of all knowledge and power, for leading me on this trip. Your love, Tolerance and everlasting support of my cherished family have been my pillars of strength. Your support has been my pillar and I dedicate this work to you all.

ACKNOWLEDGEMENTS

I want to thank God almighty for his awesome love, mercy and strength.

I also wish to express my profound gratitude to my supervisor **Dr. (Mrs) G. E. Ogbeide** for her consistent Support, Advice and Guidance during the course of this project, and also my Head of Department **Professor A.A Enuneku**. I would also like to acknowledge Some lecturers whose encouragement and support was instrumental and necessarily needed to complete this project, These lecturers include My course advisor Dr. O. Osarenotor and Dr. Jeff Ogbebor

I would also like to acknowledge my parents (Mr. and Mrs. P. Salubi) for their support financially, spiritually, emotionally and My siblings whose love and assistance was constant during the course of this project and all through my stay in the university, I don't know what I would have done without their help.

My acknowledgement also goes out to my friends: Okafor Stephen, Dikko Emmanuel, Mentie Anita, Aluma Deborah and SCAM LORDS who kept me company during the hard times of this project and a big part of the reason I had an awesome time in this school.

I would like to say that I love you all and I honestly appreciate all your love, support and may the good gracious God that I serve bless you all.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ABSTRACT

This study was carried out to determine the concentrations of some heavy metals such as nickel, cadmium, mercury and oxidative stress status of tomatoes sold in local markets in Benin City, Edo State, Nigeria. Fresh tomatoes samples were brought from Uselu and Ikpoba hill market in Benin City. The samples were oven-dried at 105°C for 24 hour and grinded to powder. The powdered samples were dissolved in deionized water, filter through whatman filter paper and the volume was made up to 25 ml using 10 ml of 98% nitric acid. The resulting solution was analysed for the presences of heavy metal using using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer model 2130). The results showed that the nickel concentration present fresh tomatoes samples obtained from the two markets ranged from 0.13 mg/kg to 0.16 mg/kg, while the cadmium concentration ranged from 0.11 mg/kg to 0.13 mg/kg. However when compared to W.H.O standard the heavy metal concentration were slightly above the recommended value of 0.10 mg/kg. Also oxidative stress analysis showed that the superoxide dismutase (SOD) value ranged from 3.02 U/g to 3.48 U/g, Catalase (CAT) value ranged from 0.08 U/g to 0.10 U/g , Glutathione peroxidase (GPx) value ranged from 4.96 U/g to 5.73 U/g, while Malondialdehyde (MDA) value ranged from 0.50 U/g to 0.64 U/g respectively. These findings were indicative of environmental pollution due to industrial and vehicular emissions and also the mode of handing and processing of the samples. Although the heavy metals detected in this study are not beneficial to man and plant, low concentration can prove detrimental to health.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The effect of heavy metal contamination of fruit and vegetables cannot be underestimated as these foodstuffs are important components of human diet. Fruit and vegetables are rich sources of vitamins, minerals, and fibers and also have beneficial antioxidative effects (Barraza *et al.,* 2017). However, the intake of heavy metal-contaminated fruit and vegetables may pose a risk to human health; hence the heavy metal contamination of food is one of the most important aspects of food quality assurance. Heavy metals, in general, are not biodegradable, have long biological half-lives, and have the potential for accumulation in different body organs, leading to unwanted side effects (Castro *et al.,* 2015). Plants take up heavy metals by absorbing them from airborne deposits on the parts of the plants exposed to the air from the polluted environments as well as from contaminated soils through root systems. Also, the heavy metal contamination of fruit and vegetables may occur due to their irrigation with contaminated water. Gupta *et al.* (2019) have investigated the concentrations of some heavy metals in different vegetables grown in various parts of Nigeria. The levels of heavy metals (lead, cadmium, copper, and zinc) have been examined in selected fruits and vegetables sold in local Nigerian markets. Murad-Basha *et al.* (2017) studied the contents of heavy metals in vegetables grown in an industrial area of Northern Nigeria.

Marín *et al.* (2018) investigated the concentrations of some heavy metals in fruits and leafy vegetables from selected markets in Lagos, Nigeria. Based on their persistence and cumulative behavior as well as the probability of potential toxicity effects, the absorption of heavy metals in

human diets as a result of the consumption of vegetables and fruits means that there is a requirement for the analysis of food items to ensure that the levels of trace heavy metals meet the agreed international standards. This is particularly important for farm products from parts of the world where only limited data on the heavy metal content are available (Stancic *et al.,* 2016).

Heavy metals (HMs) constitute one group of known hazardous substances; in some cases, these elements are naturally present in soils, as they originate from the erosion of rocks or volcanic activity. Additionally, anthropogenic activities like mineral processing; chemical, metallurgic, petrochemical, and textile industries; and fuel combustion, among others, have increased HM concentrations. In both cases, these contaminants are readily available for intake by plants because they are present mostly in the soil surface, along with plant nutrients (Strungaru *et al.,* 2018). There is worldwide concern about some metals, such as cadmium (Cd), iron (Fe), and zinc (Zn), because they have the capacity to translocate into plant shoots; other metals like arsenic (As), chromium (Cr), and lead (Pb) bioaccumulate in most plant organs: roots, stem, leaves, and fruits. In addition, HMs are nonbiodegradable, and they can move through food webs to ultimately be consumed by humans, which may result in various health risks due to their acute or chronic toxicity (Sterckeman *et al.,* 2018). This concern has led researchers to test and apply different methodologies for sample preparation and metal quantification in food samples, including acid calcination, microwave-assisted acid digestion and the use of absorbents and nanoparticles to extract HMs before quantification (Aguilar *et al.,* 2018).

In crop cultivation, organic waste is commonly used as a source of nutrients, but long-term application can increase HM concentrations in soils. In cultivable soils, Cd presence is assumed to occur from the soils' own natural volcanic composition, but it can also follow when some fertilizers (e.g., phosphorous derivatives) are used. Once consumed, this metal is retained in the

human body, particularly in the kidneys, producing highly toxic effects and increasing the risk of kidney failure and cancer (Cherfi *et al.,* 2014). For nonsmoking populations, food consumption is the main source of Cd exposure. Pb is another harmful HM that can damage the nervous, skeletal, circulatory, enzymatic, endocrine, and immune systems. The presence of metals in vegetables is influenced by many factors, principally by the crop species and its metabolism, and by others such as the soil's initial concentration of contaminants, pH, organic matter availability, and presence of other ions and molecules (Liang *et al.,* 2018). The kinetics of HMs' uptake depends on the mechanisms of their ion toxicity, including the blocking of functional groups in biomolecules and the replacing of essential metal ions in biomolecules. Crops that are located close to vehicular traffic and factories also have an increased likelihood of HM presence.

Considering that vegetables are an important dietary source of essential nutrients, especially in terms of their high content of protein, vitamins, minerals, and fibers as well as beneficial antioxidant effects, their consumption is generally recommended. Nevertheless, vegetables may also contain elevated concentrations of HMs due to high transfer from the soil to the harvested crop (Bakkali *et al.,* 2019). Further, since the early 20th century, human exposure to TMs from natural food consumption has increased because of their intensified use in industrial processes and products.

1.2 Statement of the Problem

Many plants contain both essential and toxic metals over wide range of concentration. It is well known that plant takes up metals by absorbing them from contaminated soil as well from deposits on part of the plant exposed to the air from polluted environment; heavy metals contamination may occur due to irrigation with contaminated water, the addition of fertilizer,

metals based on pesticides, industrial emission transportation, harvesting process storage and sale the contamination of vegetables with heavy metals in tomatoes is associate with etiology of a number of disease especially cardiovascular kidney nervous system and bones diseases.

1.3 Justification of the study

Tomatoes constitute an essential dietary component by contributing vitamins, iron, calcium and other micronutrient which are usually in short supply, they also act as buffering agent for acidic substance produced during digestion process. However, they contain both essential and toxic element over a wide a range of concentration metals accumulation in vegetable may pose a direct threat to human health hence this study tends to assess the presence of heavy metals presence in tomatoes sold in local markets in Benin City, Edo State, Nigeria.

1.5 Aim and Objectives

The aim of this study is to determine the concentration of some heavy metals such as nickel, cadmium, mercury and oxidative stress status of tomatoes sold in local markets in Benin City, Edo State, Nigeria.

The specific objective of the study is to:

1. determine the concentration nickel, cadmium and mercury in tomatoes sold in Uselu and Ikpoba hill markets in Benin City.
2. determine the oxidative stress level in tomatoes sold in Uselu and Ikpoba hill markets in Benin City.
3. determine the Health Risk Index (HRI) of the heavy metals accumulated.

CHAPTER TWO

LITERATURE REVIEW

The metal contamination in food has raised public and scientific interest due to their dangerous effects on human health. This has led researchers all over the world to study the pollution with heavy metals in air, water, and foods to avoid their harmful effects and to determine their permissibility for human consumption (de Oliveira *et al.,* 2017). Tomato is one of the most widely cultivated crops in the world. Tomatoes contribute to a healthy, well-balanced diet. They are rich in minerals, vitamins, essential amino acids, sugars and dietary fibres. Tomato contains much vitamin B and C, iron and phosphorus. Tomato fruits are consumed fresh in salads or cooked in sauces, soup and meat or fish dishes. Tomatoes (*Solanum lycopersicum* L.), which are frequently included in the African diet and are widely consumed as vegetables, play an important role in nutrition because of their well-established health benefits (Romero-Estévez *et al.,* 2019). Tomatoes are used in many processed food products such as sauces, salads, soups, and pastes. Common nutrients reported to be present in tomatoes are vitamins, minerals, fiber, protein, essential amino acids, monounsaturated fatty acids, carotenoids and phytosterols. These nutrients perform various body functions including constipation prevention, reduction in high blood pressure, stimulation of blood circulation, maintenance of lipid profile and body fluids, detoxification of body toxins and maintaining bone structure as well as strength (Behbahani *et al.,* 2016).

2.1 Tomatoes

Tomatoes are also an excellent source of nutrients and bioactive compounds, commonly known as secondary metabolites, the concentrations of which are correlated with the prevention of human chronic degenerative diseases, such as cardiovascular disease (CVD), cancer, and

neurodegenerative diseases (Behbahani *et al.,* 2016). Due to the high concentrations of different natural antioxidant chemicals, such as carotenoids (β-carotenoids and lycopene), ascorbic acid (vitamin C), tocopherol (vitamin E) and bioactive phenolic compounds (quercetin, kaempferol, naringenin and lutein, as well as caffeic, ferulic and chlorogenic acids), tomatoes can help ameliorate many diseases, especially chronic diseases (Sedghi *et al.,* 2015). These compounds play beneficial roles in inhibiting reactive oxygen species (ROS) by scavenging free radicals, inhibiting cellular proliferation and damage, inhibiting apoptosis as well as metal chelation, modulation of enzymatic activities, cytokine expression and signal transduction pathways. The main carotenoid in tomato is lycopene, which is responsible for its red color. The pharmacological activities of lycopene and other phenolic compounds include anticancer, anti-inflammatory, antidiabetic, anti-allergenic, anti-atherogenic, antithrombotic, antimicrobial, antioxidant, vasodilator and cardioprotective effects (da Rosa Couto *et al.,* 2018). In addition to having good nutritive value and health promoting activities, the polyphenolic compounds and carotenoids also contribute to sensory activities including maintaining good aroma, taste, and texture.

Tomato is an important dietary source of both soluble and insoluble dietary fibers, namely cellulose, hemicelluloses and pectins. In general, these fibers are resistant to intestinal digestion in the large intestine and are believed to ameliorate bowel disorders, cancer, diabetes, CVDs, and obesity (Nordberg *et al.,* 2018). Important proximate composition parameters for tomatoes include sugar content, pH, energy, acidity and reducing sugar contents. The proximate compositions help in the characterization and identification of tomato nutrients. The combination of vitamins, minerals, amino acids, and fats all together contribute to making tomato part of a balanced diet. Phytosterols, which are involved in the prevention of colon cancer and heart

disease, are present in tomatoes in lower amounts than that found in other fruits and vegetables. Among the phytosterols, β-sitosterol, campesterol and stigmasterol are the main ones. The antioxidant compounds predominately present in tomato consist of several different types of carotenoids, vitamin C, vitamin E, and phenolic compounds that confer their antioxidant activities by neutralizing reactive oxygen species (ROS) and protecting the cell membrane against lipid peroxidation (Romero-Estévez *et al.,* 2019).

Nutritional composition of tomato varies based on the tomato cultivar, extraction procedures, analysis methods and environmental conditions. During the processing of tomato products, up to 30% of their original weight are turned into waste, which may still contain some nutritive values (Zhou *et al.,* 2018. For example, the seeds and the peel are the main waste product of tomato, which are rich in protein, dietary fibers, bioactive compounds and lycopene. The by-products are used as food additives especially in the meat industries. Nevertheless, although the waste products of tomato are a rich source of nutrients, proper research should be undertaken before their consumption. In spite of having health benefits, tomatoes demonstrate some undesired effects on the body when consumed in large amounts or in abnormal body conditions. The adverse effects of tomato intake are associated with renal problems, allergies, arthritis, heartburn, and migraine (Nieboer and Richardson, 2018).

2.2 Taxonomical Classification of Tomatoes

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Solanales

Family – Solanaceae

Genius – *Solanum*

Species – *S. lycopersicum*

2.3 Types of Tomatoes

There are many variants of tomatoes and each provides the benefits listed above and more, here are the five most common types of tomatoes

2.3.1 Globe Tomatoes

These are your standard variant of tomatoes and are often referred to as beefsteak. Tomatoes or slicing tomatoes they are easily identifiable because they are large, round, and red. They can also weigh up to two pounds (Dala-Paula *et al.,* 2018).

2.3.2 Cherry Tomatoes

These tomatoes belongs to the cluster variant, cherry tomatoes tend to be just about the same size as a cherry tomatoes. But they are much sweeter and juicier than the large varieties, such as Globe Tomatoes.

2.3.3 Roma Tomatoes

Also known as plum tomatoes, they are the least juicy of all tomatoes, because they are thick and contain fewer seeds than other tomatoes variants. Roma tomatoes are a fovote pasta sauces (Corradini *et al.,* 2018).

2.3.4 Heirloom Tomatoes

This variety is gaining in popularity with government chiefs. This is because heirloom tomatoes come in large variety of shapes and colors. Their biggest downfall is that they tend to remain ripe for a very short period of time.

2.3.5 Pear Tomatoes

Another member of the cluster tomatoes variant, pear tomatoes gets their name from their shape, which resembles a pear. They are only the size of cherry tomatoes but without the high juice levels treatment of high blood pressure (França *et al.,* 2017).

2.4 Heavy Metal

The term "Heavy metals" refers to any metallic chemical element that has a relatively high density and is toxic or poisonous as low concentration. Example of heavy metals of which this project is based on includes lead, chromium, cadmium and copper, heavy metals are natural component of the earth. They cannot be degraded or destroyed to a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals are essential to maintain the metabolism of the human body. However at high concentration they can lead to poisoning (Ercilla-Montserrat *et al.,* 2018). Heavy metals poisoning could result for instance, from drinking water contamination (e.glead pipes) high ambient air concentrations near emission sources or intake via the foodchain. Heavy metals can enter a water supply by industrial and consumer waste or even from acidic rain breaking down soil and releasing heavy metals into streams, lakes, and ground waters. This implies that heavy metals have both health and environmental risks (Mohod, 2015).

2.5 Toxic Effect of Some Heavy Metals

Heavy metals are commonly found in the environment and diet. In small amounts they are required for maintaining good health but in larger amounts they can become toxic or dangerous. Heavy metal toxicity can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition and other important organs (Labhade, 2015). Long-term exposure can lead to gradually progressing physical, muscular, and neurological degenerative processes that imitate diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease and muscular dystrophy. Repeated long-term exposure of some metals and their compounds may even cause cancer. The toxicity level of a few heavy metals can be just above the background concentrations that are being present naturally in the environment (Hadayat *et al.,* 2018)

Heavy metals have effects on human body. Thus the importance of heavy metals is significant to man as it affect man’s health. Arsenic causes skin disease and leukemia while antimony tartrate causes tropical disease. The arsenic compounds are used as pesticides and for wood preservation. The phytotoxic effects of arsenic compounds made them as herbicide. But when the concentration of the metals arevey high beyond limits they become toxic. Nio-accumulation is a process whereby the heavy metals are stored up in living systems, acquired from their surrounding into their chelation process (Silva *et al.,* 2017).

2.6 The Study of Some Heavy Metals and their Toxicity

2.6.1 Cadmium

Cadmium occurs naturally in ores together with zinc, lead and copper. Cadmium compounds are used as stabilizers in polyvinyl chloride products, colored pigment, several alloys and, now most commonly, in re-chargeable nickel-cadmium batteries. Metallic cadmium has mostly been used

as an anti-corrosion agent. Cadmium is also present as a pollutant in phosphate fertilizers. EU cadmium usage has decreased considerably during the 1990s, mainly due to the gradual phase-out of cadmium products other than Ni-Cd batteries and the implementation of more stringent environmental legislation (Vallverdú-Queralt *et al.,* 2015). Notwithstanding these reductions in Europe, however, cadmium production, consumption and emissions to the environment worldwide have increased dramatically during the 20th century. Cadmium containing products are rarely re-cycled, but frequently dumped together with household waste, thereby contaminating the environment, especially if the waste is incinerated. Natural as well as anthropogenic sources of cadmium, including industrial emissions and the application of fertilizers and sewage sluge to farm land, may lead to contamination of soils, and to increased cadmium uptake by crops and vegetables, grown for human consumption (Barraza *et al.,* 2017). The uptake process of soil cadmium by plants is enhanced at low pH. Cigarette smoking is a major source of cadmium exposure. Biological monitoring of cadmium in the general population has shown that cigarette smoking may cause significant increases in blood cadmium levels, the concentrations in smokers being on average 4-5 times higher than those in non-smokers. Despite evidence of exposure from environmental tobacco smoke, however, this is probably contributing little to total cadmium body burden (Castro *et al.,* 2015).

Food is the most important source of cadmium exposure in the general nonsmoking population in most countries. Cadmium is present in most foodstuffs, but concentrations vary greatly, and individual intake also varies considerably due to differences on dietary habits. Women usually have lower daily cadmium intakes, because of lower energy consumption than men. Gastrointestinal absorption of cadmium may be influenced by nutritional factors, such as iron status (Gupta *et al.,* 2019). Generally reflects current exposure, but partly also lifetime body

burden, being proportional to the kidney concentration. Smokers and people living in contaminated areas have higher urinary cadmium concentrations, smokers having about twice as high concentrations as non-smokers.

Acute pulmonary effects and deaths are uncommon, sporadic cases still occur. Cadmium exposure may cause kidney damage. The first sign of the renal lesion is usually a tubular dysfunction, evidenced by an increased excretion of low molecular weight proteins (such as β2-microgloblin and α1 microglobulin) or enzymes (such as NAcetyl-β -D-glucosamindase). It has been suggested that the tabular damage is reversible, but there is overwhelming evidence that the cadmium induced tubular damage is indeed irreversible (Murad-Basha *et al.,* 2017). WHO estimated that a urinary excretion of 10 nmol/mmol creatinine would constitute a 'critical limit' below which kidney damage would not occur However, WHO calculated that circa 10% of individuals with this kidney concentration would be affected by tubular damage. Several reports have since shown that kidney damage and/or bone effects are likely to occur at lower kidney cadmium levels. European studies have shown signs of cadmium induced kidney damage in the general population at urinary cadmium levels around 2-3 µg Cd/g creatinine (Marín *et al.,* 2018). The initial tubular damage may progress to more severe kidney damage, and already in 1950 it was reported that some cadmium exposed workers had developed decreased glomerular filtration rate.

This has been confirmed in later studies of occupationally exposed workers. An excess risk of kidney stones, possibly related to an increased excretion of calcium in urine following the tubular damage, had been shown in several studies (Stancic *et al.,* 2016. Recently, an association between cadmium exposure and chronic renal failure was shown. Using a registry of patients, who had been treated for uremia, the investigators found a double risk in persons living close to

(<2km) industrial cadmium emitting plants as well as in occupationally exposed workers. Long-term high cadmium exposure may cause skeletal damage, first reported from Japan, where the itai-itai (ouch-ouch) disease (a combination of osteomalacia and osteoporosis) was discovered in the 1950s. The exposure was caused by cadmium contaminated water used for irrigation of local rice fields. A few studies outside Japan have reported similar findings (Strungaru *et al.,* 2018). During recent years, new data have emerged suggesting that also relatively low cadmium exposure may give rise to skeletal damage, evidenced by low bone mineral density (osteoporosis) and fracture. Animal experiments have suggested that cadmium may be a risk factor for cardiovascular disease, but studies of humans have not been able to confirm this. However, a Japanese study showed an excess risk of cardiovascular mortality in cadmium exposed persons with signs of tubular kidney damage compared to individuals without kidney damage (Sterckeman *et al.,* 2018).

2.6.2 Nickel

Nickel is a chemical element with symbol Ni and atomic number 28. It is a silvery-white lustrous metal with a slight golden tinge. Nickel belongs to the transition metals and is hard and ductile. Pure nickel, powdered to maximize the reactive surface area, shows a significant chemical activity, but larger pieces are slow to react with air under standard conditions because an oxide layer forms on the surface and prevents further corrosion (passivation) (Aguilar *et al.,* 2018). Even so, pure native nickel is found in Earth's crust only in tiny amounts, usually in ultramafic rocks, and in the interiors of larger nickel iron meteorites that were not exposed to oxygen when outside Earth's atmosphere. Meteoric nickel is found in combination with iron, a reflection of the origin of those elements as major end products of supernova nucleosynthesis. An iron nickel mixture is thought to compose Earth's inner core.

Use of nickel (as a natural meteoric nickel iron alloy) has been traced as far back as 3500 BCE. Nickel was first isolated and classified as a chemical element in 1751 by Axel Fredrik, who initially mistook the ore for a copper mineral, in the cobalt mines of Los, Hälsingland, Sweden. The element's name comes from a mischievous sprite of German miner mythology, Nickel, that personified the fact that copper-nickel ores resisted refinement into copper (Cherfi *et al.,* 2014). An economically important source of nickel is the iron ore limonite, which often contains 1-2% nickel. Nickel's other important ore minerals include garnierite, and pentlandite. Major production sites include the Sudbury region in Canada which is thought to be of meteoric origin, New Caledonia in the Pacific, and Norilsk in Russia (Liang *et al.,* 2016).

Nickel is slowly oxidized by air at room temperature and is considered corrosion-resistant. Historically, it has been used for plating iron and brass, coating chemistry equipment, and manufacturing certain alloys that retain a high silvery polish, such as German silver. About 6% of world nickel production is still used for corrosion-resistant pure-nickel plating. Nickel-plated objects sometimes provoke nickel allergy. Nickel has been widely used in coins, though its rising price has led to some replacement with cheaper metals in recent years.

Nickel is one of four elements (iron, cobalt, nickel, and gadolinium) that are ferromagnetic at approximately room temperature (Bakkali *et al.,* 2019). Alnico permanent magnets based partly on nickel are of intermediate strength between iron-based permanent magnets and rare-earth magnets. The metal is valuable in modern times chiefly in alloys; about 60% of world production is used in nickel-steels (particularly stainless steel). Other common alloys and some new superalloys comprise most of the remainder of world nickel use, with chemical uses for nickel compounds consuming less than 3% of production. As a compound, nickel has a number of niche chemical manufacturing uses, such as a catalyst for hydrogenation. Nickel is an essential

nutrient for some microorganisms and plants that have enzymes with nickel as an active site. The global production of nickel is presently used as follows: 46% in nickel steel; 34% nonferrous alloys and superalloys; 14% electroplating, and 6% other uses (de Oliveira et *al.,* 2017).

Nickel is used in many specific and recognizable industrial and consumer products, including stainless steel, alnico magnets, coinage, rechargeable batteries, electric guitar strings, microphone capsules, plating on plumbing fixtures, and special alloys such as permalloy, elinvar, and invar. It is used for plating and as a green tint in glass. Nickel is an alloy metal, and its chief use is in nickel steels and nickel cast irons, in which it typically increases the tensile strength, toughness, and elastic limit. It is widely used in many other alloys, including nickel brasses and bronzes and alloys with copper, chromium, aluminium, lead, cobalt, silver, and gold (Romero-Estévez *et al.,* 2002). Because it is resistant to corrosion, nickel was occasionally used as a substitute for decorative silver. Nickel was also occasionally used in some countries after 1859 as a cheap coinage metal, but in the later years of the 20th century was replaced by cheaper stainless steel (i.e., iron) alloys, except in the United States and Canada.

Nickel is an excellent alloying agent for certain precious metals and is used in the fire assay as a collector of platinum group elements (PGE). As such, nickel is capable of fully collecting all 6 PGE elements from ores, and of partially collecting gold. High-throughput nickel mines may also engage in PGE recovery (primarily platinum and palladium); examples are Norilsk in Russia and the Sudbury Basin in Canada (Behbahani *et al.,* 2016). Nickel foam or nickel mesh is used in gas diffusion electrodes for alkaline fuel cells. Nickel and its alloys are frequently used as catalysts for hydrogenation reactions. Raney nickel, a finely divided nickel-aluminium alloy, is one common form, though related catalysts are also used, including Raney type catalysts.

Although not recognized until the 1970s, nickel is known to play an important role in the biology of some plants, eubacteria, archaebacteria, and fungi. Nickel enzymes such as urease are considered virulence factors in some organisms (Sedghi *et al.,* 2015). Urease catalyzes the hydrolysis of urea to form ammonia and carbamate. The [NiFe]-hydrogenases can catalyze the oxidation of H_2 to form protons and electrons, and can also catalyze the reverse reaction, the reduction of protons to form hydrogen gas. A nickel-tetrapyrrole coenzyme, cofactor F430, is present in methyl coenzyme M reductase, which can catalyze the formation of methane, or the reverse reaction, in methanogenic archaea. One of the carbon monoxide dehydrogenase enzymes consists of an Fe-Ni-S cluster (da Rosa Couto *et al.,* 2018). Other nickel-bearing enzymes include a rare bacterial class of superoxide dismutase and glyoxalase I enzymes in bacteria and several parasitic eukaryotic trypanosomal parasites (in higher organisms, including yeast and mammals, this enzyme contains divalent Zn^{2+}).

Dietary nickel may affect human health through infections by nickel-dependent bacteria, but it is also possible that nickel is an essential nutrient for bacteria residing in the large intestine, in effect functioning as a prebiotic. The U.S. Institute of Medicine has not confirmed that nickel is an essential nutrient for humans, so neither a Recommended Dietary Allowance (RDA) nor an Adequate Intake have been established (Nordberg *et al.,* 2018). The Tolerable Upper Intake Level of dietary nickel is 1000 µg/day as soluble nickel salts. Dietary intake is estimated at 70 to 100 µg/day, with less than 10% absorbed. What is absorbed is excreted in urine. Relatively large amounts of nickel comparable to the estimated average ingestion above leach into food cooked in stainless steel. For example, the amount of nickel leached after 10 cooking cycles into one serving of tomato sauce averages 88 µg. Nickel released from Siberian Traps volcanic eruptions

is suspected of assisting the growth of Methanosarcina, a genus of euryarchaeote archaea that produced methane during the biggest extinction event on record (Romero-Estévez *et al.,* 2019).

The major source of nickel exposure is oral consumption. Nickel is found naturally in both food and water, and may be increased by human pollution. For example, nickel-plated faucets may contaminate water and soil; mining and smelting may dump nickel into waste water; nickel steel alloy cookware and nickel-pigmented dishes may release nickel into food. The atmosphere may be polluted by nickel metal refining and fossil fuel combustion (Zhou *et al.,* 2018). Humans may absorb nickel directly from tobacco smoke and skin contact with jewelry, shampoos, detergents, and coins. A less common form of chronic exposure is through hemodialysis as traces of nickel ions may be absorbed into the plasma from the chelating action of albumin.

The average daily exposure does not pose a threat to human health. Most of the nickel absorbed every day by humans is removed by the kidneys and passed out of the body through urine or is eliminated through the gastrointestinal tract without being absorbed. Nickel is not a cumulative poison, but larger doses or chronic exposure may be toxic, even carcinogenic, and constitute an occupational hazard. Nickel compounds, as a group, are carcinogenic and cause lung and nasal cancer (Dala-Paula *et al.,* 2018). The mechanism through which Nickel compounds are carcinogenic is not clear. Nickel refinery workers exposed to nickel compounds had a significant risk of lung cancer. If they smoked, the risk was even higher than the separate risk from smoking plus the risk from Nickel compounds. In animal studies, Nickel compounds were associated with tumors in various other sites throughout the body. In the US, the minimal risk level of nickel and its compounds is set to 0.2 µg/m3 for inhalation during 15-364 days (Corradini *et al.,* 2018).

People can be exposed to nickel in the workplace by inhalation, ingestion, and contact with skin or eye. The Occupational Safety and Health Administration (OSHA) has set the legal limit, permissible exposure limit for the workplace at 1 mg/m3 per 8 hours workday, excluding nickel carbonyl (França *et al.,* 2017). The National Institute for Occupational Safety and Health (NIOSH) specifies the recommended exposure limit (REL) of 0.015 mg/m3 per 8-hour workday. At 10 mg/m3, nickel is immediately dangerous to life and health. Nickel carbonyl [$Ni(CO)_4$] is an extremely toxic gas. The toxicity of metal carbonyls is a function of both the toxicity of the metal and the off-gassing of carbon monoxide from the carbonyl functional groups; nickel carbonyl is also explosive in air.

Sensitized individuals may show a skin contact allergy to nickel known as a contact dermatitis. Highly sensitized individuals may also react to foods with high nickel content (Ercilla-Montserrat *et al.,* 2018). Sensitivity to nickel may also be present in patients with pompholyx. Nickel is the top confirmed contact allergen worldwide, partly due to its use in jewelry for pierced ears. Nickel allergies affecting pierced ears are often marked by itchy, red skin. Many earrings are now made without nickel or low release nickel to address this problem. The amount allowed in products that contact human skin is now regulated by the European Union. In 2002, researchers found that the nickel released by 1 and 2 Euro coins was far in excess of those standards. This is believed to be the result of a galvanic reaction (Mohod, 2015). Nickel was voted Allergen of the Year in 2008 by the American Contact Dermatitis Society. In August 2015, the American Academy of Dermatology adopted a position statement on the safety of nickel: "Estimates suggest that contact dermatitis, which includes nickel sensitization, accounts for approximately $1.918 billion and affects nearly 72.29 million people. Reports show that both the nickel-induced activation of hypoxia-inducible factor (HIF-1) and the up regulation of hypoxia

inducible genes are caused by depletion of intracellular ascorbate. The addition of ascorbate to the culture medium increased the intracellular ascorbate level and reversed both the metal-induced stabilization of HIF-1- and HIF-1α-dependent gene expression (Labhade, 2015).

2.6.3 Mercury

Mercury is a chemical element; it has symbol Hg and atomic number 80. It is also known as quicksilver and was formerly named hydrargyrum (/haɪˈdrɑːrdʒərəm/ hy-DRAR-jər-əm) from the Greek words hydor (water) and argyros (silver). A heavy, silvery d-block element, mercury is the only metallic element that is known to be liquid at standard temperature and pressure; the only other element that is liquid under these conditions is the halogen bromine, though metals such as caesium, gallium, and rubidium melt just above room temperature (Hadayat *et al.,* 2018). Mercury occurs in deposits throughout the world mostly as cinnabar (mercuric sulfide). The red pigment vermilion is obtained by grinding natural cinnabar or synthetic mercuric sulfide. Exposure to mercury and mercury-containing organic compounds is toxic to the nervous system, immune system and kidneys of humans and other animals; mercury poisoning can result from exposure to water-soluble forms of mercury (such as mercuric chloride or methylmercury) either directly or through mechanisms of biomagnification.

Mercury is used in thermometers, barometers, manometers, sphygmomanometers, float values, mercury switches, mercury relays, fluorescent lamps and other devices, although concerns about the element's toxicity have led to the phasing out of such mercury-containing instruments (Silva *et al.,* 2017). It remains in use in scientific research applications and in amalgam for dental restoration in some locales. It is also used in fluorescent lighting. Electricity passed through mercury vapor in a fluorescent lamp produces short-wave ultraviolet light, which then causes the phosphor in the tube to fluoresce, making visible light.

Contamination of soils by Hg is often due to the addition of this heavy metal as part of fertilizers, lime, sludges, and manures. The dynamics between the amount of Hg that exist in the soil and its uptake by plants is not linear and depends on several variables (e.g., cation-exchange capacity, soil pH, soil aeration, and plant species). The uptake can be reduced when the soil's pH is high and/or there is an abundance of lime and salts (Vallverdú-Queralt *et al.,* 2015). Another factor affecting the level of accumulation of Hg is the species and the variety as a matter of fact; at least 45 plant families include metal-accumulating species. Most of the plants that uptake Hg tend to accumulate it on the roots, and some are even able to accumulate moderate amounts in the shoots either due to translocation or direct absorption of the vapour form. The work done by Barraza *et al.* (2017) showed that plants exposed to Hg0 can uptake and accumulate it in shoots, but there is no translocation to the roots. Toxic metal ions are thought to enter plant cells by the same uptake process as micronutrients, competing with these elements for absorption. Hg, which is a class B metal, preferentially binds with sulphur and nitrogen ligands and is thought to enter the cell through ionic channels competing with other heavy metals like cadmium or essential metals like zinc, copper and iron. However, this information is mostly based on experiments in animal cells and the authors believed that the uptake of Hg can occur via other processes which still remain unclear (Castro *et al.,* 2015).

Mercury can be absorbed through the skin and mucous membranes and mercury vapors can be inhaled, so containers of mercury are securely sealed to avoid spills and evaporation. Heating of mercury, or of compounds of mercury that may decompose when heated, should be carried out with adequate ventilation in order to minimize exposure to mercury vapor. The most toxic forms of mercury are its organic compounds, such as dimethylmercury and methylmercury. Mercury can cause both chronic and acute poisoning. Preindustrial deposition rates of mercury from the

atmosphere may be about 4 ng per 1 L of ice deposited. Volcanic eruptions and related natural sources are responsible for approximately half of atmospheric mercury emissions (Gupta *et al.,* 2019). Atmospheric mercury contamination in outdoor urban air at the start of the 21st century was measured at 0.01–0.02 μg/m^3. A 2001 study measured mercury levels in 12 indoor sites chosen to represent a cross-section of building types, locations and ages in the New York area. This study found mercury concentrations significantly elevated over outdoor concentrations, at a range of 0.0065 – 0.523 μg/m^3. The average was 0.069 μg/m^3. Half of mercury emissions are attributed to mankind. The sources can be divided into the following estimated percentages, Due to the health effects of mercury exposure, industrial and commercial uses are regulated in many countries (Marín *et al.,* 2018). The World Health Organization, Occupational Safety and Health Administration (OSHA), and National Institute for Occupational Safety and Health (NIOSH) all treat mercury as an occupational hazard; both OSHA and NIOSH, among other regulatory agencies, have established specific occupational exposure limits on the element and its derivative compounds in liquid and vapor form. Environmental releases and disposal of mercury are regulated in the U.S. primarily by the United States Environmental Protection Agency.

Toxic effects include damage to the brain, kidneys and lungs. Mercury poisoning can result in several diseases, including acrodynia (pink disease), Hunter-Russell syndrome, and Minamata disease (Stancic *et al.,* 2018). Symptoms typically include sensory impairment (vision, hearing, speech), disturbed sensation and a lack of coordination. The type and degree of symptoms exhibited depend upon the individual toxin, the dose, and the method and duration of exposure. Case–control studies have shown effects such as tremors, impaired cognitive skills, and sleep disturbance in workers with chronic exposure to mercury vapor even at low concentrations in the range 0.7–42 μg/m^3.

A study has shown that acute exposure (4–8 hours) to calculated elemental mercury levels of 1.1 to 44 mg/m3 resulted in chest pain, dyspnea, cough, hemoptysis, impairment of pulmonary function, and evidence of interstitial pneumonitis. Acute exposure to mercury vapor has been shown to result in profound central nervous system effects, including psychotic reactions characterized by delirium, hallucinations, and suicidal tendency. Occupational exposure has resulted in broad-ranging functional disturbance, including erethism, irritability, excitability, excessive shyness, and insomnia (Strungaru *et al.,* 2018). With continuing exposure, a fine tremor develops and may escalate to violent muscular spasms. Tremor initially involves the hands and later spreads to the eyelids, lips, and tongue. Long-term, low-level exposure has been associated with more subtle symptoms of erethism, including fatigue, irritability, loss of memory, vivid dreams and depression.

2.7 Oxidative Stress Induced by Heavy Metals in Plants

Exposure of plants to heavy metals leads to the overproduction of reactive oxygen species (ROS) due to their ability to change mitochondrial membrane permeability and restrict the action of ROS clearance enzymes in the cellular antioxidant system. The interaction of ROS with cellular membranes, heavy-metal-induced interactions directly or indirectly with different macromolecules, and signaling pathways leads to the accumulation of environmental pollutants and oxidative stress in exposed organisms (Sterckeman *et al.,* 2018). The heavy metal–ROS–cell signaling axis affects various pathological processes such as ATP depletion, excess ROS production, mitochondrial respiratory chain damage, decoupling of oxidative phosphorylation, and mitochondrial death.

Molecules that possess at least one atom of oxygen and have unpaired electrons are referred to as reactive oxygen species (ROS). These contain singlet hydroxyl, oxygen, and hydroperoxyl radicals (Aguilar *et al.,* 2018). ROS are formed due to the incomplete decomposition of molecular oxygen like hydroxyl radicals (OH), hydrogen peroxide (H2O2), superoxide radical anion (O_2^-), and ozone (O3). ROS, being essential for various signaling pathways, are generated within cells produced via a wide range of physiological and biochemical processes. In aerobic life, reactive active species tolerate an essential chemical entity. In plants, any variations in enzymes and cellular structure, nucleic acid, or proteins, increase the production of ROS. In plants, ROS scavenging pathways include enzymatic and non-enzymatic action regulating the production of ROS (Cherfi *et al.,* 2014). Therefore, under unfavorable conditions, the higher plants are induced to produce ROS to promote an extensive range of physiological variations. Production of ROS under the impact of drought, salinity, heavy metal toxicity, etc., leads among others, to the degradation of antioxidants, ultimately induces the gene expression of antioxidative response genes, and may damage macromolecules as lipids (lipid peroxidation) or the DNA.

Anthropogenic activities like urbanization and industrialization are accompanied by the release of heavy metal pollutants into the environment, which in turn disturbs plant development and physiology by phytotoxicity (Liang *et al.,* 2018). Additionally, secondary metabolites counterattack ROS to maintain balance. For example, vitamins, terpenes, and polyphenols are chief secondary metabolites to counterattack ROS and inhibit oxidative strain in plants. Presently the heavy metal toxicity to plant physio-chemical activities are highly concerning due to its toxicity accumulation in the food chain. To reduce oxidative stress due to heavy metals, plants have evolved several mechanisms such as increased root extraction of metals, prohibiting metal entrance into the plant, preventive toxic metal accretion, chelation by organic compounds

sequestration in vacuoles, and metal binding in the cell wall to stop the entry and ROS are converted to lesser toxic compounds (Bakkali *et al.,* 2019).

Heavy metals naturally exist in the Earth's crust with varying concentrations and are not easily broken down into less toxic compounds through metabolic processes. When present in the soil, they persist for extended periods, causing harmful impacts on both the environment and human health. In spite of these external effects, heavy metals alter many physiological processes, nutritional status of minerals, rate of photosynthesis and respiration, enzymatic processes, and many biochemical processes (de Oliveira *et al.,* 2017). However, on exposure of plants to toxic concentrations of heavy metals, more production of reaction oxygen species (ROS) is considered the most prompt effect so far. Heavy metals interact with various components in the electron transport chain thereby altering its activity and leading to ROS generation mainly in chloroplast and mitochondria. With the rise in ROS level, the membrane potential gets imbalanced further inducing leakage of ion channels, lipid peroxidation, and destruction of macromolecules. Moreover, the toxicity of heavy metals liberating ROS on subcellular organelles may alter depending upon certain factors like duration of stress, developmental stage, dose/concentration of particular heavy metal, and plant organ involved (Behbahani *et al.,* 2016).

Apart from these unfavorable conditions, ROS are also produced in plants at a certain basal rate which does not account for any toxic effect because of scavenging via different antioxidant mechanisms. Due to the immobile nature of plants, all the available resources including environmental contaminants mainly heavy metals are taken up. Further, they are distributed in different tissues and hence contribute to negligible toxicity inside the plant (Sedghi *et al.,* 2015). Due to the consumption of plants contaminated with heavy metals, animals and even humans also accumulate them in different tissues at high toxic levels. Hence, understanding the

mechanism or the pathways that contribute to the limited uptake of heavy metals in plants is of paramount concern (Romero-Estévez *et al.,* 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Uselu and Ikpoba hill market in Benin City. It is situated approximately 40 kilometres north of the Benin River and 320 kilometres by road east of Lagos. Benin City is the centre of Nigeria's rubber industry, and oil production is also a significant industry. The indigenous people of Benin City are the Edo people (the Benin People), and they speak the Edo language or Bini Language. The people of the city have one of the richest dress cultures on the African continent and are known for their beads (the beads stand for royalty and usually stand out during the traditional marriage of the Benin people), body marks, bangles, anklets, raffia work and the subsistence farming of yam, plantain and cassava.

3.2 Collection of samples

Fresh tomatoes were brought from Uselu and Ikpoba hill market in Benin City, Edo State. The fresh tomatoes products were kept in sterile zip luck bags and transported to the laboratory for heavy metal and oxidative stress levels in the tomatoes samples.

3.3 Sample Preparation and Treatment

Fresh tomatoes were taken at random from the purchased samples and were processed for analysis by the dry-ashing method. The samples were first oven-dried at 105°C for 24 h. The dried samples were then powdered manually in a grinder and were subjected to analysis for their heavy metal content. The powdered samples with three replicates taken for each market item, and were accurately weighed and placed in a silica crucible, and few drops of concentrated nitric

acid were added to the powdered samples to aid ashing. The dry-ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550°C and the samples left to ash at this temperature for 6 h. The ash was kept in desiccators and then rinsed with 3 N hydrochloric acid. The ash suspension was filtered into a 50 mL volumetric flask through Whatman No. 1 filter paper, and the volume was made up to the mark with 3 N hydrochloric acid.

3.4 Heavy Metal Analysis

All the samples were oven-dried at 70–80°C for 24 hrs, to remove all moisture. Dried samples were milled into a fine powder of 80 micron. 1.0 g of the dried sample was weighed into a digestion tube and 10ml of 98% nitric acid was added. This was then placed in a water bath and allowed to boil for about 72 hours. The resulting pale yellow solution was made up to 25ml with de-ionized water for each sample and stored. The solutions were analyzed for nickel, cadmium, mercury using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer model 2130). A certified standard reference material was used to ensure accuracy and the analytical values were within the range of certified value.

3.5 DETERMINATION OF OXIDATIVE STRESS LEVEL IN TOMATOES SAMPLES

3.5.1 Determination of Catalase (CAT)

Catalase (CAT) activity was estimated by the method described by Cohen et al., (1970).

Reagents

1. Hydrogen peroxidase (H_2O_2)
2. Suphuric acid (6M) H_2SO_4

Preparation of reagents

0.01M $KMnO_4$ was prepared by distilling 0.158g of $KMnO_4$ in 100ml of distilled water. Phosphate buffer (pH 7.4) 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 was weighed and dissolved in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

Procedure

To an unknown volume of plasma (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5ml of 6M H_2SO_4 and 7ml of 0.01M $KMnO_4$. These were mixed by inversion and allowed to stand for 10min. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as μmoles of H_2O_2 decomposed/min/mg/protein.

Calculation

$$\text{Activity} = \frac{\text{OD/min x V}}{\text{M x V x L x Y}}$$

Where OD = Absorbance

L= Light path

V= Total volume of reaction sample

M= Molar coefficient of H_2O_2 (40/m/cm)

V= Volume of sample

Y= mg protein in the sample

3.5.2 Estimation of Superoxide Dismutase Activity (SOD)

This was determined according to the methods of Masra and Fridorich (1972)

Principle

Adrenaline undergoes auto oxidation rapidly to adrenochrome whose concentration can be determined at 420nm with the aid of a spectrophotometer. The auto oxidation of adrenaline depends on the presence of superanions. Superoxide dismutase inhibits the auto-oxdiation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na_2CO_3, 0.2604g $NaHCO_3$ and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide.

Hydrochloric acid (0.005M): This was prepared by adding 0.044 concentration of HCL to 99.96mls of distilled water.

Adrenaline solution (0.3mM): This was prepared by dissolving 0.01098g of adrenaline in 100mls of 0.005M HCL solution.

Plasma volume of 100ml was mixed with 125ml of carbonate buffer and 150ml of adrenaline solution. 100ml of distilled water was mixed with 1.25ml of carbonate buffer as reference sample. These were mixed and absorbance read at 420nm.

These were mixed and read at 420nm

%inhibition= (O.D test - ODref) x 100
OD test

Enzyme concentration can thus be calculated

unit/mg protein = % inhibition
50 x Y

Where Y = mg of protein in the volume of sample used

3.5.3 Estimation of Gluthathione Peroxidase (GPx)

This was determined according to Nyman (1959)

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

Reagent and preparation

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100mls of distilled water.

Procedures

To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculations

$$\text{Activity} = \frac{\text{OD/min} \times vt \times Df}{E \times Vs \times Y}$$

OD= Absorbance of test

Vt= Total volume of reaction mixture

Df= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs= Volume of sample

Y= mg of protein used

3.5.4 Determination of Malondialdehyde (MDA)

Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978)

Principle

Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula:

$$\text{MDA (mol/mg protein)} = \frac{A \times V \times 100}{M \times V \times Y}$$

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

3.6 Health Risk Index (HRI)

Daily Intake of Metals (DIM) and reference oral dose (RfD) would be used to obtain the health risk index, according to US-EPA (2002) methods. The following formula would be used for the calculation of HRI.

HRI = DIM ÷ RfD

Where; RfD = Reference oral Dose;

The highest amount of a metal in milligrams per day that the body can be exposed to without yielding a hazardous outcome during a lifetime (IRIS, 2003).

3.7 Data Analysis

Analysis of variance (ANOVA) and Dunnet's method were employed for data evaluation; $p<0.05$ was taken as statistically significant. The software package, SPSS v16 was used for data analysis.

Plate 3.1: The researcher analyzing the tomatoes samples in the laboratory.

CHAPTER FOUR

RESULTS

The heavy metal analysis of fresh tomatoes samples obtained from Uselu and Ikpoba Hill market compared to W.H.O standard. The nickel concentration present fresh tomatoes samples obtained from the two markets ranged from 0.13 mg/kg to 0.16 mg/kg, while the cadmium concentration ranged from 0.11 mg/kg to 0.13 mg/kg respectively. When compared to W.H.O standard the heavy metal concentration were slightly above the recommended value (Table 4.1).

Table 4.1: Heavy metal analysis of fresh tomatoes samples obtained from Uselu and Ikpoba Hill market compared to W.H.O standard.

	NICKEL (mg/kg)	**CADMIUM (mg/kg)**	**MERCURY (mg/kg)**
Uselu	0.16	0.13	0.0
Ikpoba Hill	0.13	0.11	0.0
W.H.O Standard	0.10	0.10	0.1

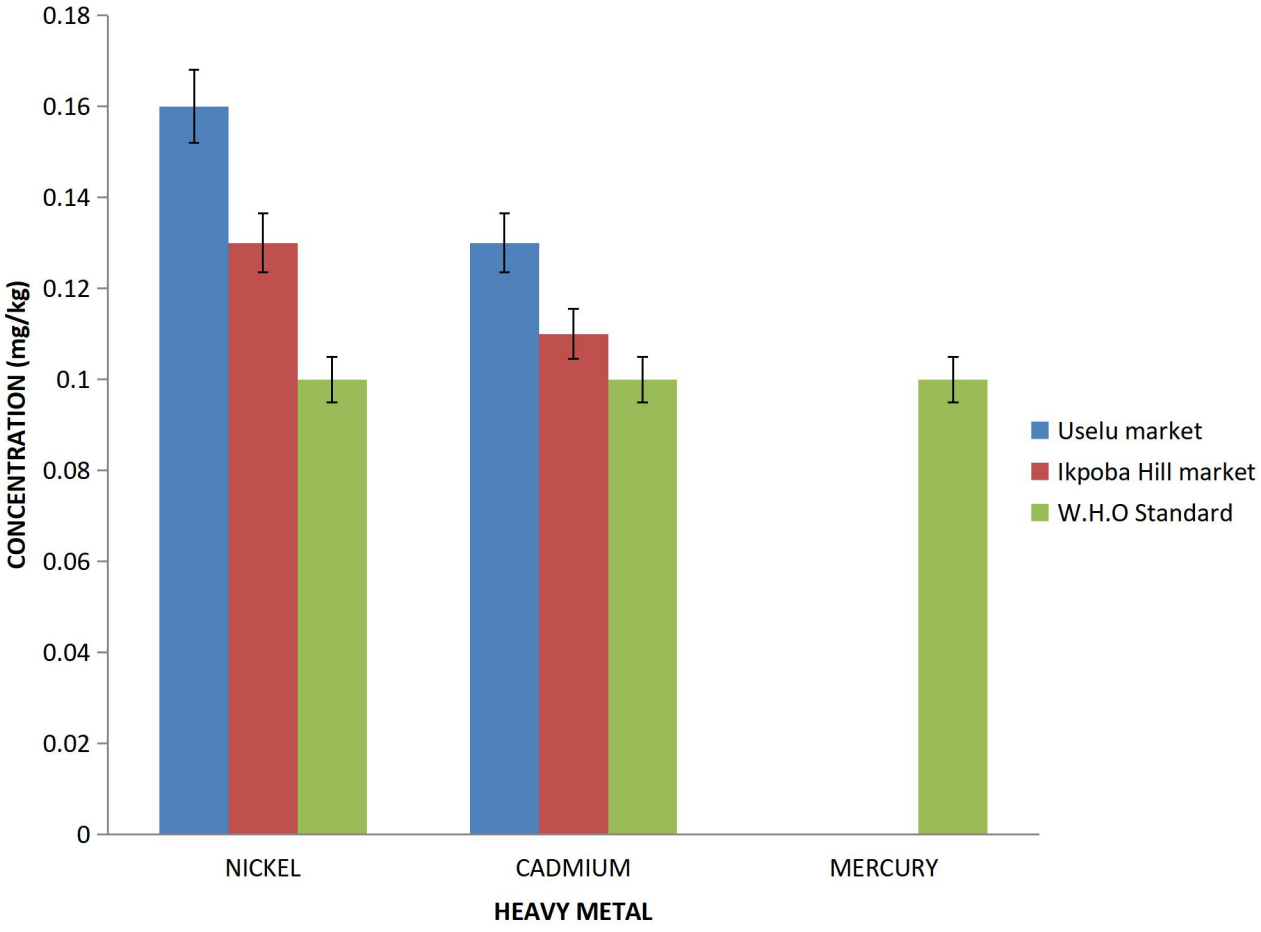

Figure 4.1: Heavy metal analysis of fresh tomatoes samples obtained from Uselu and Ikpoba Hill market compared to W.H.O standard. ($p<0.05$)

The oxidative stress analysis of fresh tomatoes samples obtained from Uselu and Ikpoba Hill market. The superoxide dismutase (SOD) value ranged from 3.02 U/g to 3.48 U/g, Catalase (CAT) value ranged from 0.08 U/g to 0.10 U/g , Glutathione peroxidase (GPx) value ranged from 4.96 U/g to 5.73 U/g, while Malondialdehyde (MDA) value ranged from 0.50 U/g to 0.64 U/g respectively (Table 4.2).

Table 4.2: Oxidative stress analysis of fresh tomatoes samples obtained from Uselu and Ikpoba Hill market

MARKET	SOD (U/g)	CAT (U/g)	GPx (U/g)	MDA(U/g)
Uselu	3.48	0.10	4.96	0.64
Ikpoba Hill	3.02	0.08	5.73	0.50

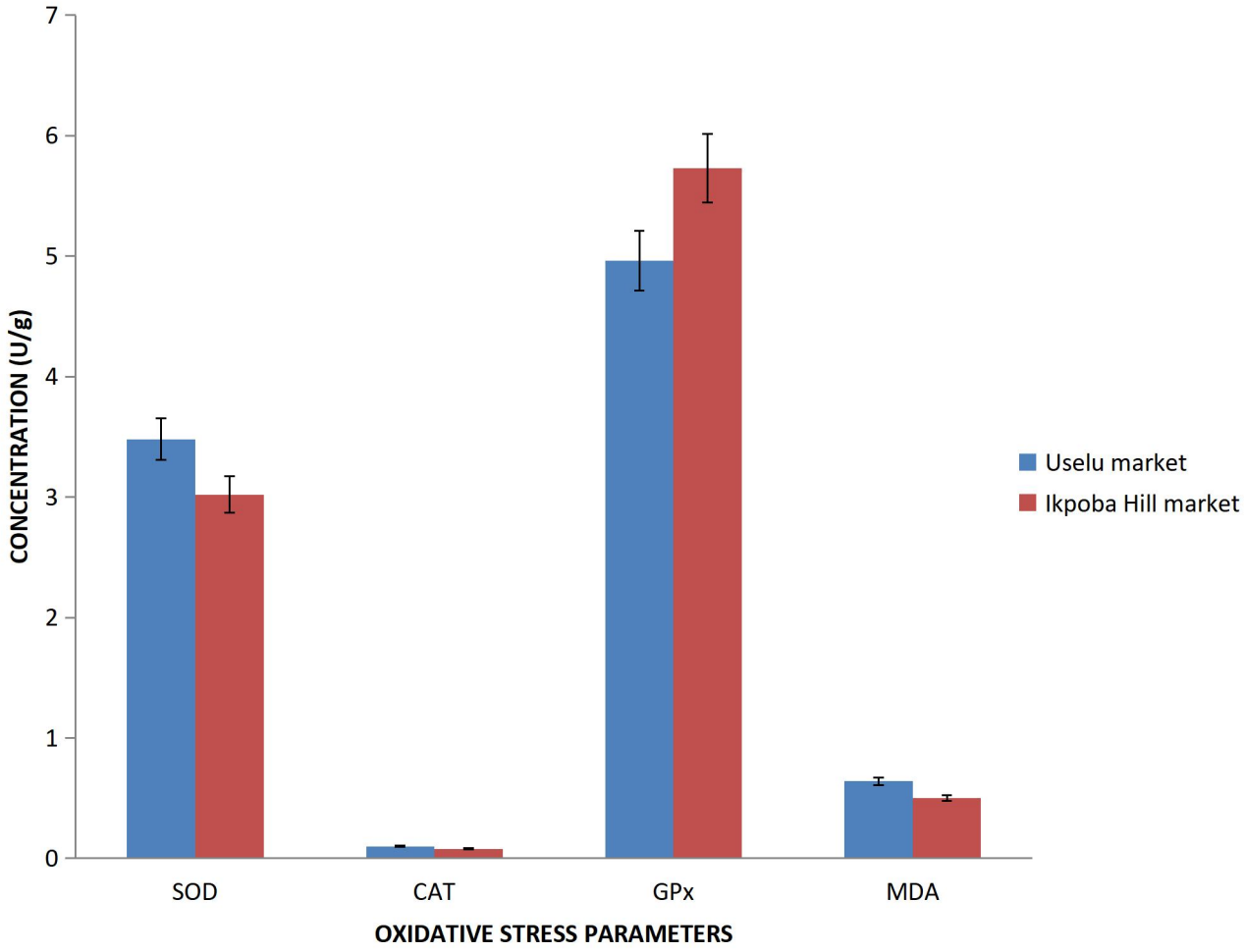

Figure 4.2: Oxidative stress analysis of fresh tomatoes samples obtained from Uselu and Ikpoba Hill market. ($p<0.05$)

Key:- Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Malondialdehyde (MDA)

CHAPTER FIVE

DISCUSSION

The results of heavy metal concentrations in in fresh tomatoes samples obtained from Ikpoba Hill market and Uselu market are depicted Table 1 and 2. The results reveal that cadmium and nickel were present in the fresh tomatoes samples, and are of potentially human carcinogens if consumed in excess. The nickel level in the fresh tomatoes samples ranged from 0.10 mg/kg to 0.22 mg/kg. While the cadmium level in the fresh tomatoes samples ranged from 0.10 mg/kg to 0.15 mg/kg. Similar results was observed in the study of Zhou *et al.* (2018) who reported that the average concentration for each heavy metal in avocado samples ranged between 0.45 - 1.40 mg/g, 2.45 - 4.80 mg/kg, 3.10 - 14.05 mg/kg and 1.20 - 2.70 mg/g for nickel, cadmium, iron, and manganese respectively. Furthermore, they explained that samples from heavy traffic areas had average concentration range of between 0 - 0.10mg/g and 0 - 0.06mg/g for nickel and cadmium.

The high content nickel present in the tomatoes samples in the present study can lead to brain damage, paralysis, anaemia and gastrointestinal symptoms. Stancic *et al.* (2016) reported that the major source of nickel exposure is oral consumption, as nickel is essential to plants. They added that can be nickel is found naturally in both food and water and may be increased by human pollution. Atmospheric pollution may occur through nickel ore refining and fossil fuel combustion. Humans may also absorb nickel directly from tobacco smoke and skin contact with jewelry, shampoos and detergents (Romero-Estévez *et al.,* 2019). A less common form of chronic exposure is through hemodialysis as traces of nickel ions may be absorbed into the plasma from the chelating action of albumin. Nieboer and Richardson (2018) reported that the average daily exposure does not pose a threat to human health, due to the fact that most of the nickel absorbed daily by humans is removed by the kidneys and passed out of the body through

urine or is eliminated through the gastrointestinal tract without being absorbed (Marín *et al.,* 2018).

Liang *et al.* (2018) reported that cadmium is a highly poisonous metal, affecting almost every organ and system in the human body. At airborne levels of 100 mg/m^3, it is immediately dangerous to life and health as most ingested lead is absorbed into the bloodstream. The primary cause of its toxicity is its predilection for interfering with the proper functioning of enzymes. It does so by binding to the sulfhydryl groups found on many enzymes, or mimicking and displacing other metals which act as cofactors in many enzymatic reactions (Gupta *et al.,* 2019). cadmium can cause severe damage to the brain and kidneys and, ultimately, death. By mimicking calcium, lead can cross the blood-brain barrier. It degrades the myelin sheaths of neurons, reduces their numbers, interferes with neurotransmission routes, and decreases neuronal growth. Symptoms of lead poisoning include nephropathy, colic-like abdominal pains, and possibly weakness in the fingers, wrists, or ankles. Small blood pressure increases, particularly in middle-aged and older people, may be apparent and can cause anemia (Ercilla-Montserrat *et al.,* 2018). Several studies, mostly cross-sectional, found an association between increased lead exposure and decreased heart rate variability. In pregnant women, high levels of exposure to lead may cause miscarriage. Chronic, high-level exposure has been shown to reduce fertility in males.

On the other hand the highest concentration of cadmium is absorbed in the kidneys of humans, and up to about 30 mg of cadmium is commonly inhaled throughout human childhood and adolescence (de Oliveira *et al.,* 2017). Cadmium is under preliminary research for its toxicity in humans, potentially affecting mechanisms and risks of cancer, cardiovascular disease, and osteoporosis. Cadmium is also an environmental hazard as human exposure is primarily from fossil fuel combustion, phosphate fertilizers, natural sources, iron and steel production, cement

production and related activities, nonferrous metals production, and municipal solid waste incineration. Bread, root crops, and vegetables also contribute to the presences of cadmium in human populations as human consume these food sources all year round (da Rosa Couto *et al.,* 2018).

More so the oxidative stress levels in fresh tomatoes samples obtained from Ikpoba Hill market and Uselu market are depicted Table 3 and 4. The superoxide dismutase (SOD) levels in fresh tomatoes samples ranged from 0.851614 U/g to 5.011948 U/g. Also the catalase (CAT) activity ranged from 0.016798 U/g to 0.299908 U/g. Furthermore the glutathione peroxidase (GPx) activity ranged from 1.058129 U/g to 8.866339 U/g. And malondialdehyde (MDA) activity ranged from 0.19741 mol/g to 1.064974 mol/g. Similar results was obtained in work carried out by () who stated that oxidative stress parameters observed in fresh pepper samples from local farms were in the following: catalase activity ranged from 1.98 U/g to 2.08 U/g, glutathione peroxidase activity ranged from 1.29 U/g to 3.39 U/g and malondialdehyde (MDA) activity ranged from 7.41 mol/g to 9.74 mol/g. According to Barraza *et al.* (2017) in plants, superoxide dismutase enzymes (SODs) act as antioxidants and protect cellular components from being oxidized by reactive oxygen species (ROS). Bakkali *et al.* (2019) stated that ROS can form as a result of drought, injury, herbicides and pesticides, ozone, plant metabolic activity, nutrient deficiencies, photoinhibition, temperature above and below ground, toxic metals, and UV or gamma rays. To be specific, molecular O_2 is reduced to O^{-}_2 (superoxide) when it absorbs an excited electron released from compounds of the electron transport chain. Superoxide is known to denature enzymes, oxidize lipids, and fragment DNA. SODs catalyze the production of O_2 and H_2O_2 from superoxide (O^{-}_2), which results in less harmful reactants (Aguilar *et al.,* 2018). Furthermore when acclimating to increased levels of oxidative stress, SOD concentrations

typically increase with the degree of stress conditions. The compartmentalization of different forms of SOD throughout the plant makes them counteract stress very effectively (Labhade, 2015).

According to Vallverdú-Queralt *et al.* (2015) in plants, catalase scavenges H_2O_2 generated during mitochondrial electron transport, β-oxidation of the fatty acids, and most importantly photorespiratory oxidation. Accumulating evidence indicates that catalase plays an important role in plant defense, aging, and senescence (Sterckeman *et al.,* 2018). Catalase activity is influenced by other factors such as salicylic acid and nitric oxide. Salicylic acid nonselectively inhibits the activity of all catalases or protects them from inactivation, possibly depending on the redox status of the cell (Silva *et al.,* 2017). The study of Nordberg *et al.* (2018) explained that plant glutathione peroxidase (GPx) are similar to animal GPXs, but their active region contains cysteine instead of selenocysteine. Plant GPXs are monomeric proteins that are linked to thioredoxin (Trx) and glutathione (GSH) detoxification pathways. They employ Trx more effectively than GSH to decrease H_2O_2 and organic peroxides (Mohod, 2015). GPX keeps the thiol-disulfide balance in check and also collaborates with peroxiredoxins and glutathione-S-transferases (GSTs) to maintain redox homeostasis. GPX plays an important role in response to abiotic and biotic stresses and during plant growth and development processes. Furthermore GPXs regulate plant growth and development under normal as well as in unfavorable conditions. The relevance of GPX(L)s in growth and development came to light after reports of the high transcript amount of *GPX(L)* genes in *O. sativa* and *A. thaliana* plants and that their expressions are dependent on tissues and developmental stages (Marín *et al.,* 2018).

Similarly Dala-Paula *et al.* (2018) reported that malondialdehyde (MDA), a substance produced by membrane lipids in response to reactive oxygen species (ROS), can be used as a drought

indicator to evaluate the degree of plasma membrane damage and the ability of plants to drought stress tolerance. The more the plant is damaged, the higher its MDA content, as found in studies that focused on plant responses to abiotic and biotic stresses (Sedghi *et al.,* 2015). That is to say, plants will generate ROS under abiotic or biotic stress conditions, thereby impairing the production of biomolecules, such as lipids, proteins, and nucleic acids, which increases the MDA content and the permeability of the plasma membrane, leading to extravasation of the content of cells. It is the mechanism by which drought resistance in plants is regulated (Zhou *et al.,* 2018).

5.1 Conclusion and Recommendation

The aim of the study was successful as the research revealed that heavy metals such as Cadmium (Cd) and Nickel (Ni) were found in some of the fresh tomatoes samples obtained from Uselu market and Ikpoba Hill market in Benin City, Edo State, compared to the WHO standard (0.05mg/kg). Although the oxidative stress parameters (SOD, CAT GPX and MDA) showed that these fresh tomatoes samples had gone through mechanical damage and microbial contamination, however these findings are indicative of environmental pollution due to industrial and vehicular emissions and also the mode of handing during preparation of the samples. Although the essential elements are not beneficial to man and plant but when found in excessive amount well above the recommended standard it can prove detrimental to health.

REFERENCES

Aguilar, P., Mondaca, R., Ginocchio, K., Vidal, S., Sauvé, A. and Neaman, M. (2018). Comparison of exposure to trace elements through vegetable consumption between a mining area and an agricultural area in central Chile. *Environmental Science and Pollution Research,* **25**: 19114–19121.

Bakkali, N., Ramos, B., Souhail, E. and Ballesteros, K. (2019). Characterization of trace metals in vegetables by graphite furnace atomic absorption spectrometry after closed vessel microwave digestion. *Food Chemistry,* **116**: 590–594.

Barraza, E., Schreck, T., Lévêque, G., Uzu, F., López, J., Ruales, J., Prunier, A., Marquet, L. and Maurice, (2017). Cadmium bioaccumulation and gastric bioaccessibility in cacao: a field study in areas impacted by oil activities in Ecuador. *Environmental Pollution,* **229**: 950–963.

Behbahani, J., Abolhasani, M.M., Amini, O., Sadeghi, F., Omidi, A., Bagheri, M. and Salarian, M. (2015). Application of mercapto ordered carbohydrate-derived porous carbons for trace detection of cadmium and copper ions in agricultural products. *Food Chemistry,* **173**: 1207–1212.

Behbahani, Y., Bide, S., Bagheri, M., Salarian, F., Omidi, M., Reza Nabid, M. (2016). A pH responsive nanogel composed of magnetite, silica and poly(4-vinylpyridine) for extraction of Cd(II), Cu(II), Ni(II) and Pb(II). *Microchim Acta,* **183**: 111–121.

Castro, A.A.F., de Almeida, C.P., Pirovani, G.S.M., Reis, N.M., Almeida, P.A.O. and Mangabeira, A.V. (2015). Morphological, biochemical, molecular and ultrastructural changes induced by Cd toxicity in seedlings of *Theobroma cacao* L. *Ecotoxicology and Environmental Safety*, **115**: 174–186.

Cherfi, S., Abdoun, O. and Gaci, A. (2014). Food survey: levels and potential health risks of chromium, lead, zinc and copper content in fruits and vegetables consumed in Algeria. *Food and Chemical Toxicology,* **70**: 48–53.

Corradini, A., Correa, M.S., Moyano, P., Sepúlveda, C. and Quiroz, F. (2018). Nitrate, arsenic, cadmium, and lead concentrations in leafy vegetables: expected average values for productive regions of Chile. *Archives of Agronomy and Soil Science,* **64**: 299–317.

da Rosa Couto, J.J., Comin, M., Souza, F.K., Ricachenevsky, M.A., Lana, L.C., Gatiboni, C.A., Ceretta, G. and Brunetto, H. (2018). Should heavy metals be monitored in foods derived from soils fertilized with animal waste. *Frontiers in Plant Science,* **9**: 732-743.

Dala-Paula, F.B., Custódio, E.A.N., Knupp, H.E.L., Palmieri, J.B.B., Silva, M.B.A. and Glória, B.M. (2018). Cadmium, copper and lead levels in different cultivars of lettuce and soil from urban agriculture. *Environmental Pollution,* **242**: 383–389.

de Oliveira, J.Á., Peres, M.L., Felsner, K.C. and Justi, T.M. (2017). Direct determination of Pb in raw milk by graphite furnace atomic absorption spectrometry (GF AAS) with electrothermal atomization sampling from slurries. *Food Chemistry,* **229**: 721–725.

Ercilla-Montserrat, P., Muñoz, J.I., Montero, X., Gabarrell, J. and Rieradevall, M. (2018). A study on air quality and heavy metals content of urban food produced in a Mediterranean city (Barcelona). *Journal of Cleaner Production,* **195**: 385–395.

França, A.M.A., Albuuerque, A.C., Almeida, P.B., Silveira, C.A., Filho, C.A., Hazin, E.V. and Honorato, F.C.S.S. (2017). Heavy metals deposited in the culture of lettuce (*Lactuca sativa* L.) by the influence of vehicular traffic in Pernambuco, Brazil. *Food Chemistry,* **215**: 171–176.

Gupta, K.K., Yadav, V., Kumar, S., Kumar, R.P., Chadd, A. and Kumar, N. (2019). Trace elements in soil-vegetables interface: translocation, bioaccumulation, toxicity and amelioration: A review. *Science of the Total Environment,* **651**: 2927–2942.

Hadayat, L.M., de Oliveira, E., da Silva, L., Han, M., Hussain, X., Liu, L.Q. and Ma, N. (2018). Assessment of trace metals in five most-consumed vegetables in the US: conventional vs. organic. *Environmental Pollution,* **243**: 292–300.

Labhade, K.R. (2015). Assessment of heavy metal contamination in vegetables grown in and around Nashik City, Maharashtra State, India. *IOSR Journal of Applied Chemistry,* **5**: 9–14.

Liang, W., Wu, Y., Zhang, S., Zhou, C. and Long, H. (2018). Levels, temporal trend and health risk assessment of five heavy metals in fresh vegetables marketed in Guangdong Province of China during 2014-2017. *Food Control,* **92**: 107–120.

Marín, O., Pardo, A., Sánchez, Y., Sanchis, D., Vélez, V., Devesa, G., Font, V. and Yusà, N. (2018). Assessment of metal levels in foodstuffs from the Region of Valencia (Spain), *Toxicology Reports*, **5**: 654–670.

Mohod, C.V. (2015). A review on the concentration of the heavy metals in vegetable samples like spinach and tomato grown near the area of Amba Nalla of Amravati City. *International Journal of Innovative Research in Science, Engineering and Technology,* **4**: 2788–2792.

Murad-Basha, N., Yasovardhan, S.V., Satyanarayana, G.V., Subba-Reddy, A. and Vinod-Kumar, A. (2017). Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. *Toxicology Reports*, **4**: 181–187.

Nieboer, D.H.S. and Richardson, E. (2018). The replacement of the nondescript term heavy metals by a biologically and chemically significant classification of metal ions. *Environmental Pollution,* **242**: 13–26.

Nordberg, A., Bernard, G.L., Diamond, J.H., Duffus, P., Illing, M., Nordberg, I.A., Bergdahl, T., Jin, S. and Skerfving, G.F. (2018). Risk assessment of effects of cadmium on human health. *Pure and Applied Chemistry,* **90**: 755–808.

Romero-Estévez, G.S., Yánez-Jácome, K., Simbaña-Farinango, H. and Navarrete, D. (2019). Content and the relationship between cadmium, nickel, and lead concentrations in Ecuadorian cocoa beans from nine provinces. *Food Control,* 106: 1067-1080.

Romero-Estévez, G.S., Yánez-Jácome, K., Simbaña-Farinango, P.Y., Vélez-Terreros, H. and Navarrete, D. (2019). Evaluation of two sample preparation methods for the determination of cadmium, nickel and lead in natural foods by Graphite Furnace Atomic Absorption Spectrophotometry. *Universitas Scientiarum,* 24: 497–521.

Sedghi, M., Shojaee, M., Behbahani, M.R. and Nabid, R. (2015). Application of magnetic nanoparticles modified with poly(2-amino thiophenol) as a sorbent for solid phase extraction and trace detection of lead, copper and silver ions in food matrices. *RSC Advances* **5**: 67418–67426.

Silva, P., Silva, H., Oliveira, I., Gaivão, M., Matos, O., Pinto-Carnide, C. and Santos, S. (2017). Pb low doses induced genotoxicity in Lactuca sativa plants. *Plant Physiology and Biochemistry,* **112**: 109–116.

Stancic, D., Vujević, A., Gomaz, S., Bogdan, D. and Vincek, S. (2016). Detection of heavy metals in common vegetables at Varaždin City Market, Croatia. *Archives of Industrial Hygiene and Toxicology,* **67**: 340–350.

Sterckeman, L., Gossiaux, S., Guimont, C., Sirguey, Z. and Lin, T. (2018). Cadmium mass balance in French soils under annual crops: scenarios for the next century. *Science of The Total Environment,* **639**: 1440–1452.

Strungaru, M., Nicoara, C., Teodosiu, E., Baltag, C., Ciobanu, G. and Plavan, S.A. (2018). Patterns of toxic metals bioaccumulation in a cross-border freshwater reservoir. *Chemosphere,* **207**: 192–202.

Vallverdú-Queralt, R.M. and Lamuela-Raventós, A. (2015). Foodomics: a new tool to differentiate between organic and conventional foods. *Electrophoresis,* **13**: 1–11.

Zhou, Z., Chen, H., Pan, B., Sun, D., Zeng, L., He, R., Yang, G. and Zhou, Z. (2018). Cadmium contamination in soils and crops in four mining areas. *Geochemical exploration in China,* **192**: 72–84.

APPENDIX

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.489	5	2.498	1.534	.251
Within Groups	19.536	12	1.628		
Total	32.025	17			

VAR00002

Duncan

Groups	N	Subset for alpha = 0.05
4	3	5.4367
3	3	6.2800
5	3	6.9200
2	3	6.9433
6	3	7.6633
1	3	7.9300
Sig.		.051

Means for groups in homogeneous subsets are displayed.

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	264.444	5	52.889	4.481	.016
Within Groups	141.620	12	11.802		

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	264.444	5	52.889	4.481	.016
Within Groups	141.620	12	11.802		
Total	406.064	17			

VAR00002

Duncan

Groups	N	Subset for alpha = 0.05		
4	3	35.8333		
2	3	40.8000	**40.8000**	
3	3		**42.3000**	**42.3000**
1	3		**42.9667**	**42.9667**
5	3		**45.1333**	**45.1333**
6	3			**48.3000**
Sig.		.102	**.177**	**.070**

Means for groups in homogeneous subsets are displayed.

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.712	5	7.142	1.465	.271
Within Groups	58.493	12	4.874		
Total	94.205	17			

VAR00002

Duncan

Groups	N	Subset for alpha = 0.05
4	3	11.4667
3	3	12.6333
5	3	13.6000
2	3	14.7000
6	3	15.1000
1	3	15.4000
Sig.		.072

Means for groups in homogeneous subsets are displayed.

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81793.778	5	16358.756	2.477	.092
Within Groups	79259.333	12	6604.944		
Total	161053.111	17			

VAR00002

Duncan

Groups	N	Subset for alpha = 0.05	
5	3	247.6667	
6	3	335.3333	**335.3333**

4	3	373.6667	**373.6667**
1	3	387.0000	**387.0000**
2	3		**441.3333**
3	3		**445.6667**
Sig.		.075	**.155**

Means for groups in homogeneous subsets are displayed.

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	126.624	5	25.325	.578	.717
Within Groups	526.067	12	43.839		
Total	652.691	17			

VAR00002

Duncan

Groups	N	Subset for alpha = 0.05
2	3	70.9000
4	3	71.4667
3	3	71.8667
5	3	73.6667
6	3	74.9000
1	3	78.6667
Sig.		.217

Means for groups in homogeneous subsets are displayed.

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	60.444	5	12.089	.307	.900
Within Groups	473.173	12	39.431		
Total	533.618	17			

VAR00002

Table 4.1: Heavy metal analysis of fresh tomatoes samples obtained from Uselu market

Sample Code	UNIT	NICKEL	CADMIUM	MERCURY
UT1	Mg/kg	Not Detected	Not Detected	Not Detected
UT2	Mg/kg	Not Detected	Not Detected	Not Detected
UT3	Mg/kg	Not Detected	Not Detected	Not Detected
UT4	Mg/kg	0.22	0.15	Not Detected
UT5	Mg/kg	Not Detected	Not Detected	Not Detected
UT6	Mg/kg	Not Detected	Not Detected	Not Detected
UT7	Mg/kg	Not Detected	Not Detected	Not Detected
UT8	Mg/kg	0.18	0.1	Not Detected
UT9	Mg/kg	Not Detected	Not Detected	Not Detected
UT10	Mg/kg	0.12	Not Detected	Not Detected
UT11	Mg/kg	0.20	Not Detected	Not Detected
UT12	Mg/kg	Not Detected	Not Detected	Not Detected
UT13	Mg/kg	Not Detected	Not Detected	Not Detected
UT14	Mg/kg	0.10	Not Detected	Not Detected
UT15	Mg/kg	0.15	Not Detected	Not Detected

Table 4.2: Heavy metal analysis of fresh tomatoes samples obtained from Ikpoba Hill market

Sample Code	UNIT	NICKEL	CADMIUM	MERCURY
IT1	Mg/kg	0.10	Not Detected	Not Detected
IT2	Mg/kg	Not Detected	Not Detected	Not Detected
IT3	Mg/kg	Not Detected	Not Detected	Not Detected
IT4	Mg/kg	Not Detected	Not Detected	Not Detected
IT5	Mg/kg	0.10	Not Detected	Not Detected
IT6	Mg/kg	0.18	0.11	Not Detected
IT7	Mg/kg	Not Detected	Not Detected	Not Detected
IT8	Mg/kg	Not Detected	Not Detected	Not Detected
IT9	Mg/kg	0.15	Not Detected	Not Detected
IT10	Mg/kg	Not Detected	Not Detected	Not Detected
IT11	Mg/kg	Not Detected	Not Detected	Not Detected
IT12	Mg/kg	Not Detected	Not Detected	Not Detected
IT13	Mg/kg	Not Detected	Not Detected	Not Detected
IT14	Mg/kg	0.22	0.10	Not Detected
IT15	Mg/kg	Not Detected	Not Detected	Not Detected

Table 4.3: Oxidative stress levels in fresh tomatoes samples obtained from Uselu market

Sample Code	SOD U/g Prot	CAT U/g Prot	GPx U/g Prot	MDA mol/g Prot
UT1	2.838725	0.180568	3.91762	0.209768
UT2	4.247049	0.094647	5.789587	0.73096
UT3	5.011948	0.112196	8.866339	0.365134
UT4	3.376884	0.078342	5.094836	0.948171
UT5	3.356282	0.076649	5.290235	0.424779
UT6	4.239514	0.085082	5.722042	1.064974
UT7	0.851614	0.016798	1.058129	0.19741
UT8	3.541456	0.071246	4.919152	0.705086
UT9	3.271356	0.061168	4.248109	0.831406
UT10	4.385455	0.089613	5.712392	0.745482
UT11	4.250763	0.081558	5.596601	0.692233
UT12	3.508564	0.069996	5.781601	0.468722
UT13	2.483883	0.056287	3.439862	0.52989
UT14	3.217686	0.06681	3.957353	0.744912

UT15	3.613731	0.299908	5.036941	0.975074

Key:- superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA)

Table 4.4: Oxidative stress levels in fresh tomatoes samples obtained from Ikpoba Hill market

Sample Code	SOD U/g Prot	CAT U/g Prot	GPx U/g Prot	MDA mol/g Prot
IT1	3.328418796	0.140158348	9.448605852	0.274182444
IT2	1.573257315	0.042540878	4.935622849	0.125860585
IT3	3.959585445	0.096135598	10.30043029	0.818304273
IT4	3.211614933	0.066246068	8.185278058	0.689061665
IT5	2.844382456	0.083328387	8.145349872	0.508783904
IT6	2.701662562	0.068342298	4.856016028	0.413887694
IT7	2.511978416	0.068250454	3.454975113	0.310857329
IT8	3.864451771	0.074006024	6.42644148	0.66802926
IT9	2.600327185	0.067209552	4.023762909	0.290447504
IT10	4.58083129	0.116374672	7.154710572	0.366881058
IT11	3.254576749	0.082788296	4.648823867	0.361445783
IT12	1.558431674	0.041455873	2.267920945	0.366564781
IT13	2.225745086	0.055643627	3.412809131	0.712360721
IT14	4.039597226	0.102198795	5.722041738	0.968158348

IT15	3.013197528	0.053881937	3.49252919	0.599734847

Key:- superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA)