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SCHOOL OF PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES



HEALTH RISK ASSESSMENT OF HEAVY METALS VIA CONSUMPTION OF LETTUCE (*LACTUCA SATIVA* L.) COLLECTED FROM SELECTED OPEN - MARKETS AND SUPERMARKETS IN THE ACCRA METROPOLIS

BY

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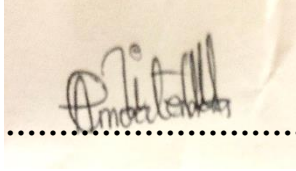
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DECLARATION

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DECLARATION

I, Afua Amoatema Aduse-Poku declare that this dissertation is the product of my study, except for references made to the work of other people that have been properly cited and that this dissertation has not been presented elsewhere for another degree either in whole or in part.



...29th January, 2023...

Afua Amoatema Aduse-Poku

Date

Student



Dr. Paul K: Botwe

...31st January, 2023....

Date

Supervisor



DEDICATION

I dedicate this work to The Almighty God for his Grace and mercies and to my parents for their endless support and encouragement throughout my program of study.



ACKNOWLEDGMENTS

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I would like to acknowledge and give my warmest thanks to my supervisor Dr. Paul Kingsley Botwe who made this work possible. His guidance and advice carried me through all the stages of writing my thesis.

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ABSTRACT

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Background: Bioaccumulation of metals in vegetables is known to harm human health. Food contamination by heavy metals has been a major public health concern since the consumption of such foods can cause deleterious effects. The study aimed to measure and compare levels of heavy metals in lettuce sold in selected supermarkets and open markets in Accra Metropolis and their implication on human health.

Objectives: The specific objectives of the study were to measure and compare concentrations of heavy metals in lettuce from markets and supermarkets in Accra Metropolis, compare concentrations of heavy metals with FAO/WHO standards and assess the potential human health risks associated with consuming lettuce with the measured heavy metal concentrations.

Methods: This was an analytical cross-sectional study conducted in Accra Metropolis. A total of 16 samples of lettuce (approximately 15kg) from two varieties (Eden and Great lakes) were selected for heavy metals analysis where two samples of each of the two varieties were randomly selected across four markets namely the (Palace Mall and Melcom Plus) and two 'open markets' (Madina and Kaneshie Markets). All analyses namely; mean, standard deviation, and ANOVA were conducted using IBM Statistical Package for Service Solutions (version 26.0).

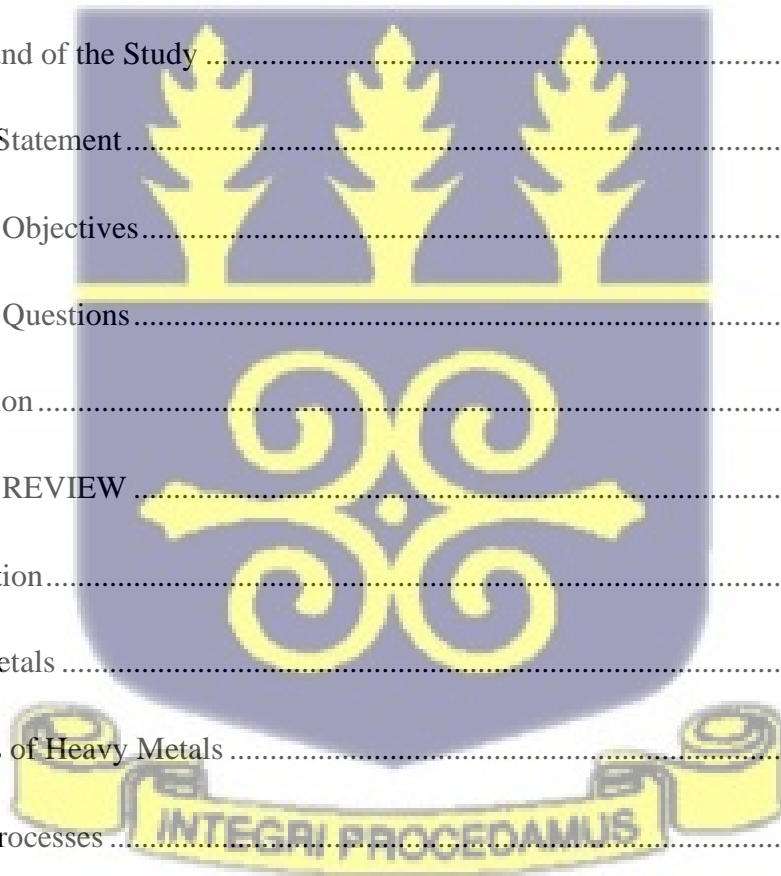
Results: The study indicates that there were relatively higher levels of metals in open-market lettuce samples than in supermarkets. Studies showed higher levels of copper and zinc in lettuce leaves as compared to Cadmium and Lead. Again, the study showed Iron, zinc, and manganese having concentrations exceeding the threshold (0.70 - Fe, 0.30 -Zn, 0.14 -Mn).

Conclusion: Out of the 16-lettuce sampled from the open – markets and the supermarkets the findings revealed that Risk level analysis associated with the use of Lettuce by humans indicates that the concentrations of Pb, Cu, and Cd found in them were below permissible limits, however, Mn, Zn, and Fe were found to have levels of concentration which were above the permissible limit and poses a potential health risk to consumers using various reference guides/standards. Even though almost all the heavy metal levels detected in this study were far below the WHO permissible limits, the essential metals detected had a relatively higher concentration. Therefore, vegetables sampled from the selected supermarkets and open markets in Accra are not hazardously contaminated by heavy metals and are largely safe for consumption.

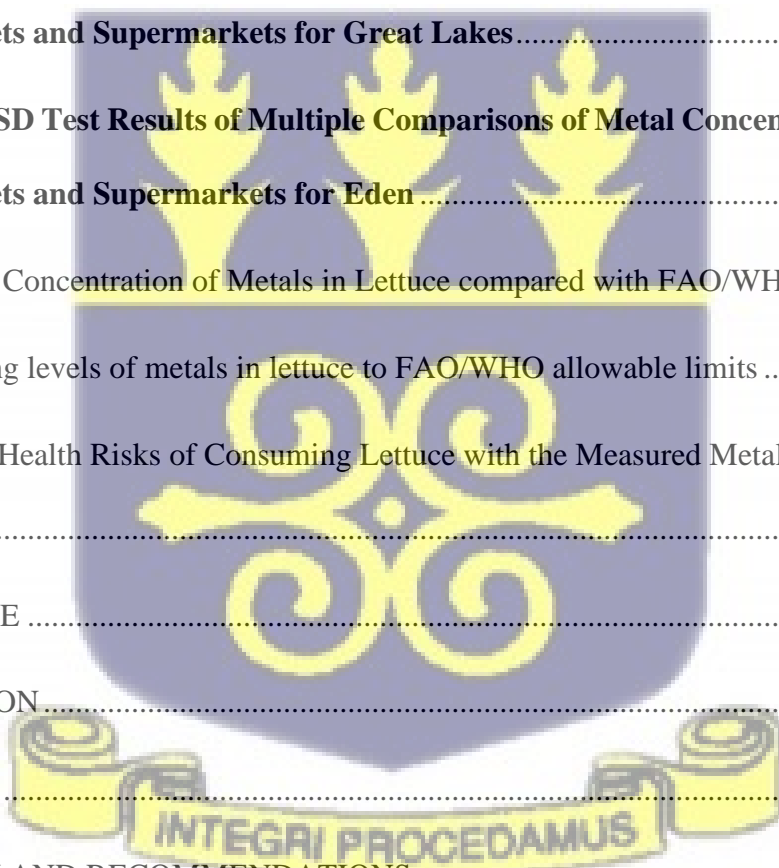
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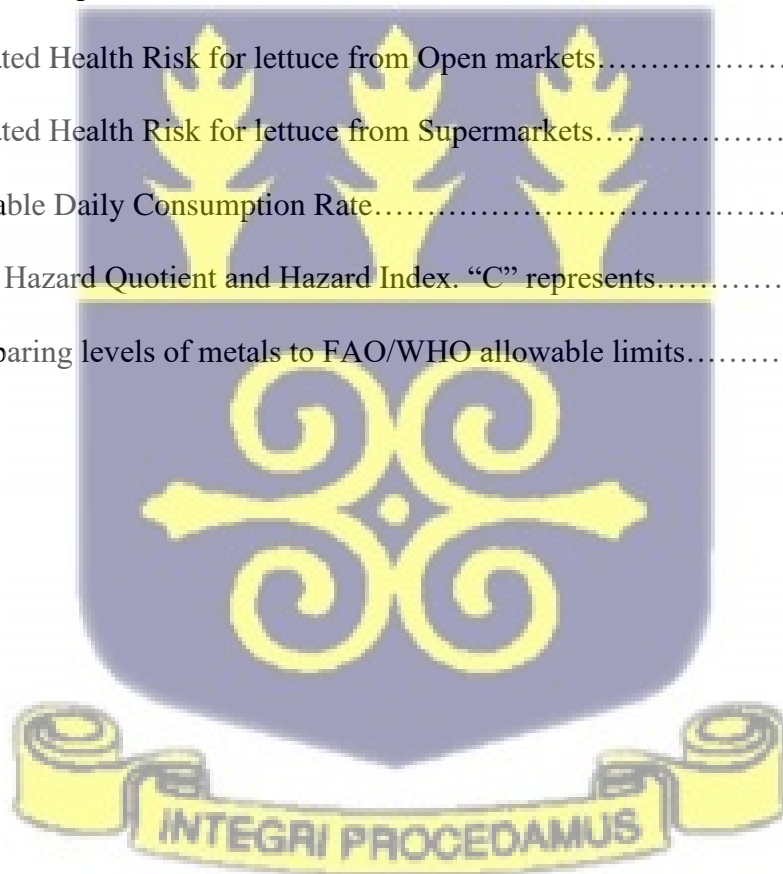
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| | |
|-------|--------------------------------|
| Cd | CADMIUM |
| Cu | COPPER |
| Fe | IRON |
| Mn | MANGANESE |
| Pb | LEAD |
| Zn | ZINC |
| F | F-RATIO |
| DF | DEGREE OF FREEDOM |
| A | EXACT STATISTIC |
| CRlim | MAXIMUM SAFE DAILY CONSUMPTION |
| RfD | REFERENCE DOSE |
| BW | BODY WEIGHT |
| C | CONCENTRATION |
| EDI | ESTIMATED DAILY INTAKE |
| ADI | AVERAGE DAILY INTAKE |
| THQ | TARGET HAZARD QUOTIENT |
| HI | HAZARD INDEX |
| EF | EXPOSURE FREQUENCY |
| ED | EXPOSURE DURATION |
| AT | AVERAGING TIME |
| IR | INGESTION RATE |
| TI | TOLERABLE INTAKE |
| C | CHILDREN |
| A | ADULT |
| FAO | FOOD/AGRICULTURE ORGANIZATION |



WHO

WORLD HEALTH ORGANIZATION

FDA

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FOOD AND DRUGS AUTHORITY

GSA

GHANA STANDARDS AUTHORITY



CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Vegetables are important for optimum human health and well-being. Among the quality assurance practices to ensure the integrity of vegetables, heavy metal contamination is an important factor (Wang et al. 2005). There is a growing interest in the safety of vegetables concerning the risk of heavy metals pollution in the environment (Abubakari and Mahunu, 2007).

Elements with comparatively elevated densities that are toxic in little quantities are considered heavy metals (Huseen et al, 2019), Typical examples of heavy metals are Arsenic, Zinc, Lead, Copper, Manganese, Cadmium, and Iron. Thus, when taken up, heavy metals are accumulated in the tissues of living organisms faster than they are broken down or excreted. In principle, the fundamental importance of heavy metal elements like copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe) cannot be overstated since they contribute to managing and balancing the appropriate working mechanisms of human membranes; and substantial deficits might compromise the immune system.

Extremely high amounts, on the other hand, could be lethal. Manganese insufficiency, for example, impairs fertility and increases newborn death rates (Burch, 1975), whereas pathologic phenomena such as iron oxide accumulation in Parkinson's disease are linked to the ingestion of excessive trace metallic ions (FDA, 2001). Toxins interact directly with humans in many ways and find their way into human tissue via channels such as food, water, air, or absorption through the skin (Vhahangwele et al, 2018).

These metal elements are not biologically degradable, possess extensive half-lives, and an increase in many human organs, causing adverse side effects (Jarup, 2003; Sathawara et al., 2004; Singh et al., 2010; Nabulo et al., 2011). Toxic metals can disrupt essential biochemical

functions within the kidney, liver, cardiovascular, neurological, and bone systems, resulting in chronic inflammatory disease and cancer (WHO 1992; Jarup 2003).

Cadmium, copper, arsenic, chromium, lead, zinc, cobalt, and nickel are among the toxic metals most commonly discovered in vegetables. Several of them are micronutrients when consumed in minute amounts, but constitute a substantial risk to human health when consumed in high concentrations or for extended periods (Sharma et al., 2009).

It is not arguable that pollution by heavy metals from agricultural chemicals like pesticides has cumulative impacts on the living components of the soil and consequently humans through consumptive pathways (Opaluwa et al., 2012; Wilson et al, 2007). Heavy metals from agricultural chemicals are transferred to humans through the food chain which is responsible for a series of health implications (Premarathna et al., 2011).

Among the harmful medical concerns induced by environmental toxins are lung cancer, renal dysfunction, osteoporosis, and cardiac arrest. Also, metal ion deposition in body tissue can impact the central nervous system, acting as a pseudo-co-factor or promotor of medical conditions like epilepsy, headaches, and coma. Both adults and children are estimated to suffer from these substances (Ahmed et al., 2021).

As a result, continuous review of concentrations of heavy metals in soils and crops is critical to comprehend the concentrations and design measures to mitigate pollution threats to health. Heavy metal studies were limited in Ghana due to the perception of decreased public health importance. Heavy metal contamination, on the other hand, has been demonstrated to have serious environmental and health consequences in less developed nations such as Zimbabwe, Zambia, and Nigeria (Mapanda et al., 2005; Ogwuegbu & Muhanga 2005; Eriyamremu et al., 2005).

Studies have investigated heavy metals in various food items (Odai et al., 2008; Bempah et al, 2011; Larbie et al., 2014), however, the literature is not comprehensive on the topic. Despite

the limitations of these investigations, the information on heavy metal build-up in vegetables is nevertheless relevant in terms of human health. Moreover, there are records of uncontrolled application of high doses of agrochemicals in farms both local and international, that supply vegetables to our markets both the open markets (an open place where goods and services are sold especially foodstuffs) and supermarkets (enclosed areas where food and services are sold) in Accra and other parts of the country.

Whilst open markets are exposed to aerosols, human activities, and vehicular emissions due to the nature of the markets, the same cannot be said of the supermarkets posing the question of whether there is a difference in goods and services, (vegetables specifically lettuce in this case) offered on these two markets. It is against this odd that this study sought to investigate heavy metal concentrations in lettuce (*Lactuca sativa L.*) from selected open markets and supermarkets, and its associated health risks in the Accra metropolis.

1.2 Problem Statement

People's eating habits have changed dramatically over time, with a growing interest in the nutritional benefits of plant-based foods like vegetables and fruits. Vegetables are important for human nutrition and health because they include bioactive nutrient molecules including dietary fiber, vitamins, and minerals, as well as non-nutritive phytochemicals (phenolic compounds, flavonoids, bioactive peptides, etc). These dietary and non-nutrient molecules diminish chronic illnesses like cardiovascular disease, diabetes, certain cancers, and obesity (Pennington et al., 2009; September-Malaterre et al., 2018).

However, the significant threat that can reduce the benefits accrued from vegetables is the indiscriminate use of agrochemicals during their cultivation (Barau et al., 2018). Contamination of vegetables not only reduces the wholesomeness of the vegetables and their

associated benefits but poses a significant threat to food security, economic livelihood, and human health.

There are differences in maximum limits or allowable levels of heavy metals in the edible parts of plants such as vegetables. However, both national and international regulations on food quality have been in favor of lowering the maximum allowable levels of heavy metals in food materials due to the increased risk of human contamination (Barau et al., 2018; Sharma et al., 2009). Meanwhile, not much attention has been focused on the international and national regulations of food quality when it comes to vegetables consumed in developing countries. There are records of discriminate and uncontrollable application of high doses of agrochemicals in farms both local and international, that supply vegetables to supermarkets in Accra and other parts of the country. The increased and uncontrolled use, coupled with a weak supervisory and monitoring framework can lead to health issues in the country.

The newness of the research is to offer a critical look at the contamination levels of heavy metals in lettuce that make their way into supermarkets and open markets in the city of Accra for consumption and the health implications associated with them. This study sought to do a comparative analysis between the two places of selling and buying lettuces i.e., supermarkets and open markets, and which option provided a better health option for consumption.

1.3 Research Objectives

To assess contamination levels of heavy metals in lettuce sold in selected supermarkets and open markets in Accra Metropolis and their implication on human health.

The specific objective is to;

1. Measure and compare concentrations of heavy metals in lettuce from markets and supermarkets in Accra Metropolis.
2. Compare concentrations of heavy metals in lettuce with FAO/WHO standards.

3. Assess the potential human health risks associated with consuming lettuce with the measured heavy metal concentrations.

1.4 Research Questions

To achieve the objective of this study, several questions have been set. These are;

1. Does lettuce from the open market have higher concentrations of heavy metals than those from the supermarket?
2. How do the levels of heavy metals in the lettuce compare with FAO/ WHO recommended limits?
3. What are the potential health risks of consuming lettuce with the measured heavy metals concentration in humans?

1.5 Conceptual Framework

According to (Creswell & Plano-Clark, 2011), a conceptual framework is a visual or written product, one that “explains, either graphically or in narrative form, the main things to be studied the key factors, concepts, or variables and the presumed relationships among them”. The term “conceptual framework” can also be used in a broader sense that includes the actual ideas and beliefs that the researcher holds about the phenomena studied whether these are written down or not (Creswell & Plano-Clark, 2011). (Bryman, 2016), defined a conceptual framework as a hypothesized model identifying the model under study and the relationship between the dependent and independent variables. (Teye, 2008), defined an independent variable also known as the explanatory variable as the presumed cause of the changes of the dependent variable, while a dependent variable refers to the variable which the researcher wishes to explain. The goal of the conceptual framework is to categorize and describe concepts relevant to the study and map relationships among them. Such a framework helps the researcher to define the concept, map the research terrain or conceptual scope, systematize relations among

concepts, and identify gaps in literature (Castles et al., 2014). The conceptual framework of the study was based on key concepts of the study and literature review. The conceptual framework was then used to analyze the results of the research.

Figure 1.1 is a conceptual framework, which illustrated how heavy and essential metals when accumulated can affect humans.

Figure 2.3 Conceptual framework of heavy and essential metals

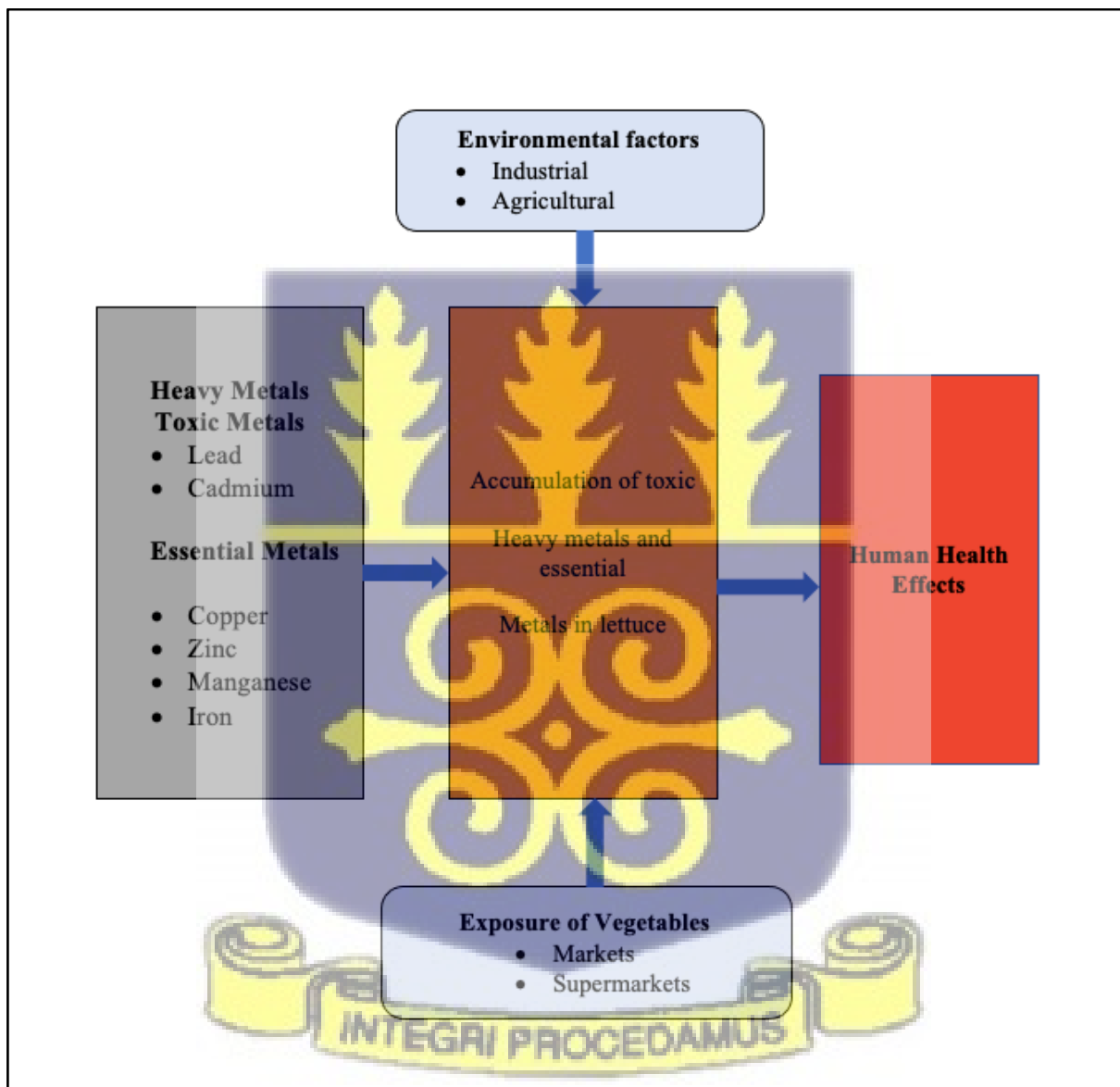


Figure 1: Source: Author's construction (2022)

The conceptual framework has five components. The first components list heavy metals and essential metals, the second talks about the accumulation of toxic heavy metals and essential metals in vegetables, and the third and four components illustrated the environmental factors and the exposure of vegetables at the markets and supermarkets. The last component of the conceptual framework points out the effects of contaminated vegetables on humans. These various components of the conceptual framework illustrate how heavy and essential metals when accumulated can affect humans. The framework was guided by the literature, theories, and earlier conceptual framework that attempted to relate pinpoint sources of heavy and essential metals in humans.

Humans are exposed to contaminated vegetables from our farms. Both anthropogenic and natural processes release a huge number of toxic substances such as heavy metals in the soils and the use of pesticides releases a huge number of toxic substances in vegetables. When these vegetables are consumed, they can have harmful health effects on humans.

1.6 Justification

Heavy metals discharged into the environment quickly create bonds with particulate matter and sink into the soil (Hedge et al., 2009; Naser, 2013). Vegetables are essential in providing people in developing nations with a regular and consistent number of vitamins, minerals, and fiber. Owing to the dependence of humans on vegetables for micronutrients, heavy metal concentrations in them may have a direct impact on human health. As a result, it is critical to monitor the concentration of heavy metals in vegetables to verify that they are safe (Naser, 2013; Zhou et al., 2008).

Again, there is a growing interest in the area of contamination levels of heavy metals in vegetables from supermarkets and open markets in Ghana see (Affum et al., 2020; Ametepey et al., 2018; Amoah et al., 1998) Therefore, the outcome of this study will be valuable in

influencing policy formulation, public health education, and strategic actions to prevent high levels of heavy metals in vegetables in the country.



CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Lettuce (*Lactuca sativa* L.) is a good source of antioxidants, Vitamins A, and C, and anticarcinogenic phytonutrients. It also contains dietary fiber, carbs, protein, and a little fat. In tropical markets farms, such as Ghana, early maturing iceberg-type lettuce with three notable varieties (Eden, Trinity, and Great Lakes) is commonly cultivated. The productivity and quality of these lettuces are affected by growth, yield, and fertilizers.

For cultivating vegetable crops, both commercial and subsistence farming in Ghana have relied mostly on the usage of chemical fertilizers (Lampkin, 1990). This is because they are readily assimilated and consumed by plants.

2.2 Vegetable Consumption in Ghana

With an upsurge in the middle-class population and increased mindfulness of the health benefits of consuming vegetables, Ghana's domestic vegetable has seen growth (Lente et al., 2014). Cultivation of fresh vegetables in Ghana is undertaken all over the country and this is a result of the country's precise climate and market distribution. Additionally, the increase in this sector is also attributed to the expansion of irrigated agriculture along the Volta River and Lake Volta (Lente et al., 2014; Sophie Leonie, 2020).

Chilies, onions, tomatoes, okra, beans, and other vegetables are among the most often grown in the country. Ghanaian fruits and vegetables continue to be mostly imported by the United States and the European Union. Fresh veggies are in high demand, mostly for traditional household markets and supermarkets. Vegetable intake in Ghana is predicted to be at 50.1 kg/person/per year (roughly 137.3 g/person/day) (Azupogo et al., 2018). This is low as compared to the recommendation of the World Health Organization (WHO), that consuming

about 400g of vegetables a day will help prevent chronic diseases such as obesity, diabetes, and heart disease (World Health Organization, 2010).

Urbanization in major cities in Ghana has reduced the available farmland for vegetable cultivation, for, instance, Accra and Tamale recorded a 36%-50% decline in urban vegetable farming between 2006 to 2014 (Drechsel et al., 2014). The pathogenic load of water used for urban irrigation and uncontrolled use of pesticides has also resulted in the decline in vegetable production and export in Ghana, as some of these vegetables are rejected by importing countries due to the presence of some organisms that are harmful (Gonzalez et al., 2014). This shortfall in vegetables has resulted in the importation of vegetables by supermarkets in Ghana where both locally cultivated vegetables are sold alongside imported ones. For instance, it is estimated Ghana's import of vegetables amounted to \$10.4 billion in 2019. As a result, about 5,300 street food sellers and 800,000 daily consumers benefit from both locally produced and imported vegetables within major cities in Ghana (Drechsel et al., 2014).

2.3 Heavy Metals

According to Huseen et al. (2019), a metal with a comparatively elevated density that is toxic in little quantity is considered a heavy metal. Typical heavy metals include arsenic (As), lead (Pb), mercury (Hg), cadmium (Cu), chromium (Cr), thallium (Tl), and more. These metals are vital to maintaining the body's metabolism, but become toxic at high concentrations (Huseen et al., 2019).

Whenever heavy metals are ingested, they remain absorbed in organisms quicker than they should be broken down (metabolized) or eliminated (Huseen et al., 2019). Even though some of these metals are important micronutrients, toxins from all of them cause metabolic interference and mutagenesis in living organisms. Heavy metal toxins have a wide range of effects on living creatures, ranging from decreased fitness to interference with reproduction, which can lead to cancer and death (Huseen et al., 2019; Vhahangwele et al., 2018).

2.3.1 Sources of Heavy Metals

Heavy metals are known environmental pollutants with adverse health effects, and their production and emission globally have been on the increase. Numerous studies indicate splitting heavy metal sources into two categories: natural and man-made sources. Natural sources comprise sedimentary rocks, volcanic eruptions, soil formation, and rock weathering. Industry, agriculture, mining, and home effluents are examples of anthropogenic sources (Roobahaniry, 2015).

2.4 Natural processes

Heavy metals can be found in a variety of natural sources, each with its own set of environmental circumstances. Volcanic eruptions, sea-salt sprays, forest fires, rock weathering, biogenic sources, and wind-borne soil particles are among them. When it comes to heavy metals' natural sources, igneous and sedimentary rocks are the most prevalent. They contain higher levels of heavy metals than other rock types (Bradl, 2005). The determination of heavy metals in these rocks depends on the rock type and the condition of the ecosystem in the surrounding (Sharma, R.K.; Agrawal, 2005). Additionally, heavy metals can also be found in the form of hydroxides, oxides, sulfides, sulfates, phosphates, silicates, and organic compounds.

2.4.1 Anthropogenic processes

Anthropogenic activities have been associated with the contamination of the environment by heavy metals. These activities include emissions from mines and illegal mining activities (galamsey operation), discharge of effluents, smelteries, agricultural runoffs, and inappropriate dumping of domestic waste. According to (Cui et al., 2005), Electronic waste (e-waste), spent batteries, and paints are examples of waste products. The number of heavy metals discovered at disposal sites and, invariably, in the environment increases as a result of trash (Bortey-Sam et al., 2015).

Because of the sluggish leaching of heavy metals into the acidic environment, they may wind up in water bodies and soils, polluting our fish and plants in the long term (Monferran et al., 2016).

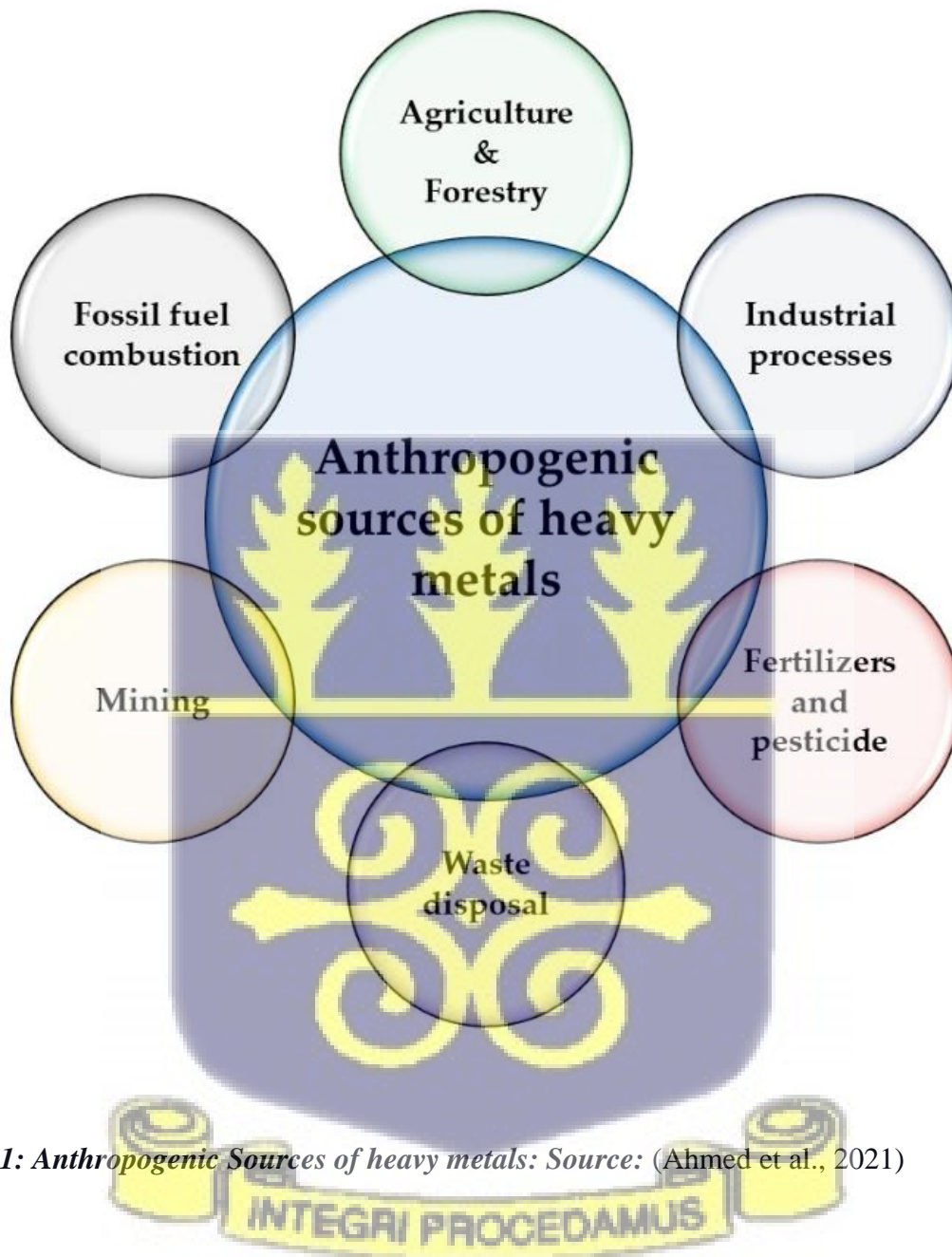


Figure 1: Anthropogenic Sources of heavy metals: Source: (Ahmed et al., 2021)

2.5 Lead

Another known metal is Lead; found in the earth's crust and released in small amounts into the environment (Juberg, 2000). Lead is non-biodegradable and can maintain a high level of

toxicity over time if accumulated in the environment (Flora et al., 2012; Huseen & Mohammed, 2019). Lead can be found in “batteries, petrol additives, rolled and extruded products, alloys, pigments and compounds, cable sheathing, shot, and ammunition” (Gupta, 2013). Lead can also originate from the environment through both natural and human-induced sources.

Humans can be exposed to Pb through drinking water, food such as vegetables, air, soil, and dust from old paint. Its ingestion in living beings can cause the tubular structures of the kidneys to malfunction, leading to chronic renal illness. Immense consequences on the reproductive health of humans, for instance, women may get miscarriages, stillbirths, and even sterility, and men can also display signs of reduced semen quality when they are poisoned by Pb (Juberg et al., 2000). Furthermore, shell-burst lead ammunition could well be a supplementary source of lead in human-eating meats. Whenever these bullets are employed in hunting animals, this happens, exposure to meat poisoned by lead bullets can have adverse effects on human health (Jarzyńska & Falandysz, 2011).

2.5.1 Iron

About 5g of iron can be found and it is the most abundant d-metal ion in the human body. Iron occurs in the oxidation states (+2) and (+3) (Saad et al., 2016). These ionic compounds become soluble in biofluids and produce harmful hydroxyl radicals when exposed to oxygen (Saad et al., 2016). Iron balance is a delicate procedure, as several distinct enzymes react to a variety of stimuli, including hypoxia, anemia, and inflammation (Saad et al., 2016).

The iron balance in humans is predominantly managed by the amount of dietary absorption of iron in the duodenum. The study of hereditary abnormalities, both in people and animals, leading to various iron diseases has yielded a wealth of information about the proteins involved in iron absorption and regulation of iron homeostasis in recent years (Saad et al., 2016; Zoroddu et al., 2019). Despite iron is important for survival, it can sometimes be hazardous if there is too much of it. Iron has the potential to affect the gastrointestinal system. Iron poisoning causes

nausea, vomiting, diarrhea, and stomach pain. Over time, iron can build up in the organs, causing serious liver or brain disorders (Zoroddu et al., 2019).

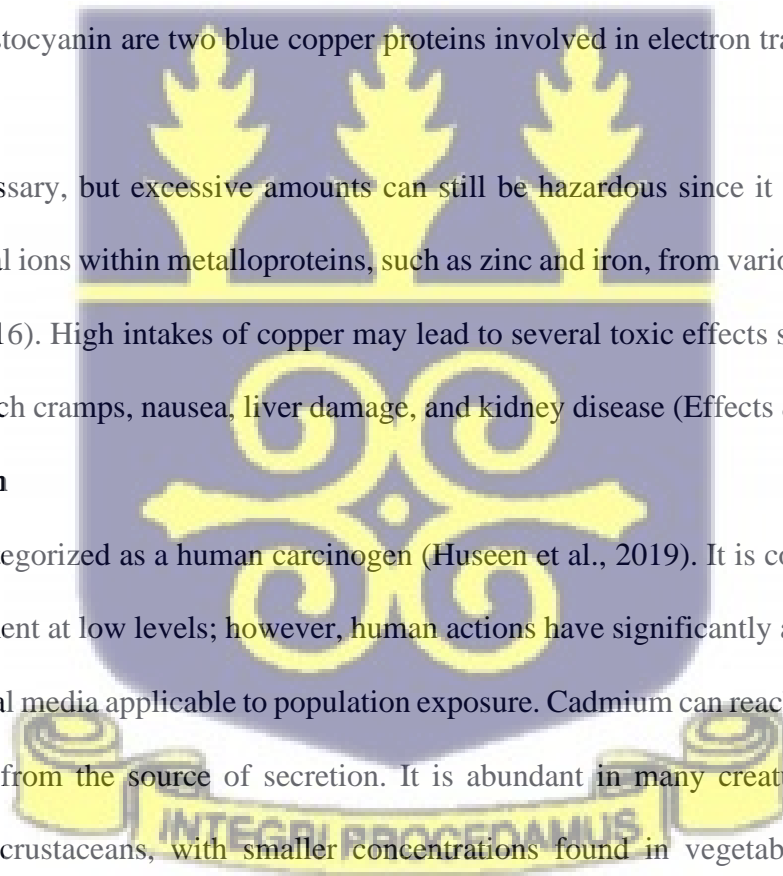
2.4.2 Copper

Approximately 100 mg of it is present in our body. Copper is one of the essential d-metals and the third most abundant (Saad et al., 2016). The daily recommended intake of copper is around a few milligrams. Copper can be found in several foods such as meats, mushrooms, nuts, and more (Saad et al., 2016). Cu (II) and Cu (I) have the highest oxidation values in living organisms (Saad et al., 2016). Several key enzymes in oxidation reactions, such as cytochrome c oxidase, ascorbate oxidase, and superoxide dismutase, require copper as a cofactor. Copper is often used in biological systems for electron flow in complement to its catalytic activity. Azurin and plastocyanin are two blue copper proteins involved in electron transport (Zoroddu et al., 2019).

Copper is necessary, but excessive amounts can still be hazardous since it can displace less aggressive metal ions within metalloproteins, such as zinc and iron, from various sites of action (Saad et al., 2016). High intakes of copper may lead to several toxic effects such as vomiting, diarrhea, stomach cramps, nausea, liver damage, and kidney disease (Effects & Family, 2000).

2.4.3 Cadmium

Cadmium is categorized as a human carcinogen (Huseen et al., 2019). It is commonly present in the environment at low levels; however, human actions have significantly augmented levels in environmental media applicable to population exposure. Cadmium can reach larger distances by air transfer from the source of secretion. It is abundant in many creatures, particularly mollusks, and crustaceans, with smaller concentrations found in vegetables, cereals, and starchy roots (Huseen et al., 2019). Humans can be exposed to cadmium through improper recycling of electronic and electrical trash (e-waste), as well as toys, jewelry, and plastics



containing the metal. Contaminated food, active and passive cigarette smoke inhalation and inhalation by personnel in several sectors are the main causes of population exposure.

Exposure of humans to cadmium exerts toxic can have effects on the kidneys as well as the skeletal and respiratory systems. Other indicators that could be utilized to identify cadmium accumulation in the body's organs include blood and urine. As a result, both blood and urine can be used to determine the amount of cadmium in the body. Cadmium's lethality is exacerbated by its bioaccumulative properties (Cui et al., 2005; Monferran et al., 2016). According to several scholars, when cadmium remains in its basic state within the environment for an extended period, its concentrations in humans can indeed exceed the allowed limits. Cadmium has a life span ranging from 10 to thirty years. (Rudy, 2009).

According to (Ihedioha, J. N., & Okoye, 2013), heavy metal concentration occurrences above acceptable parameters do not only have consequences on animal or fish life but could have a poisonous effect on human health as discussed above. Particularly once ingestion is consistent and sufficient.

2.5 Exposure and effect of Heavy Metal Toxicity

It is established in the field of toxicology that “excess of everything is bad “(Ali et al., 2019). Therefore, heavy metal build-up in soft tissues makes them toxic depending on the dose and length of exposure. Supplementary heavy metals (Cd, Pb, and Hg) and metalloids (As, etc.) may be toxic even at relatively little concentrations (Barau et al., 2018). Essential heavy metals on the other hand are required in trace amounts in the body but become toxic outside certain limits or threshold concentrations. Factors that affect the toxicity of heavy metals include the method of exposure, age, gender, genetics of an exposed person as well as the chemical species of the heavy metals (Sun, Y., Zhou, Q., Xie, X., & Liu, 2010). The scrutinizing and investigation of heavy metal absorptions in the environment are needed for pollution valuation and control. This will help offer valuable data about circulation, principal sources, and the

outcome of these elements in the environment and their bioaccumulation in the food chain (Barau et al., 2018).

Health effects accompany the exposure to heavy metals such as Cd, Pb, and Hg, and exhaust the main antioxidants of cells by infecting the characteristic antioxidant defenses of cells which can result in a condition called “oxidative stress”. Higher concentrations of toxic heavy metals such as Cr, Cd, and Pb, have been found in patients with cancer and diabetes (Ahmed et al., 2021; Ali et al., 2019; Barau et al., 2018).

2.6 Vegetable Farms and Market Conditions

Urban vegetable farming in Ghana dates back to the colonial era when vegetables were grown in gardens around the castles and forts along Ghana’s Gold Coast (La Anyane, 1963). Additionally, according to La Anyane (1963), during the Second World War, agriculture was stimulated in all parts of the country to help nourish the allied troops on the Gold Coast. During post-independence Ghana, owing to the economic crunches in the country, as part of a national agenda (Operation Feed Yourself) the government then encouraged and supported urban agriculture to meet the population’s food demands (Danso et al., 2014).

In the late 1990s, the decentralization of the Ministry of food and Agriculture postulated urban farming with transformed provision, as each district including cities received its own Agricultural Directorate with extension staff (Amoah et al., 1998). Even though urban farming was encouraged for the past decade urban farmers were still confronted with a lot of challenges, for instance, they were not trained in the peculiarities of urban farming. With all these challenges, backyard gardening vestiges as a well-accepted activity communally, particularly in the middle-income group (Amoah et al., 1998; Danso et al., 2014).

2.6.1 Accra Urban Farming

The Greater Accra region seems to have reached a saturation point as it has shown coherent regressions in the almanac growth of its urban population from 6.1% in 1960 to 3.5% in 2010 (GSS 2012). About 60% of Accra's population lives in informal settlements in the center of the city while the middle and upper classes prefer its periphery. Natural drainage systems in Accra include several streams, ponds, and lagoons. About 60% of the city's urban area drains into the Odaw River which passes the Korle Lagoon before flowing into the sea (Danso et al., 2014). The wastewater and solid waste that Odaw receives constitute a major environmental disaster as shown in figure 2.2.

Figure 2.2 Odaw River in Accra



Source: Danso et al. (2014)

Irrigated urban vegetable production takes place on more than six large sites within Accra central. In 2005 about 47 hectares of this land were under vegetable farms and with seasonal variation, about 251 hectares were under mixed cereal-vegetable systems. It is estimated that about 60% of these vegetable farmers in the city produce exotic crops such as lettuce, cabbage, spring onions, and cauliflowers, and 40% indigenous local or traditional vegetables such as

tomatoes, okoro, ayoyo (*Corchorus* sp.), garden eggs (aubergine) and hot pepper (Amoah et al., 1998).

The most common water source is raw large or diluted wastewater that is channeled into shallow reservoirs (dug-outs), while other farmers also use pipe-borne water, stream water, or water from treatment ponds. In most of these sites, mutual agreements were formalized with the local authorities and institutions for farming in the areas as a way of maintaining the lands and preventing any nonagricultural encroachment.

Figure 2.3 Parts of the Dzorwulu site



Source: Danso et al. (2014)



CHAPTER THREE

METHODOLOGY

3.1 Study Design

The study was an analytical cross-sectional, intending to determine the quantities of trace metals in lettuce from Accra's "open markets" and supermarkets. An open site or region where products and services are sold and given is referred to as an "open market."

3.2 Description of the study sites

The study was conducted in the grocery sections of two major supermarkets (Palace Mall and Melcom Plus) and two 'open markets' (Madina and Kaneshie Markets) in the Accra Metropolis as shown in Figures 3 and 4 below.

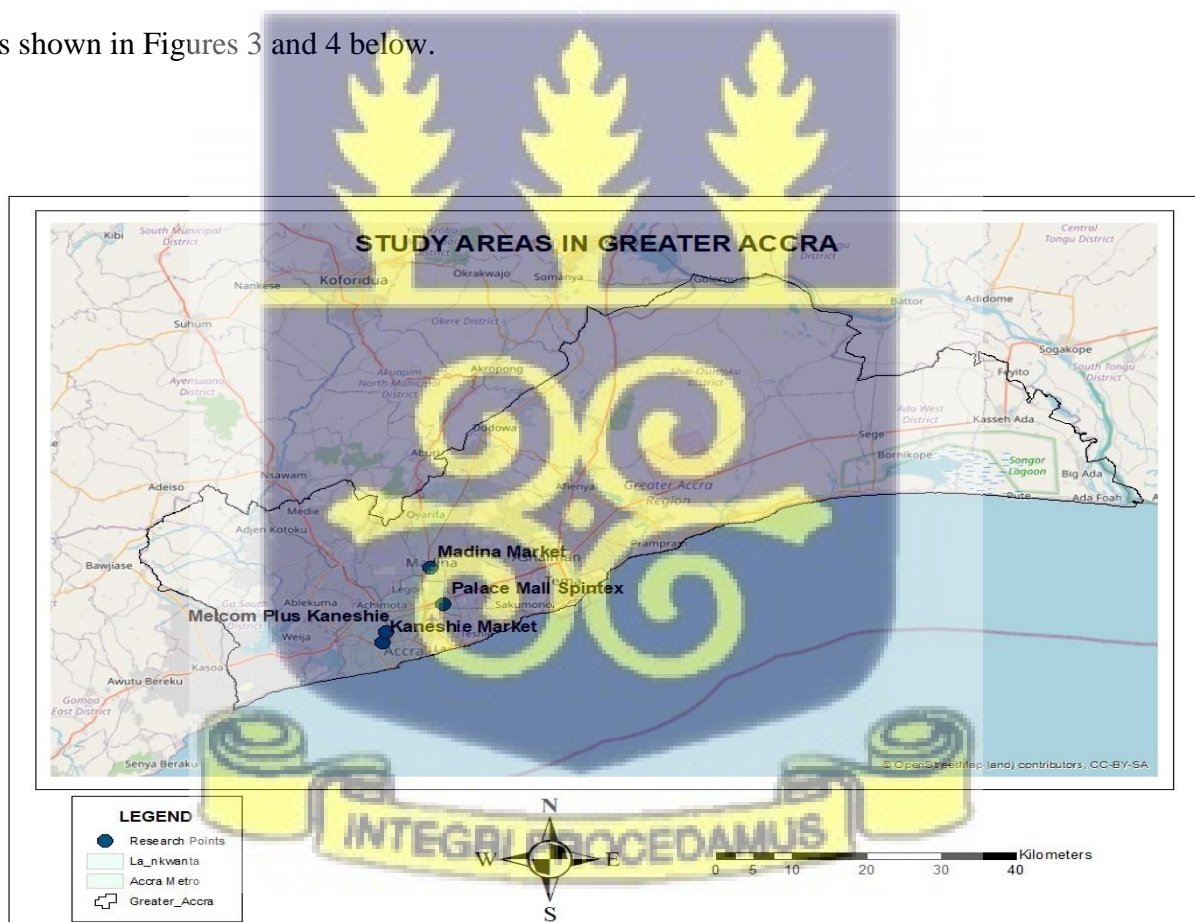


Figure 3: selected study sites

These markets were selected because they served a high number of shoppers from diverse socio-demographic backgrounds. Additionally, these markets made vegetables available all year round for shoppers.

3.3. Sampling technique

This study examined metal concentrations in leafy vegetable; lettuce. Lettuce was selected because it is commonly cultivated and most widely consumed in Ghana among the exotic leafy vegetables (Drechsel et al., 2014). Exotic leafy vegetables seem to be more popular, particularly among urban inhabitants, even though indigenous leafy vegetables are mostly cheaper and even more nutritious (Darkwa, 2014).

A total of 16 samples of lettuce (approximately 15kg) from two varieties (Eden and Great lakes) [Fig. 5 & 6] were selected for heavy metals analysis. Two samples of each of the two varieties were randomly selected across the four markets. All samples were packaged in well-labeled zip-lock bags and transported to the laboratory for processing and analysis.

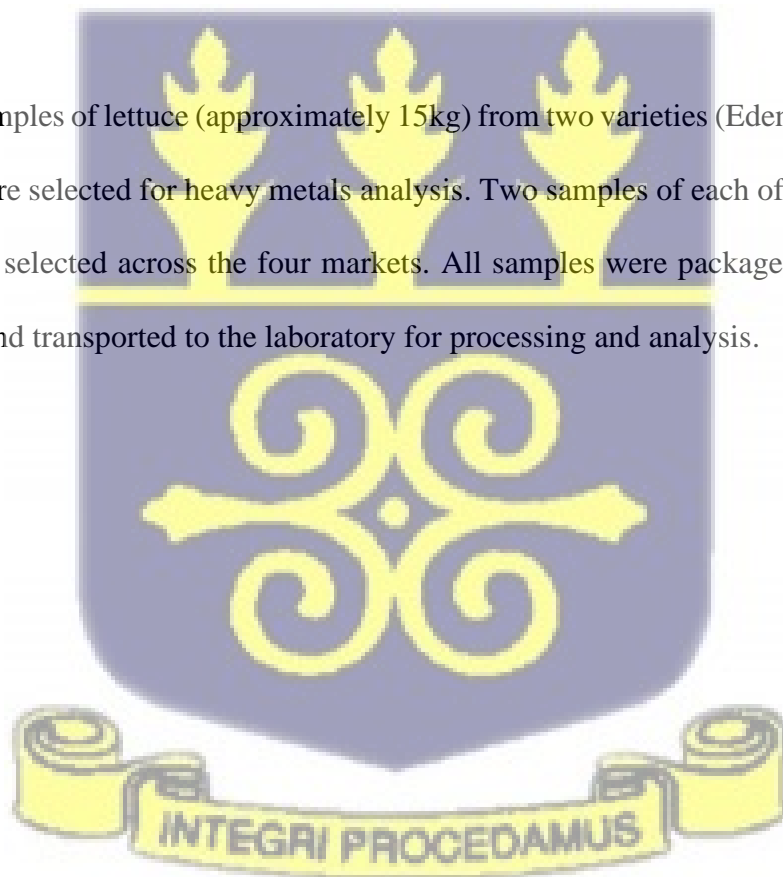




Figure 5 (A)



Figure 6 (B)

The varieties of lettuce selected for this study: Figure 5 represents Eden and Figure 6 represents Great Lakes.

3.4 Preparation of Samples

The sampling preparation protocol of (Barau et al., 2018) was used in this study. In the laboratory, samples from each market were washed with clean water and carefully rinsed with deionized water to remove any soil particles or debris that must have been attached to the plants. They were then dried at 80° C to obtain a constant weight. The dried samples of each variety of lettuce from each market were pulverized and sieved through a mesh size of 2 mm to obtain sample finer lettuce particles for each variable across the markets.

3.5 Sample Analysis

3.5.1 Heavy Metal Analysis of vegetable Samples

One kilogram of the sample was homogenized thoroughly with a laboratory blender and sampled into a well-labeled sample cup. Five milliliters of Nitric acid and 3ml of hydrogen peroxide were added into each sample and covered tightly. The samples were then arranged in the microwave digester with a temperature of 170°C, a pressure of 50 bar, and 1000 energy. After the sample was cooled for 20 minutes and was poured into their well-labeled centrifuge tubes with deionized water to the 25ml mark. The digestion thus ended in 45 minutes on the tube which led to the analysis using VARIAN AA 240FS-Atomic Absorption Spectrometer under recommended instrument parameters (British-Adopted European Standard., 2003).



Figure 7. Atomic Absorption Spectrometer used for trace metal extraction in this study





Figure 8. Microwave digester

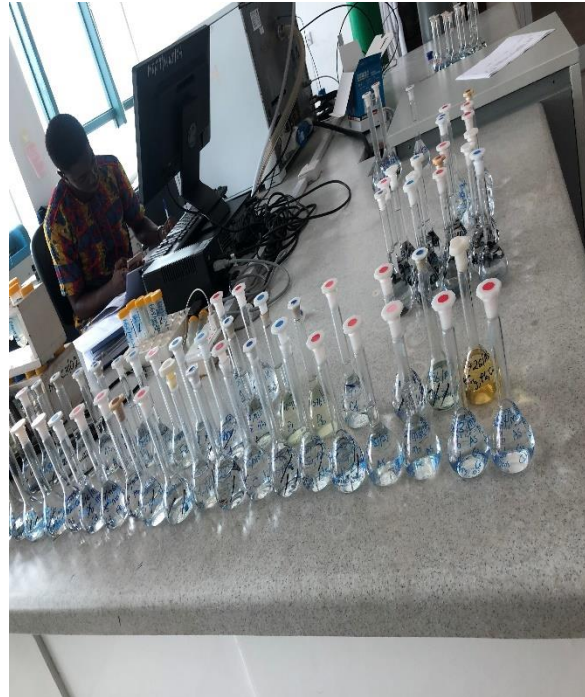


Figure 9. Volumetric flask

3.5.2 Statistical Analysis

All analyses were conducted using IBM Statistical Package for Service Solutions (version 26.0) (<https://www.ibm.com/analytics/spss-statistics-software>)

3.5.2.1 Comparing concentrations of heavy metals in lettuce across markets

To compare the concentration of metals in lettuce across sites, descriptive statistics (e.g., means, standard deviations) and Analysis of variance (ANOVA) were used to compare the concentration of heavy metals across sites was used.

3.5.2.2 Comparing concentrations of heavy metals in lettuce with FAO/WHO standards

To compare the concentration of metals in lettuce across sites with FAO/ WHO standards, descriptive statistics (e.g., means, standard deviations). T-tests and Analysis of variance (ANOVA) were used to compare the concentration of heavy metals across sites with FAO/WHO standards.

3.5.2.3 Potential human health risk assessment of metals in lettuce

The estimated Average Daily Intake (ADI) per meal size of lettuce was determined based on Eq. (1) where C_{let} is the heavy metal concentration in lettuce (mg/kg, on a constant weight basis), I_{let} the daily average consumption of lettuce (kg/day) and BW is the body weight of the exposed individual (kg) (Zhuang et al., 2009). The technique of the European Food Safety Authority was chosen, which is based on the assessment of dangerous ingestion limits represented in terms of actual meals (EFSA, 2016).

All consumption limits and risk factors were calculated assuming for adults (> 18 years old), a meal size of 0.182 kg/day and for children (6 years old), a meal size of 0.118 kg/day (Nabulo et al., 2011). The BW of an adult Ghanaian was estimated as 60 kg (>18 years old), 30 kg (7-13 years), and 15 kg (0-6 years) (Vuvor & Fabea, 2017). The ingested dosage (Intake Rate) was presumed to be equivalent to the absorption toxin dose, and heating had no impact on the pollutants, according to USEPA (2015) guidance (Chien et al., 2002)

Equation 1:

$$ADI = \frac{C_{let} \times I_{let}}{BW}$$

3.6.1. Allowable daily consumption limits of metals for lettuce (i.e., critical limit)

Equation 2:

$$CRLim = BW \times RfD$$

→ → → C



Where CRLim is the maximum safe daily consumption rate of lettuce (kg day), RfD is the reference dose for each trace metal (mg kg⁻¹ day), BW is the average consumer body weight

(in kg), 60kg for adults and 30kg for children and C is the concentration of the chemical in the edible portion of lettuce (mg kg⁻¹).

3.7 Target Hazard Quotient (THQ)

The target hazard quotient (THQ) [Equation 3] is defined as the ratio of toxic element exposure to the reference dose (which is the greatest dose at which no adverse health effects are expected) [Johann et al., 2017]. There is a reference dosage for each trace element being evaluated. The THQ determines whether or not the hazardous material poses a non-carcinogenic health risk. If the THQ is less than or equal to 1, no non-carcinogenic health impacts are expected. However, if the THQ is larger than one, there is a danger of negative health consequences. A THQ of more than one does not imply a statistically significant risk of harmful non-carcinogenic health consequences (Johann et al., 2017). The THQ was estimated based on the risk-based concentration table for Region III and a methodology published by the United States Environmental Protection Agency (US EPA).

Equation 3:

$$THQ = \frac{E_{FR} \times Ed \times F_{IR} \times C}{RFD \times BW \times ATn}$$

Where E_{FR} is the trace element exposure frequency [or the number of exposure events per year of exposure (ranging from 365 days for persons who eat lettuce seven times a week to 52 days for people who eat lettuce once a week)], Ed is the exposure duration (70 years for adults and 6 years for children), F_{IR} is the vegetable ingestion rate in grams per day for the respective vegetable type (lettuce), 0.182g for adults and 0.118g for children, C is the trace element

concentration in mg/kg per day, BW_a is the reference body weight of 60 kg for adults and 30 kg for children, and AT_n is the averaged exposure time [equal to EF ED]. (365 days * 70)

3.7.2 Hazard Index (HI)

A Quotient defined by USEPA (1989), which is the entire sum of all Hazard Quotients, was used to calculate the Hazard Index (HI), which is the potential danger of heavy metals to health owing to more than one heavy metal. This is given as;

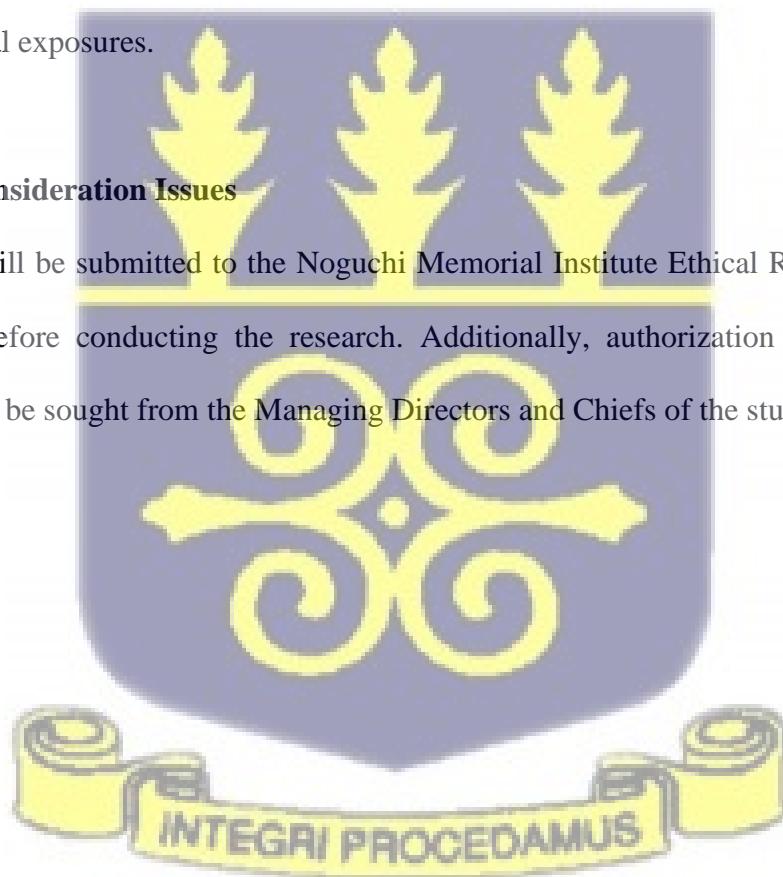
Equation 4:

$$HI = SHQ = HQ_{Cd} + HQ_{Pb} + HQ_{Fe} + HQ_{Cu} + HQ_{Mn} + HQ_{Zn}$$

The hazard index posits that the amount of the negative effect is proportional to the total number of metal exposures.

3.8 Ethical Consideration Issues

The research will be submitted to the Noguchi Memorial Institute Ethical Review Board for endorsement before conducting the research. Additionally, authorization and well-versed permission will be sought from the Managing Directors and Chiefs of the study areas.



CHAPTER FOUR

RESULTS

4.1 Descriptive Statistics

This section presents the descriptive statistics (mean and standard deviation) of selected heavy metals in lettuce sampled from the open markets and the supermarkets as well as the different varieties sampled from the markets.

4.1.1. Comparing concentrations of heavy metals in lettuce across markets

Results from *Table 4.1* below show the concentration of Iron, Zinc, and Manganese from the various markets sampled for the study. From the results on the concentration of iron, Kaneshie Market (18.02 ± 5.83) and Madina Market (14.00 ± 2.92) had the highest concentrations. Palace Mall recorded the lowest iron concentration of 4.70 ± 1.03 . Lettuce from the Kaneshie market recorded the highest zinc concentration of 2.09 ± 0.85 with lettuce from Melcom Plus recording the least zinc concentration of 0.50 ± 0.58 . Manganese concentration in lettuce was highest at the Madina market at 6.56 ± 2.65 followed by Kaneshie market, Palace Mall, and Melcom Plus in descending order with 3.22 ± 2.14 , 2.01 ± 0.95 , and 1.21 ± 0.16 respectively.

There was a statistically significant difference ($F(9, 24.488) = 19.284$, $p = 0.0001$; Wilks' Lambda = 0.006; partial eta squared = 0.817) between the concentration of metals in lettuce from the markets and lettuce from the supermarkets as shown in *Table 4.2* below.

From *Table 4.2*, it can be observed that the concentration of iron differed significantly between the Madina market and Palace; and between the Kaneshie market and both Palace and Melcom Plus. Zinc concentration differed significantly between Madina market and Palace, and Kaneshie market and Melcom Plus. There was a significant difference in manganese concentration between Madina market and both Palace mall and Melcom Plus but no difference between Kaneshie market and any of the supermarkets.

Table 4.1. Heavy metal concentration in lettuce across sites

| Metal | Site/Market Name | Mean | Std. Deviation |
|----------------|-------------------------|-------------|-----------------------|
| Iron (Fe) | Palace Mall | 4.70 | 1.03 |
| | Melcom Plus | 7.24 | 2.48 |
| | Madina Market | 14.00 | 2.92 |
| | Kaneshie Market | 18.02 | 5.83 |
| Zinc (Zn) | Palace Mall | 2.01 | 0.51 |
| | Melcom Plus | 0.50 | 0.58 |
| | Madina Market | 0.65 | 0.06 |
| | Kaneshie Market | 2.09 | 0.85 |
| Manganese (Mn) | Palace Mall | 2.01 | 0.95 |
| | Melcom Plus | 1.21 | 0.16 |
| | Madina Market | 6.56 | 2.65 |
| | Kaneshie Market | 3.22 | 2.14 |

Note: The metals' concentrations used for this analysis were Iron, Zinc, and Manganese. This was because Lead, Cadmium, and Copper produced constant metal concentrations in all the open markets as well as supermarkets hence having constant means and 0 standard deviations.

Table 4.2.

| Metal | Open Market | Supermarket | p-value | 95% Confidence Interval | |
|--------------|--------------------|--------------------|----------------|--------------------------------|--------------------|
| | | | | Lower Bound | Upper Bound |
| Iron (Fe) | Madina Market | Palace Mall | 0.013* | 1.900 | 16.710 |

| | | | | | |
|----------------|-----------------|-------------|---------|--------|--------|
| | | Melcom Plus | 0.078 | -0.645 | 14.165 |
| | Kaneshie Market | Palace Mall | 0.0001* | 5.920 | 20.730 |
| | | Melcom Plus | 0.005* | 3.375 | 18.185 |
| Zinc (Zn) | Madina Market | Palace Mall | 0.026* | -2.561 | -0.149 |
| | | Melcom Plus | 0.982 | -1.057 | 1.356 |
| | Kaneshie Market | Palace Mall | 0.997 | -1.126 | 1.286 |
| | | Melcom Plus | 0.010* | 0.378 | 2.790 |
| Manganese (Mn) | Madina Market | Palace Mall | 0.016* | 0.836 | 8.264 |
| | | Melcom Plus | 0.005* | 1.631 | 9.059 |
| | Kaneshie Market | Palace Mall | 0.768 | -2.499 | 4.929 |
| | | Melcom Plus | 0.411 | -1.704 | 5.724 |

* The concentration is significant at the 0.05 level

4.2 Tukey HSD Test Results of Multiple Comparisons of Metal Concentration between Open Markets and Supermarkets for Great Lakes

The results presented in Table 4 on the differences in metal concentration in Great Lakes across the open markets as compared to the supermarkets revealed that there was no statistically significant difference in the concentration of any of the metals found in Great Lakes from any of the markets.

Table 4.

| Dependent Variable | Open Market | Supermarket | p-value | 95% Confidence Interval |
|--------------------|-------------|-------------|---------|-------------------------|
|--------------------|-------------|-------------|---------|-------------------------|

| | | | | Lower | Upper |
|-------------------|-----------------|-------------|------|--------------|--------------|
| | | | | Bound | Bound |
| Iron (Fe) | Madina Market | Palace Mall | .687 | -5.365 | 11.837 |
| | | Melcom Plus | .962 | -7.206 | 9.997 |
| | Kaneshie Market | Palace Mall | .809 | -6.016 | 11.185 |
| | | Melcom Plus | .994 | -7.857 | 9.346 |
| Zinc (Zn) | Madina Market | Palace Mall | .584 | -9.012 | 3.541 |
| | | Melcom Plus | .949 | -7.408 | 5.145 |
| | Kaneshie Market | Palace Mall | .944 | -5.107 | 7.446 |
| | | Melcom Plus | .573 | -3.504 | 9.043 |
| Manganese (Mn) | Madina Market | Palace Mall | .966 | -5.761 | 7.893 |
| | | Melcom Plus | .664 | -9.484 | 4.170 |
| | Kaneshie Market | Palace Mall | .527 | -3.627 | 10.027 |
| | | Melcom Plus | .996 | -7.349 | 6.304 |

4.3 Tukey HSD Test Results of Multiple Comparisons of Metal Concentration between Open Markets and Supermarkets for Eden

The results presented in Table 5 revealed that there is a statistically significant concentration of Manganese in the Eden variety of lettuce from the Madina market than in both Palace mall and Melcom Plus ($p < 0.05$).

Table 5.

| Metals | Open Markets | Supermarket | p-value | 95% Confidence | |
|----------------|-----------------|-------------|---------|----------------|-------------|
| | | | | Lower Bound | Upper Bound |
| Iron (Fe) | Madina Market | Palace Mall | .989 | -13.48191 | 10.93591 |
| | | Melcom Plus | .999 | -11.70466 | 12.71316 |
| | Kaneshie Market | Palace Mall | .998 | -11.53791 | 12.87991 |
| | | Melcom Plus | .932 | -9.76066 | 14.65716 |
| Zinc (Zn) | Madina Market | Palace Mall | .595 | -3.29301 | 8.24301 |
| | | Melcom Plus | .975 | -6.56751 | 4.96851 |
| | Kaneshie Market | Palace Mall | .262 | -1.99501 | 9.54101 |
| | | Melcom Plus | .994 | -5.26951 | 6.26651 |
| Manganese (Mn) | Madina Market | Palace Mall | .013* | .80499 | 6.96551 |
| | | Melcom Plus | .003* | 1.67174 | 7.83226 |
| | Kaneshie Market | Palace Mall | .896 | -2.35601 | 3.80451 |
| | | Melcom Plus | .449 | -1.48926 | 4.67126 |

Table 5

*. The mean difference is significant at the .05 level.

4.4 Levels of Concentration of Metals in Lettuce compared with FAO/WHO Limit

This research objective sought to find out whether there was a statistically significant difference in metals in lettuce for this study as against the FAO/WHO recommended limits. This analysis was done in two parts. The first part considered the graphical representation of the concentration of metals in the lettuce as against the FAO/WHO limits. This was shown in Figure 4.1. The second section contains the results of the one-sample t-test at a 0.05 level of significance to determine whether there was a statistically significant difference in the mean

concentration of metals in the lettuce and the FAO/WHO limits. The concentration of each of the metals in the lettuce was compared to the FAO/WHO limit the concentration of each other metals. The results are presented in the figure below:

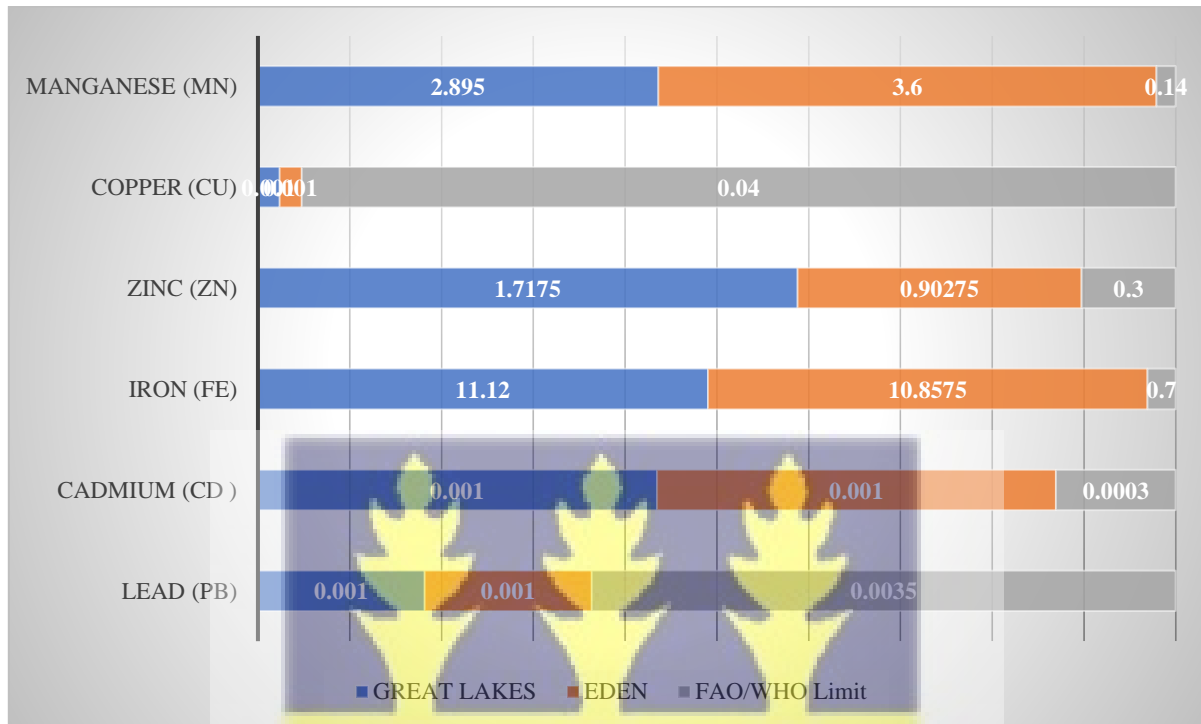


Figure 20: Metal Concentration in Lettuce Compared to FAO/WHO Limits

Note: Each metal has a different scale

4.5 Comparing levels of metals in lettuce to FAO/WHO allowable limits

From Table 6 below, the negative t-value ($t(16) = -26.667, df(15), p = 0.000$) indicates that the concentration of Lead (0.001) in the lettuce from the study sites is less than the FAO/WHO limit (0.0035). Since the p-value is less than the 0.05 level of significance, there is a statistically significant difference (decrease) in Lead concentration in lettuce from the study sites and the concentration of Lead according to FAO/WHO limits. In the same vein, the negative t-value (t

(16) = -388.849, df (15), p = 0.000) indicates that the concentration of copper (0.001) in the lettuce from the study sites is less than the FAO/WHO limit (0.04). Since the p-value is less than the 0.05 level of significance, there is a statistically significant difference (decrease) in copper concentration in lettuce from the study sites and the concentration of copper according to FAO/WHO limits. In contrast, the positive t-value ($t(16) = 5.08$, df (15), p = 0.000) indicates that the concentration of Cadmium (0.001) in the lettuce from the study sites is higher than the FAO/WHO limit (0.0003). Since the p-value is less than the 0.05 level of significance, there is a statistically significant difference (increase) in Cadmium concentration in lettuce from the study sites and the concentration of Cadmium according to FAO/WHO limits. Additionally, the positive t-value ($t(16) = 6.518$, df (15), p = 0.000) indicates that the concentration of Iron (10.99) in the lettuce from the study sites is higher than the FAO/WHO limit (0.7). Since the p-value is less than the 0.05 level of significance, there is a statistically significant difference (increase) in Iron concentration in lettuce from the study sites and the concentration of Iron according to FAO/WHO limits. Again, the positive t-value ($t(16) = 4.398$, df (15), p = 0.001) indicates that the concentration of Zinc (1.31) in the lettuce from the study sites is higher than the FAO/WHO limit (0.3). Since the p-value is less than the 0.05 level of significance, there is a statistically significant difference (increase) in Zinc concentration in lettuce from the study sites and the concentration of Zinc according to FAO/WHO limits. Finally, the positive t-value ($t(16) = 4.718$, df(15), p = 0.000) indicates that the concentration of Manganese (3.25) in the lettuce from the study sites is higher than the FAO/WHO limit (0.14). Since the p-value is less than the 0.05 level of significance, there is a statistically significant difference (increase) in Cadmium concentration in lettuce from the study sites and the concentration of Lead according to FAO/WHO limits.

Table 6.

| Metals | | | | | FAO/WH | | Df | p-value |
|-----------------------|-------|-------|-------|-------|---------------------|----------|----|---------|
| | MM | MP | PM | KM | O | T | | |
| | | | | | Concentration Limit | | | |
| | 0.001 | 0.001 | 0.001 | 0.001 | 0.0035 | -26.667 | 15 | 0.000 |
| Lead (Pb) | | | | | | | | |
| Copper (Cu) | 0.001 | 0.001 | 0.001 | 0.001 | 0.04 | -388.849 | 15 | 0.000 |
| Cadmium (Cd) | 0.001 | 0.001 | 0.001 | 0.001 | 0.0003 | 5.08 | 15 | 0.000 |
| Iron (Fe) | 14.00 | 7.24 | 4.70 | 18.02 | 0.7 | 6.518 | 15 | 0.000 |
| Zinc (Zn) | 0.65 | 0.50 | 2.01 | 2.09 | 0.3 | 4.398 | 15 | 0.001 |
| Manganese (Mn) | 6.56 | 1.21 | 2.01 | 3.22 | 0.14 | 4.718 | 15 | 0.000 |

Source: Fieldwork (2021)

Note: Df = Degrees of freedom



4.6 Potential Health Risks of Consuming Lettuce with the Measured Metals Concentrations

Table 7: Estimated Health Risk for lettuce from Open markets

| Heavy Metals | ADULTS | CHILDREN |
|--------------|--------|--|
| | | $ADI = \frac{C_{let} \times IR_{let}}{BW}$ |
| ZINC | 0.004 | 0.005 |
| COPPER | 0.000 | 0.000 |
| LEAD | 0.000 | 0.000 |
| CADMIUM | 0.000 | 0.000 |
| MANGANESE | 53.372 | 0.019 |
| IRON | 0.049 | 0.0181 |

Table 8: Estimated health risk for lettuce from Supermarkets

| Heavy Metals | ADULTS | CHILDREN |
|--------------|--------|----------|
| ZINC | 0.004 | 0.005 |
| COPPER | 0.000 | 0.000 |
| LEAD | 0.000 | 0.000 |
| CADMIUM | 0.000 | 0.000 |
| MANGANESE | 17.554 | 0.006 |
| IRON | 0.063 | 0.023 |

The RfD is an estimate of a contaminant's daily consumption over a lifetime that is unlikely to induce harmful health consequences (USEPA, 2000). The USEPA's regional screening threshold produced RfD values of 0.003 for Cd, 0.004 for Cu, 0.14 for Mn, 0.7 for Fe, 0.0035 for Pb, and 0.3 for Zn (mg kg⁻¹ day⁻¹) (USEPA, 2015).



Table 9: Allowable Daily Consumption Rate (mg kg⁻¹)

| Sample | Site | Variety | Metals and Concentrations | | | | | | | | | | CR _{lim} | | | | | | | |
|---------|------|---------|---|-------|-------|-------|-------|------|----|---|-----|-----|-------------------|------|-------|-------|------|------|------|------|
| | | | Maximum allowable CR _{lim} for metals. Adults and Children | | | | | | | | | | | | | | | | | |
| | | | Cd | | Pb | | Fe | | Zn | | Cu | | Mn | | | | | | | |
| Lettuce | | | A | C | A | C | A | C | A | C | A | C | A | C | A | C | A | C | A | C |
| PM | GL | | 0.001 | 0.001 | 5.59 | 2.45 | 0.001 | 1.18 | 18 | 9 | 210 | 105 | 7.51 | 3.76 | 7.35 | 3.67 | 2400 | 1200 | 7.12 | 3.56 |
| PM | E | | 0.001 | 0.001 | 3.8 | 1.56 | 0.001 | 2.83 | 18 | 9 | 210 | 105 | 11.05 | 5.53 | 11.54 | 5.77 | 2400 | 1200 | 2.97 | 1.48 |
| MP | GL | | 0.001 | 0.001 | 16.53 | 0.6 | 0.001 | 1.07 | 18 | 9 | 210 | 105 | 4.47 | 2.24 | 18 | 9 | 2400 | 1200 | 1.97 | 0.99 |
| MP | E | | 0.001 | 0.001 | 11.47 | 0.7 | 0.001 | 1.35 | 18 | 9 | 210 | 105 | 8.25 | 4.13 | 18000 | 9000 | 2400 | 1200 | 0.95 | 0.47 |
| MM | GL | | 0.001 | 0.001 | 9.39 | 1 | 0.001 | 4.26 | 18 | 9 | 210 | 105 | 2.54 | 1.27 | 30 | 15 | 2400 | 1200 | 7.85 | 3.93 |
| MM | E | | 0.001 | 0.001 | 5.09 | 0.001 | 0.001 | 8.85 | 18 | 9 | 210 | 105 | 3.66 | 1.83 | 25.71 | 12.86 | 2400 | 1200 | 6.22 | 3.11 |
| KM | GL | | 0.001 | 0.001 | 12.97 | 2.82 | 0.001 | 5.07 | 18 | 9 | 210 | 105 | 3.24 | 1.62 | 6.38 | 3.19 | 2400 | 1200 | 1.66 | 0.83 |
| KM | E | | 0.001 | 0.001 | 23.07 | 1.35 | 0.001 | 1.37 | 18 | 9 | 210 | 105 | 1.82 | 0.91 | 13.33 | 6.67 | 2400 | 1200 | 6.13 | 3.07 |

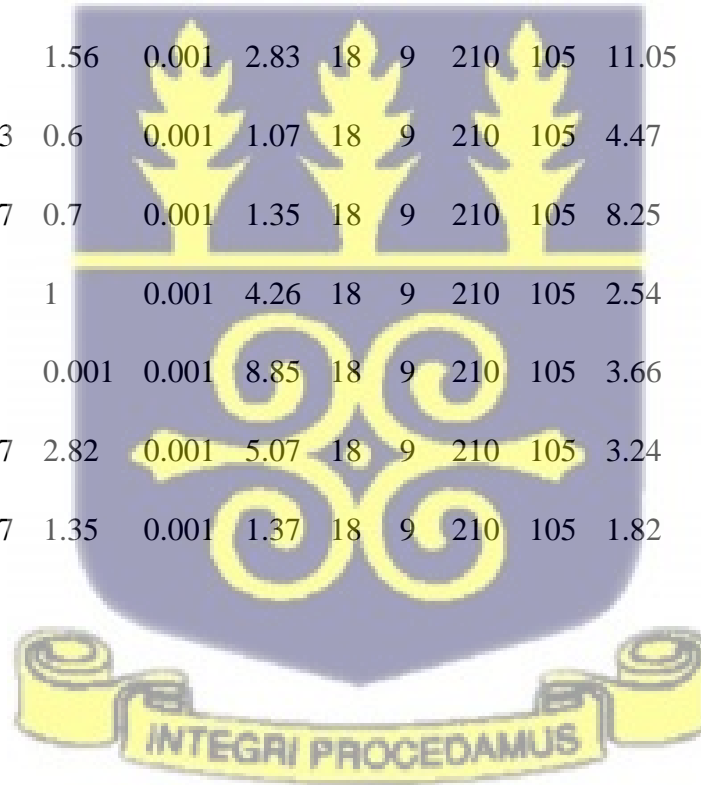


Table 4 shows the estimated daily intake of metals per meal size (EDI) for adults and children who consume lettuce leaves (edible portions). The following are the average EDI values of metals consumed by adults and children from lettuce consumption: Cu N Cd N Pb \approx Cu N Cd N Pb Cu N Cd N Pb. Pb Cu Cd for both adults and children, EDI levels were lower than those recommended by the joint FAO/WHO Expert Committee on Food Additives (JECFA, 2009), however for Fe, Zn, and Mn, the value was greater than the tolerated intake (TI in g kg⁻¹ day⁻¹).



Table 10: Target Hazard Quotient and Hazard Index. “C” represents

| Specie (Lettuce) | Exposure time (d) | Target Hazard Quotient (THQ) | | | | | | | | | | | | Hazard Index (HI) | |
|---------------------|----------------------|------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------------------|----------|
| | | Zn | | Cu | | Pb | | Cd | | Mn | | Fe | | A | C |
| Lettuce | | A | C | A | C | A | C | A | C | A | C | A | C | A | C |
| | 1 | 0.003 | 0.004 | 0.00002 | 0.00003 | 0.00022 | 0.0003 | 0.0025 | 0.0033 | 0.018 | 0.023 | 0.012 | 0.015 | 0.0355 | 0.046 |
| | 7 | 0.023 | 0.030 | 0.0001 | 0.0002 | 0.00152 | 0.0020 | 0.018 | 0.023 | 0.123 | 0.160 | 0.083 | 0.108 | 0.249 | 0.323 |



CHAPTER FIVE

5.0. DISCUSSION

This study aimed to assess and compare the heavy metal (Zinc, Copper, Lead, Manganese, Cadmium, and Iron) concentrations in Lettuce from both supermarkets and open markets. This chapter discusses the findings of this study concerning the study objectives and in light of the extant literature.

5.1. Concentrations of heavy metals in lettuce from markets and supermarkets in Accra Metropolis.

The open markets recorded higher concentrations of heavy metals in lettuce. This may be due to the nature of the markets where goods were not covered. The supermarkets were not exposed to the ambient environment whereas open markets were exposed to the ambient environment. Waste materials such as electronic items may release toxic materials into the atmosphere (Bortey-Sam et al., 2015) which may settle on food items in the open markets. Another reason for the difference may be the level of scrutiny that farm produce is subjected to before display and then consumption. For supermarkets, farm produce is obtained from farms that practice safe farming practices whereas, for the open markets, farm produce is brought without any form or level of scrutiny. For instance, farmers supplying supermarkets may be more likely to use clean water in watering while farmers bringing wares to the open markets may resort to using any available water such as a stream or creek which may contain leached toxic materials from nearby waste (Monferran et al., 2016).

5.2. Comparison of heavy metal concentrations in lettuce with FAO/WHO standards

The second objective of the study sought to determine whether heavy metal concentrations in lettuce were within the FAO/WHO limits (0.3mg/kg for Zinc (Zn), 0.003mg/kg for Cadmium

(Cd), 0.0035mg/kg for Lead (Pb), 0.04mg/kg for Copper (Cu), 0.7mg/kg for Iron (Fe) and 0.14mg/kg for Manganese (Mn) (FAO/WHO., 2015), for safe consumption. Results from the study showed that lettuce from all locations had lower levels of lead, copper, and cadmium (Pb, Cu, Cd) concentrations. Iron (Fe), Zinc (Zn), and Manganese (Mn) were however relatively higher in lettuce from both supermarkets and Open Markets. Iron recorded the highest concentration followed by manganese and zinc respectively.

5.3. Human health risks associated with consuming lettuce with the measured heavy metal concentrations.

One of the most important health risk assessment tools is estimating the metal contaminant's estimated daily intake (EDI). It considers the frequency and duration of exposure, as well as the body weight of those who are exposed. In general, the risk of metal contamination to one's health is proportional to one's dietary habits and routes of exposure mainly inhalation. For Cd, Pb, and Cu, the EDI for lettuce was within the acceptable daily intake reference ranges. The EDI for Fe, Zn, and Mn was found to be greater than the standard limit for tolerable daily consumption. The HQ calculated the noncarcinogenic risk of metals in lettuce, and the findings are displayed in the Table. If the HQ value is less than 1, the exposed consumers are thought to be safe; however, if the HQ value is equal to or greater than 1, the exposed customers are deemed to be in danger. The HQ values for Cd, Pb, Zn, Fe, Mn, and Cu were all less than one, indicating that modest eating of lettuce provides minimal health hazards from these metals. If the HI value of any heavy or critical metal in lettuce is less than 1, the exposed population is unlikely to suffer any negative health consequences.

Results obtained from fieldwork as represented in Table 4.9 showed Iron, zinc, and manganese having concentrations not exceeding the threshold (0.70 mg kg⁻¹– Fe, 0.30mg kg⁻¹ -Zn, 0.14mg kg⁻¹ -Mn) as stated by FAO/WHO (USAID., 2015). Measurements showed Iron to have

concentrations of 14.00 $\mu\text{g}/\text{kg}$, 7.24 $\mu\text{g}/\text{kg}$, 4.70 $\mu\text{g}/\text{kg}$, 18.02 $\mu\text{g}/\text{kg}$ for Madina Market, Melcom Plus, Palace mall, and Kaneshie Market respectively. Zinc had concentrations of 0.65 $\mu\text{g}/\text{kg}$, 0.50 $\mu\text{g}/\text{kg}$, 2.01 $\mu\text{g}/\text{kg}$, 2.09 $\mu\text{g}/\text{kg}$ for samples from Madina market, Melcom plus, Palace Mall, and Kaneshie Market respectively. Manganese concentrations of 6.56 $\mu\text{g}/\text{kg}$, 1.21 $\mu\text{g}/\text{kg}$, 2.01 $\mu\text{g}/\text{kg}$, 3.22 $\mu\text{g}/\text{kg}$, for the Madina market, Melcom plus, Palace Mall, and Kaneshie market respectively. Results generally suggest that iron, zinc, and manganese are present in lettuce in such amounts that are detrimental to human health. The study also showed that Cadmium, Lead, and Copper are barely present in lettuce hence posing minimum to no risk to consumers, and are wholesome for consumption. This observation was consistent with findings by Sobukola et al. (2010) and Ametepey et al. (2018) who also found reported levels of heavy metals in leafy vegetables from open markets well below the allowable limits. Lettuce consumers in Accra should be concerned about the levels of iron, zinc, and manganese in lettuce purchased from open markets and supermarkets which were above the permissible/allowable limits for human consumption (Akan, 2013; J. Hu et al., 2013; Kananke et al., 2014; Kumar et al., 2009; Sharma et al., 2008, 2009)



CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The following conclusions were drawn from the research;

Risk level analysis associated with the use of Lettuce by humans indicates that the concentrations of Pb, Cu, and Cd found in them were below permissible limits, however, Mn, Zn, and Fe were found to have levels of concentration that were below the permissible limit and does not pose a potential health risk to consumers using various reference guides/standards. Even though almost all the heavy metal levels detected in this study were far below the WHO permissible limits, the essential metals detected had a relatively higher concentration. The HQ for consumers of lettuce in Accra for Cd, Pb, Zn, Fe, Mn and Cu were less than 1 as well as HI, meaning the exposed population is unlikely to suffer any negative health consequences if consumed moderately.

Therefore, a continued and sustained approach to the monitoring of these heavy and essential metals should be enforced and instilled to protect and promote the health of all who consume this Lettuce from both the open markets and the supermarkets in the Accra metropolis.

RECOMMENDATIONS

To broaden the scope of heavy metal knowledge in Lettuce, the following suggestions are made:

Heavy metal levels found in Lettuce sampled from Accra Metropolis were below the permissible limits however some essential metals (Mn, Fe, and Zn) found were above permissible limits. To protect consumers' health against metal toxicities, continuous and frequent monitoring programs for heavy metals in food commodities should be implemented in other regions.

Lettuce is necessary to provide the body with vital nutrients, and they are usually eaten raw. It is therefore recommended that Government agencies such as the FDA, GSA, and other public

health agencies should conduct periodic analyses of vegetables sold on the markets to ascertain the safety of Lettuce being sold in the country and educate the public on the effects of prolonged exposure to heavy metals.

Produce (Lettuce) sold in the open markets should be covered with plain plastic to reduce the amount of contamination due to vehicular and human activities.

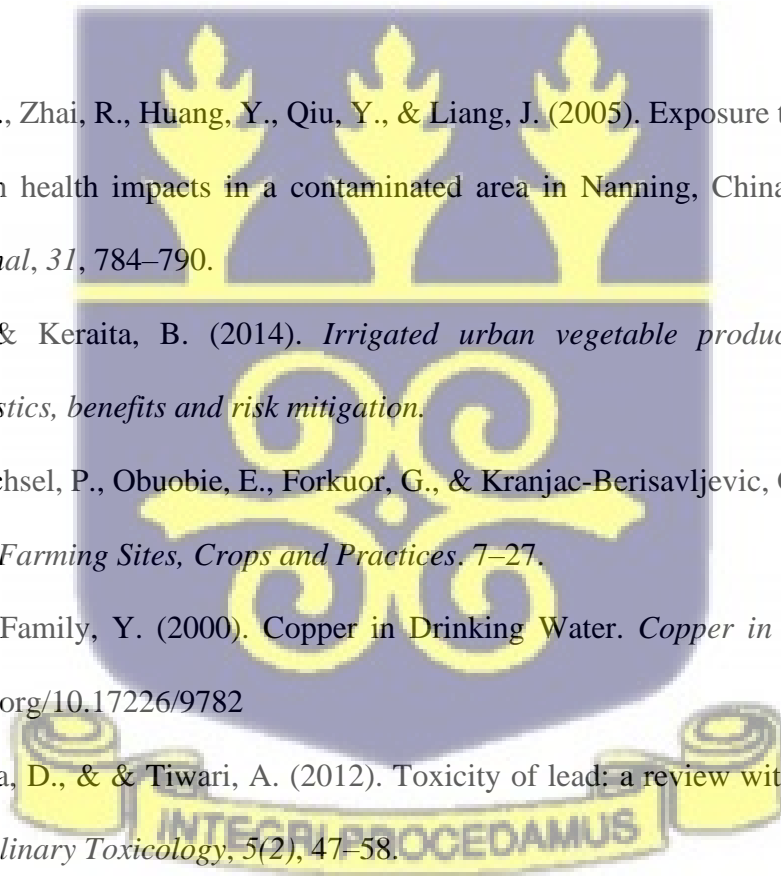
It is also recommended that further research can be done to track the supply chain of Lettuce to determine the actual sources of heavy metals in Lettuce by considering the location of production, mode of transportation, and mode of storage.



REFERENCES

- Ahmed, Alengebawy., Sara Taha, A. ., Sundas, R. Qureshi. and, & Man-Qun, W. (2021). Plants : Ecological Risks and Human Health Implications. *Toxins*, 9, 42.
- Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, 2019(Cd). <https://doi.org/10.1155/2019/6730305>
- Azupogo, F., Seidu, J. A., & Issaka, Y. B. (2018). Higher vegetable intake and vegetable variety are associated with a better self-reported health-related quality of life (HR-QoL) in a cross-sectional survey of rural northern Ghanaian women in fertile age. *BMC Public Health*, 18(1), 920. <https://doi.org/10.1186/s12889-018-5845-3>
- Affum, A. O., Osaе, S. D., Kwaansa-Ansah, E. E., & Miyittah, M. K. (2020). Quality assessment and potential health risk of heavy metals in leafy and non-leafy vegetables irrigated with groundwater and municipal-waste-dominated stream in the Western Region, Ghana. *Heliyon*, 6(12), e05829. <https://doi.org/10.1016/j.heliyon.2020.e05829>
- Ametepey, S., Cobbina, S., Akpabey, F., Duwiejuah, A., & Naangmenyele, Z. (2018). Health risk assessment and heavy metal contamination levels in vegetables from Tamale Metropolis, Ghana. *International Journal of Food Contamination*, 5. <https://doi.org/10.1186/s40550-018-0067-0>
- Amoah, P., Lente, I., Asem-hiablie, S., & Abaidoo, R. C. (1998). Quality of Vegetables in Ghanaian Urban Farms and Markets. *Quality of Vegetable, Nyanteng*, 89–103. <http://contraception.about.com/od/contraceptionglossaryef/g/Ethinyl-Estradiol-Synthetic-Estrogen.htm>.

- Barau, B. W., Abdulhameed, A., Ezra, A. G., Muhammad, M., Kyari, E. M., & Bawa, U. (2018). Heavy metal contamination of some vegetables from pesticides and the potential health risk in Bauchi, northern Nigeria. *AFRREV STECH: An International Journal of Science and Technology*, 7(1), 1–11. <https://doi.org/10.4314/stech.v7i1.1>
- Bortey-Sam, N., Nakayama, S., M., M., Ikenaka, Y., Akoto, O., Baidoo, E., Yohannes, Y., & B., ... Ishizuka, M. (2015). Human health risks from metals and metalloids via consumption of food animals near gold mines in Tarkwa, Ghana: estimation of the daily intakes and target hazard quotients (THQs). *Ecotoxicology and Environmental*, 111, 160–167.
- Bradl, H. B. (2005). Sources and origins of heavy metals. *Interface Science and Technology*, 6, 1–27.
- Cui, Y., Zhu, Y., Zhai, R., Huang, Y., Qiu, Y., & Liang, J. (2005). Exposure to metal mixtures and human health impacts in a contaminated area in Nanning, China. *Environmental International*, 31, 784–790.
- Drechsel, P., & Keraita, B. (2014). *Irrigated urban vegetable production in Ghana: characteristics, benefits and risk mitigation*.
- Danso, G., Drechsel, P., Obuobie, E., Forkuor, G., & Kranjac-Berisavljevic, G. (2014). *Urban Vegetable Farming Sites, Crops and Practices*. 7–27.
- Effects, H., & Family, Y. (2000). Copper in Drinking Water. *Copper in Drinking Water*. <https://doi.org/10.17226/9782>
- Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: a review with recent updates. *Interdisciplinary Toxicology*, 5(2), 47–58.
- Gonzalez, Y. S., Dijkxhoorn, Y., Elings, A., Glover-Tay, J., Koomen, I., van der Maden, E. C. L. J., ... & Obeng, P. (2014). *Vegetables Business Opportunities in Ghana:*



- Gupta, V. (2013). Mammalian Feces as Bio-Indicator of Heavy Metal Contamination in Bikaner Zoological Garden, Rajasthan, India. *Res. J. Animal, Veterinary and Fishery Sci*, 1(15), 10–15.
- Hedge, L., Knott, A., & Johnston, E. (2009). Dredging related metal bioaccumulation in oysters. *Mar. Pollut. Bull.* (Vol. 58, pp. 832–840).
- Huseen, H. M., & Mohammed, A. J. (2019). Heavy Metals Causing Toxicity in Fishes. *Journal of Physics: Conference Series*, 1294(6). <https://doi.org/10.1088/1742-6596/1294/6/062028>
- Ihedioha, J. N., & Okoye, C. O. B. (2013). Dietary intake and health risk assessment of lead and cadmium via consumption of cow meat for an urban population in Enugu State, Nigeria. *Ecotoxicology and Environmental Safety*, 93, 101–106.
- Jarzyńska, G., & Falandysz, J. (2011). Selenium and 17 other largely essential and toxic metals in muscle and organ meats of Red Deer (*Cervus elaphus*)--consequences to human health. *Environment International*, 37(5), 882–888.
- Johann, M. R., Leslie, A., Hoo, Fung., & Charles, N. G. (2017). Assessment of the potential health risks associated with the aluminum, arsenic, cadmium, and lead content in selected fruits and vegetables grown in Jamaica. *Elsevier*.
- Juberg, D. R. (2000). *LEAD AND HUMAN HEALTH*.
- Juberg, D. R., Ross, G. L., & Ponirovskaya, Y. (2000). Lead and Human Health. *Science*.
- Lente, I., Oforu-Anim, J., Brimah, A. K., & Atiemo, S. (2014). Heavy metal pollution of vegetable crops irrigated with wastewater in Accra, Ghana. *West African Journal of Applied Ecology*, 22(1), 41–58.
- Li, Z.; Ma, Z.; van der Kuijp, T.J.; Yuan, Z.; Huang, L. (2014). A review of soil heavy metal pollution from mines in China: Pollution and health risk assessment. *Sci. Total Environ.*, 468–469, 843–853.

La Anyane, S. (1963). *Ghana agriculture. Its economic development from early times to the middle of the twentieth century. Ghana agriculture. Its economic development from early times to the middle of the twentieth century.*

Monferran, M., V, G., L., P., , Wunderlin, D., Angeles Bistoni, M., & L, D. (2016). Potential human health risks from metals and As via *Odontesthes bonariensis* consumption and ecological risk assessments in a eutrophic lake. *Ecotoxicology and Environmental Safety*, 129, 302–310.

Nabulo, G., Black, C., R.Young, Craigon, J., & S.D., A. (2011). Does the consumption of leafy vegetables grown in peri-urban agriculture pose a risk to human health? *Environmental Pollution*, 162, 389–398.

Naser, H. A. (2013). Assessment and management of heavy metal pollution in the marine environment of the Arabian Gulf: A review. *Marine Pollution Bulletin*, 72(1), 6–13.
<https://doi.org/10.1016/j.marpolbul.2013.04.030>

Opaluwa, O. D., Aremu, M. O., Ogbo, L. O., Abiola, K. A., Odiba, I. E., Abubakar, M. M. and Nweze, N. O. (2012). Heavy metal concentrations in soil, plant leaves, and crops are grown around dumpsites in Lafiya Metropolis, Nasarawa State, Nigeria. *Advances in Applied Science Research.*, 3(2), 780–784.

Pennington, J. A. T., & Fisher, R. A. (2009). Classification of fruits and vegetables. *Journal of Food Composition and Analysis*, 22, S23–S31.
<https://doi.org/https://doi.org/10.1016/j.jfca.2008.11.012>

Premarathna, H., Hettiarachchi, G. and Indraratne, S. (2011). Trace metal concentration in crops and soils collected from intensively cultivated areas of Sri Lanka. *Sri Lanka: Pedologist*, 230–240.

- Roozbahani, M.M.; Sobhanardakani, S.; Karimi, H.; Sorooshnia, R. (2015). Natural and Anthropogenic Source of Heavy Metals Pollution in the Soil Samples of an Industrial Complex; a Case Study. *Iran. J. Toxicol*, 9, 1336–1341.
- Rudy, M. (2009). The analysis of correlations between the age and the level of bioaccumulation of heavy metals in tissues and the chemical composition of sheep meat from the region in SE Poland. *Food and Chemical Toxicology*, 47(6), 1117–1122.
- Saad, A. A. A., El-Sikaily, A., & Kassem, H. (2016). Essential, non-essential metals and human health. *Pollution Status, Environmental Protection, and Renewable Energy Production in Marine Systems, January 2016*, 87–135.
- Septembre-Malaterre, A., Remize, F., & Poucheret, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Research International*, 104, 86–99. <https://doi.org/https://doi.org/10.1016/j.foodres.2017.09.031>
- Sharma, R. K., Agrawal, M., Marshall, F. M. (2009). *Heavy metal in vegetables collected from production and market sites of the tropical urban area of India. Food Chem. Toxicol.* 47, 583–591.
- Sharma, R.K.; Agrawal, M. (2005). Biological effects of heavy metals: An overview. *J. Environ. Biol*, 26, 301–313.
- Sphie Leonie, S. (2020). *Heavy metal exposure through fish from the Korle Lagoon and Ghanaian market: implication for consumers.*
- Sun, Y., Zhou, Q., Xie, X., & Liu, R. (2010). Spatial, sources and risk assessment of heavy metal contamination of urban soils in typical regions of Shenyang, China. *Journal of Hazardous Materials*, 174(1-3).
- Vhahangwele, M., & Khathutshelo, L. (2018). *Environmental Contamination by Heavy Metals.*

Wilson, B. & Pyatt, F. B. (2007). Heavy metal dispersion, persistence, and bioaccumulation around an ancient copper mine situated in Anglesey, UK. *Ecotoxicology and Environmental Safety*, 66, 224–231.

World Health Organization. (2010). *The WHO recommended the classification of pesticides by hazard and guidelines for classification 2009*. 1–60.

Zhang, X.; Yan, L.; Liu, J.; Zhang, Z.; Tan, C. (2019). Removal of different kinds of heavy metals by novel PPG-nZVI beads and their application in simulated stormwater infiltration facility. *Appl. Sci.*, 9, 4213.

Zhou, Q., Zhang, J., Fu, J., Shi, J., & Jiang, G. (2008). *Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem*. *Anal. Chimica Acta*. 606, 135–150.

Zhuang, P., M., M. B., H., X., N., L., & Li, Z. (2009). Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Science of Total Environment*, 407, 1551–1561.

Zoroddu, M. A., Aaseth, J., Crisponi, G., Medici, S., Peana, M., & Nurchi, V. M. (2019). The essential metals for humans: a brief overview. *Journal of Inorganic Biochemistry*, 195, 120–129. <https://doi.org/https://doi.org/10.1016/j.jinorgbio.2019.03.013>

