ANALYSIS OF METALS AND PESTICIDE RESIDUES IN SELECTED BEANS SAMPLES IN PORT HARCOURT.

BY

KALU, SAMUEL NNANNA

(B.Sc. UNIPORT)

(REG NO: 20184141088)

A THESIS SUBMITTED TO

THE POST GRADUATE SCHOOL,

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc.) IN BIOCHEMISTRY

OCTOBER, 2023.

CERTIFICATION

I certify that this thesis **"ANALYSIS OF METALS AND PESTICIDE RESIDUES IN SELECTED BEANS SAMPLES IN PORT HARCOURT"** was carried out by **Kalu, Samuel Nnanna** (20184141088) in the Department of Biochemistry, Federal University of Technology, Owerri, Imo State, under the supervision of:

Prof. (Mrs) A.A. Emejulu
(Supervisor)

23/11/2023
Date

Prof. C.O. Ujowundu
(Co-Supervisor)

23/11/2023
Date

Prof. L.A. Nwaogu
(Head of Department Biochemistry)

23/11/23
Date

Prof. C.S. Alisi
(Dean, School of Biological Sciences)

23/11/23
Date

Prof. B.O. Esonu
(Dean, Postgraduate School)

Date

Prof. S.O. Eze Prof SO Eze
(External Examiner)

19/10/23
Date

DEDICATION

To my loving parents,

Surv. and Mrs Ude Kalu

ACKNOWLEDGEMENTS

My profound gratitude goes to my supervisor, Professor Mrs. A.A Emejulu for her support and contribution to the success of this work. I also thank my co-supervisor, Assoc. Prof. C.O Ujowundu for the selfless and courageous manner in which he nurtured and contributed to the success of this work.

I am grateful to all members of staff of the Department of Biochemistry, especially the Head of Department, may God bless you. My gratitude also goes to senior colleagues and friends in the faculty, particularly Mr Nmadike Ezeji-Chigbu.

My immense gratitude goes to my parents, Surv and Mrs Kalu, my brothers and sisters. I also thank my pastor, Rev Onyekachi Keshi, my friends and well-wishers for their love and various contributions to my success.

Above all, I am infinitely grateful to God almighty for His grace, mercies, guidance and protection in my life and for providing all the human and material resources I used for my research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Analysis of metals and pesticide residues were evaluated using four (4) beans (*Phaseolus vulgaris L.*) varieties. The varieties were Iron beans, Patisco beans, Brown beans and Local beans and they were grouped into raw and parboiled samples. Proximate analysis of the samples was done using standard method while metal analysis was done using Atomic Absorption Spectrometer and the analysis of pesticide residues was done using gas chromatography with mass spectrometric detector (GC - MS) after careful extraction and clean up. Results showed that all the beans samples contained metals, mercury which is a toxic metal was also detected and at a level above the maximum permissible limit of 0.0005mg/kg in all the samples. It was observed that parboiling of the beans, reduced the accumulated metals in the beans. The results of the study also showed the presence of 17 different pesticide residues in all samples of beans. 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,2-dichlorovinyl dimethyl phosphate (DDVP) were detected in all samples of beans and at levels above European Union's (EU's) MRL except in iron beans samples. Glyphosate was detected in all the samples at concentration above EU's MRL. However, pesticides in Raw iron beans such as carbofuran (0.43±0.03 mg/kg), Endosulphan (0.18±0.01 mg/kg), HCB (0.62±0.10 mg/kg) Profenos (0.55±0.04 mg/kg) and t-nonachlor (0.32±0.00 mg/kg) decreased significantly to 0.23±0.10 mg/kg, 0.09±0.01 mg/kg, 0.31±0.01 mg/kg, 0.38±0.01 mg/kg, and 0.22±0.00 mg/kg respectively in Parboiled iron beans. These results indicate reduced Health Risk Index (HRI) to pesticides of parboiled beans samples. Similar results were also recorded in other raw and parboiled beans varieties studied. Of all the samples studied, parboiled local beans had the least pesticide residue concentration. Series of soaking and washing is generally advised in preparation of beans samples. Stringent monitoring of the use of pesticides in agriculture and food storage in Nigeria should continuously be encouraged to ensure pesticide residue level does not exceed FAO/WHO limits. Parboiling of beans samples before cooking should equally be encouraged.

Keyword: Phaseolus vulgaris, heavy metals, parboiling, pesticides, health risk.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Beans (*Phaseolus vulgaris L.*) are one of the most economically and nutritionally important indigenous African grain legumes produced throughout the tropical and subtropical areas of the world. Grains, legumes or pulses are rich and low-cost sources of dietary proteins and other nutrients for a large part of world's population (Egounlety *et al*, 2003). They supplement to the lower quality cereal or root and tuber protein commonly consumed in tropical Africa (Karikari *et al*, 1999).

The bulk of beans production and consumption is in sub-Saharan Africa (SSA) particularly West and Central Africa. Nigeria produces the most quantity of cowpea grains annually at approximately 2.14 million metric tonnes (FAOStat, 2017) and consumes more than 3.0 million metric tonnes. The other major producers are Niger Republic and Burkina Faso with an average of 1.59 and 0.57 million metric tonnes, respectively.

Beans (*Phaseolus vulgaris L.*) are consumed either alone or in a combination with cereals or tubers to enhance protein value and serves as a major source of protein in the absence of sufficient animal protein for the populace. It is a subsistence legume that is very versatile and can be cooked plain or in processed dishes. In most parts of West Africa, including Nigeria cowpea beans seeds are prepared and taken in different ways, it could be cooked, made into soup or milled and mixed with onions, pepper and other ingredients fried with either palm oil or groundnut oil and eaten as "akara"or wrapped with leaves cooked and taken as "moin moin".

Nutritionally, beans (*Phaseolus vulgaris L.*) grain is more or less the same as other pulses, with a relatively low-fat content and high total protein concentration. Cowpea beans seed is considered as a nutrient dense food with low energy density. An average cowpea grain contains 23 - 32% protein (José *et al*, 2014), 50 - 60% carbohydrate (Khalid *et al.*, 2013; Kirse *et al.*, 2015) and about 1% fat in dry basis. The total protein content of cowpea beans seeds is approximately two to fourfold greater than cereal and tuber crops (Sebetha *et al*, 2014; Trehan *et al*, 2015). Moreover, compared to cereal grains, cowpea protein is a richsource of the amino acid lysine and is used as a natural complimentary food with cereals (Gonçalves *et al.*, 2016).

In agriculture, plants procure their nutrients from soil, but they do not have the ability to take up essential elements only but sometimes absorb nonessential or even toxic elements (which could be heavy metals or pesticides. Metal uptake is highly a complex process involving metal transfer from soil sap to inside cells of roots (Tangahu *et al.,* 2011). Soil sap enters the root through root hairs. Saifullah *et al.,* (2009) elaborated mechanism of metal uptake in which soil sap first enters symplast by crossing plasma membrane and then passes through cells by means of plasmodesmata. In the farm, these toxic elements (heavy metals and pesticides) get to the plants most times as a result of indiscriminate applications of fertilizers and pesticides like herbicides and insecticides. Also, there is the need to preserve the proceeds from the farm after harvest against certain pests. Thus, there is further application of pesticides on the proceeds and most times these pesticides are applied without following safe regulation limits.

Pesticides are poisons produced because they have toxic effects on one pest orthe other (Banjo *et al.,* 2010); they are intentionally applied to the environment to control unwanted organisms such as fungi, weeds, and insects (Wendie *et al.,* 2011). Organochlorine pesticides (OCPs) are chlorinated hydrocarbons used extensively from the 1940s to 1960s in agriculture, and representative compounds in this group include DDT, methoxychlor, dieldrin, chlordane, texaphene, mirex, kepone, lindane, and benzene hexachloride (BHC), all of which persist in the environment, bio-accumulate, and magnify in the food chain, and their adverse toxicity to wildlife and humans is of global concern (Jones *et al*., 1999). Most of these pesticides and their active metabolites were found to cause severe health conditions such as neurological damage, hypertension, cancer, cardiovascular diseases and skin disorders in human (Gerken *et al.,* 2001; Mansour, 2004; Parbhu *et al.,* 2009). Aside the concern of bioaccumulation of pesticides in plants, there is also the concern of heavy metal bioaccumulation.

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Fergusson, 1990). With the assumption that heaviness and toxicity are interrelated, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure (Duffus, 2002).

Although heavy metals are naturally occurring elements that are found throughout the earth's crust, most environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds (He ZL *et al.,* 2005; Goyer, 2001; Herawati *et al.,* 2000). Environmental contamination can also occur through metal corrosion, atmospheric deposition, soil

erosion of metal ions and leaching of heavy metals, sediment re-suspension and metal evaporation from water resources to soil and ground water (Nriagu, 1989).

Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation results when there is an increase in the concentration of a chemical in a biological organism over time, compared to the natural concentration of chemicals in the environment. Heavy metals may enter animals and human tissues via inhalation and diet.

Toxic elements collected in the soil can be transferred up in the food chain (soil-air-plant-animal-human).
Some metals, for instance arsenic, cadmium, chromium, cobalt, iron, lead, nickel, titanium and zinc, are known to have carcinogenic effects. Vital heavy metals are essential for organisms in certain concentrations which affect biological reactions. Therefore, these have to be taken to the bodies regularly.

According to Bojinova *et al.* (1994) beans belong to the group of crops that strongly accumulate heavy metals. Studies on heavy metals are important from public point of view, where the attention has been drawn to the necessity of measuring the accumulation of heavy metals, particularly those metals which pose serious health hazards to human (e.g Cd, Pb, Hg). In small quantities, certain heavy metals are nutritionally essential for a healthy life. Some of these are referred to as trace elements e.g. iron, copper, manganese and zinc. Human exposure to heavy metals has risen dramatically in the last 50 years, as a result of an exponential increase in the use of heavy metals in industrial processes and products. Today chronic exposure comes from chemical residues in raw foods and processed foods. In today's industrial society, there is no escaping exposure to toxic chemicals and metals.

1.2 Problem Statement

The study was designed to investigate the concentrations of heavy metals and pesticides in selected beans varieties (which are local beans, iron white beans, brown beans, and patisco beans varieties) popularly consumed in Port Harcourt, Rivers State, Nigeria. Port Harcourt, like every other settlement, has lots of beans consumers. Most of these beans' varieties are grown by farmers in the Northern part of Nigeria and outside Nigeria.

Farmers'knowledge and practices regarding certain fertilizers used in growing beans and pesticides used against the bean fly (*Ophiomyiaphaseoli*) and the associated health effects is of great public health

importance. This information is lacking among smallholder bean farmers and wholesale beans sellers in many sub-Saharan countries. This reason necessitated this research.

1.3: Aim and Objectives

1.3.1: Aim

The aim of this study was to evaluate the concentrations of heavy metals and pesticides in selected beans varieties consumed in Port Harcourt, Rivers State.

1.3.2 Objectives

The objectives of this study include:

i. To determine the concentration of the toxic metals in the samples,
ii. To determine the concentration of the essential metals in the samples,
iii. To determine pesticides and their concentration in the samples,
iv. To determine the heavy metal concentration in thesamples.

1.4: Justification and significance of the study

Beans are a common protein food source eaten in Nigeria and other countries of the world. Food consumption is one major exposure route of toxicants to humans, but few data exist for Port Harcourt, Rivers State, Nigeria. Hence, due to the unavoidable exposure of Port Harcourt residents to some food contaminants such as heavy metal and pesticides, result from this study may provide some relevant data on their quantitative levels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1: Beans

Bean (*Phaseolus vulgaris L.*), are the member of the Leguminosae, family Phaseoleae, subfamily Papilionoideae (Bressani, 1993). They have been recognized to be domesticated and originated in America on the basis of chronological, archaeological, botanical and linguistic evidences (Gepts *et al*, 1991; Papa and Gepts, 2003; Papa *et al.,* 2005) and they are now consumed in every part of the world especially by the people of low income in the developing countries (Shimelis *et al*, 2005). Beans are warm climate plants that are mainly grown in warm temperate regions. However, the beans plant is sensitive to hot weather during germination and to relative humidity and drought during flowering (Şehirali 1998).

Beans (*Phaseolus vulgarisL.),* are a major source of highly valuable plant protein and micro-nutrients (Broughton *et al*., 2003; VazPatto *et al.,* 2015), they are beneficial to the health of humans as they play certain roles in the prevention of cardiovascular disease, obesity, diabetes mellitus and cancer (Messina, 2014; Bitocchi *et al*., 2016; Jenkins, 2007; Chung *et al.*, 2008), they aid in biological nitrogen fixation in the soil, and also control weeds (Bitocchi *et al.,* 2016). In areas such as Mexico, South and Central American and in most African countries, beans are consumed as basic foods (Leterme, 2002; Luthria *et al*, 2006).

Most often, beans are mainly used as dry seeds (Lin *et al*., 2008). The important classes of dry beans include, haricot beans (Shimelis *et al*, 2005), red kidney beans (Choung *et al*., 2003), black beans (Aparicio-Fernandez *et al*., 2006), Mexican beans (Hosfield *et al.*, 2004), pinto beans, tirga beans (Amir *et al.*, 2006), great northern beans, navy beans and pink beans (Kelly *et al.*, 2003; Luthria *et al*, 2006). In this work, four beans varieties (*Phaselous vulgaris L.*) were used and they are: patasco, iron, brown, and local beans.

Nutritionally, beans are good sources of proteins, and their proteins can be 2-3 times that of cereal grains, (Siddiq *et al.*, 2010). Beans also have high dry matter content, and also contain high amounts of starch, dietary fibre, minerals and vitamins (Kutos *et al*., 2003; Costa *et al*., 2006). Furthermore, beans also contain rich variety of phytochemicals, antioxidant activity and an extensive array of flavonoids such as anthocyanins, flavonoids, proanthocyanidins, flavonols, phenolic acids and isoflavones (Choung *et al*, 2003; Aparicio-Fernadez *et al*. 2005b; Lin *et al.*, 2008; Granito *et al.*, 2008).

2.2: Nutritional Constituents of Beans

2.2.1: Carbohydrates

Carbohydrates are the major nutritional component of beans (*Phaseolus vulgaris L.*) found in variable amounts, accounting up to 50-60% of the dry matter (Vargas-Torres *et al.,* 2004; Reynoso-Camacho *et al*., 2006; Ovando-Martinez *et al*., 2011). Starch and non-starch polysaccharides constitute the major components of these carbohydrates along with little amounts of carbohydrate derivate such as oligosaccharides (Bravo *et al.,* 1998; Reynoso- Camacho *et al.,* 2006). Carbohydrate contents of different beans varieties are shown in Table 2.1. Among all carbohydrates, starch is the major carbohydrate found in beans.

Table 2.1. Composition of some beans varieties(Imran *et al.,* 2014)

Source	Carbohydrates	Protein	Lipids	Ash	References
Common beans	54.3-59.9	20.9-22.1	2.49-2.52	3.80-4	Costa et al., 2006
Pinto beans	57.3	30.1	1.8	4.9	Amir et al., 2006
Black beans	67.83-68.09	25.66-25.93	1.52-1.59	4.65-4.71	Berrios et al., 1999
Navy beans	54.30	18.15	2.63	4.14	Sai-Ut et al., 2009

2.2.2: Protein

Beans are an excellent source of dietary proteins and they play an important role in human nutrition by complementing other foods such as rice and other cereals (Butt *et al.,* 2010). Consuming beans along with cereals offers the best strategy to combat problem of protein malnutrition (Batista *et al.,* 2011). Bean seeds contain between 20 and 25% proteins, much of which is made up of the storage protein phaseolin. Phaseolin is a major determinant of both quantity and nutritional quality of proteins in bean seeds (Bliss *et al.*, 1983; Gepts *et al.*, 1984). Like other seed proteins of the legume family, phaseolin is deficient in sulphur-containing amino acids such as methionine. Seed proteins of cereals generally contain sufficient sulphuric amino acids but are themselves deficient in other essential amino acids such as lysine. Combined consumption of cereals and legumes generally alleviates these mutual deficiencies ensuring a balanced diet when cereals and legumes are consumed in the ratio of 2:1. Complementary food strategies such as this are in practice in Latin America and Eastern and Western Africa, Brazil, and most parts of the Asia (Broughton *et al.*, 2003; Siddiq *et al.*, 2010).

2.2.3: Lipids

Lipid content in beans is approximately 2% (Table 2.1) with good composition of exogenic unsaturated fatty acids (Mabaleha *et al.*, 2004). The major lipid components in beans are phospholipids and triacylglycerols, while minor amounts of diacylglycerols, hydrocarbons, steryl esters, and hydrocarbons may also present. These lipids may also take the form of Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) in beans (Yoshida *et al.*,2005).

2.2.4: Minerals and vitamins

Beans are an important source of micronutrients like minerals and vitamins and are considered superior to cereals as a source of micronutrients (Welch *et al.*, 2000). Beans like other legumes have higher micronutrients than cereals, first because legumes have a higher initial content of minerals, and second since many cereals are polished before eating (like for the production of white rice or wheat flour for white bread, etc.). As a significant proportion of the minerals are found in the seed coat (or bran) they are discarded during processing. Most legumes, including common beans, are consumed whole. As a result their mineral content is conserved.

Beans have the highest level of mineral content than other legumes. They are a vital source of minerals such as iron, zinc, copper, phosphorous, and aluminium, while other minerals are also found in considerable amounts (Broughton *et al.*, 2003; Shimelis *et al.*, 2005). When compared to other legumes, beans have the highest iron level with a range of 62.0–150 μg/g, which is mostly present in nonheme form (Vadivel *et al.*, 2000).

Beans are also vital source of vitamins and the vitamin contents vary in the different varieties of beans (Augustin *et al.*, 2000). Beans are essential source of folate, tocopherols, thiamine, riboflavin, niacin, biotin, and pyridoxaimine (Broughton *et al.*, 2003; Campos-Vega *et al.*, 2010).

2.3: Heavy metals

Heavy metal is any metal or metalloid of environmental importance (Lee *et al.*, 2017). It has since been extended to some other similarly toxic metal or metalloid, irrespective of density, such as arsenic, chromium, cobalt, nickel, copper, zinc, arsenic, selenium, silver, cadmium, antimony, mercury, thallium and lead are commonly found to be heavy metals (Duffus *et al.*, 2002; Liu *et al.*, 2018).In general, heavy metals refer to a group of metals and metalloids with an atomic density greater than 4 g/cm^3 or 5 times or more than water. Heavy metal is often referred to as trace elements as they exist in minute concentrations in biological systems (Duruibe *et al.*, 2007).They are a significant class of contaminants that affect the environment.

Heavy metal contamination in the environment is a serious problem, because of the toxicity, persistence, and bio-accumulative nature of these metals.

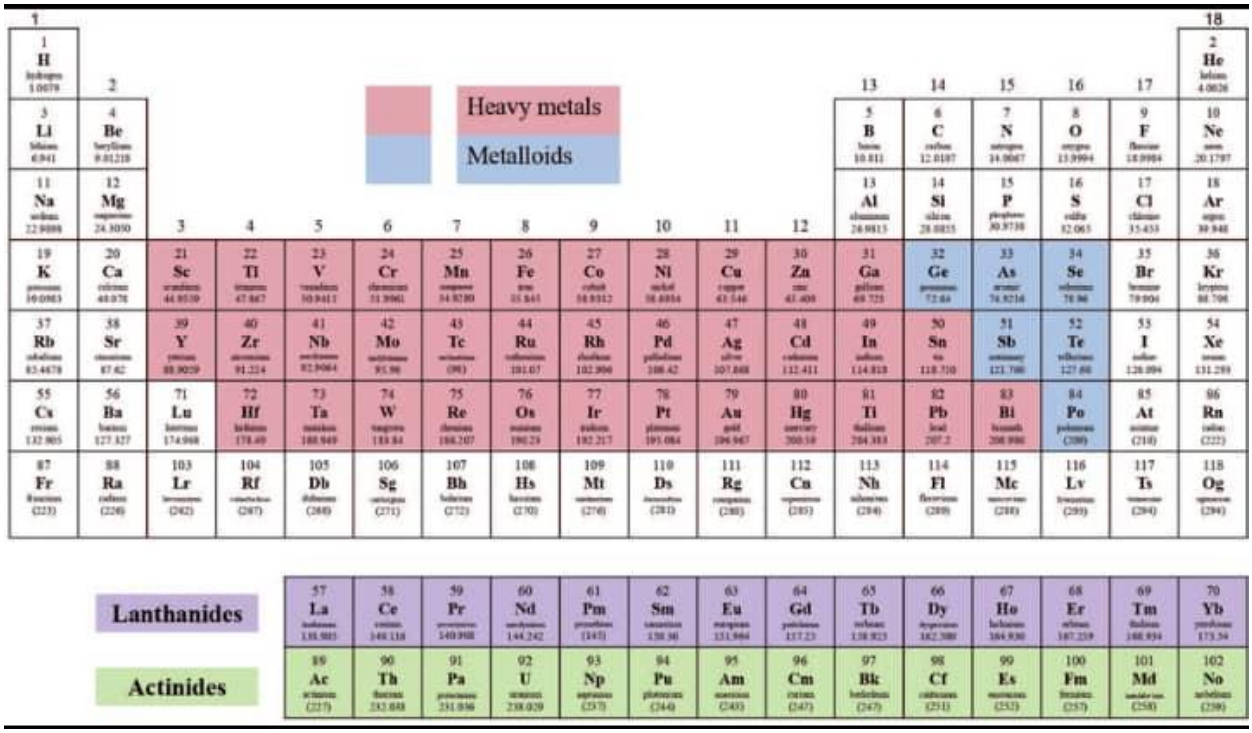

Figure 2.1: Position of heavy metals in periodic table.(Mohammad *et al.*, 2021)

2.4 Sources of Heavy Metals

Heavy metals may come from natural and anthropogenic processes and end up in various environmental compartments (soil, water, air and their interface). Figure 2.2 gives information on natural and anthropogenic sources of heavy metals.

2.4.1 Natural Sources

Natural emissions of heavy metals occur under numerous and certain environmental conditions. Volcanic eruptions, sea-salt sprays, forest fires, rock weathering, biogenic sources and particles of wind-borne soil are included in these pollutants. The release of metals from their endemic spheres to different environmental compartments will lead to natural weathering processes. In the form of hydroxides, oxides, sulphides, sulphates, phosphates, silicates and organic compounds, heavy metals can be found. Lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), arsenic (As), mercury (Hg), selenium (Se), zinc (Zn) and copper (Cu) are the most popular heavy metals. The above-mentioned heavy metals can be present in traces, but even in such amounts they still cause health problems to humans and other mammals.

2.4.2 Anthropogenic Sources

Industries, irrigation, drainage, mining and metallurgical processes, as well as runoff, also contribute to the release of pollutants into various compartments of the ecosystem. For certain metals, anthropogenic heavy metal processes have been noted to go beyond natural fluxes. In wind-blown dust, metals naturally released are mainly from industrial areas. Car exhaust that releases lead; smelting that releases arsenic, copper and zinc; insecticides release arsenic and the burning of fossil fuels that release nickel, vanadium, mercury, selenium and tin are some essential anthropogenic causes that contribute significantly to heavy metal pollution in the environment. Because of the everyday manufacture of products to meet the demands of the large population, human activities have been found to contribute more to environmental pollution.

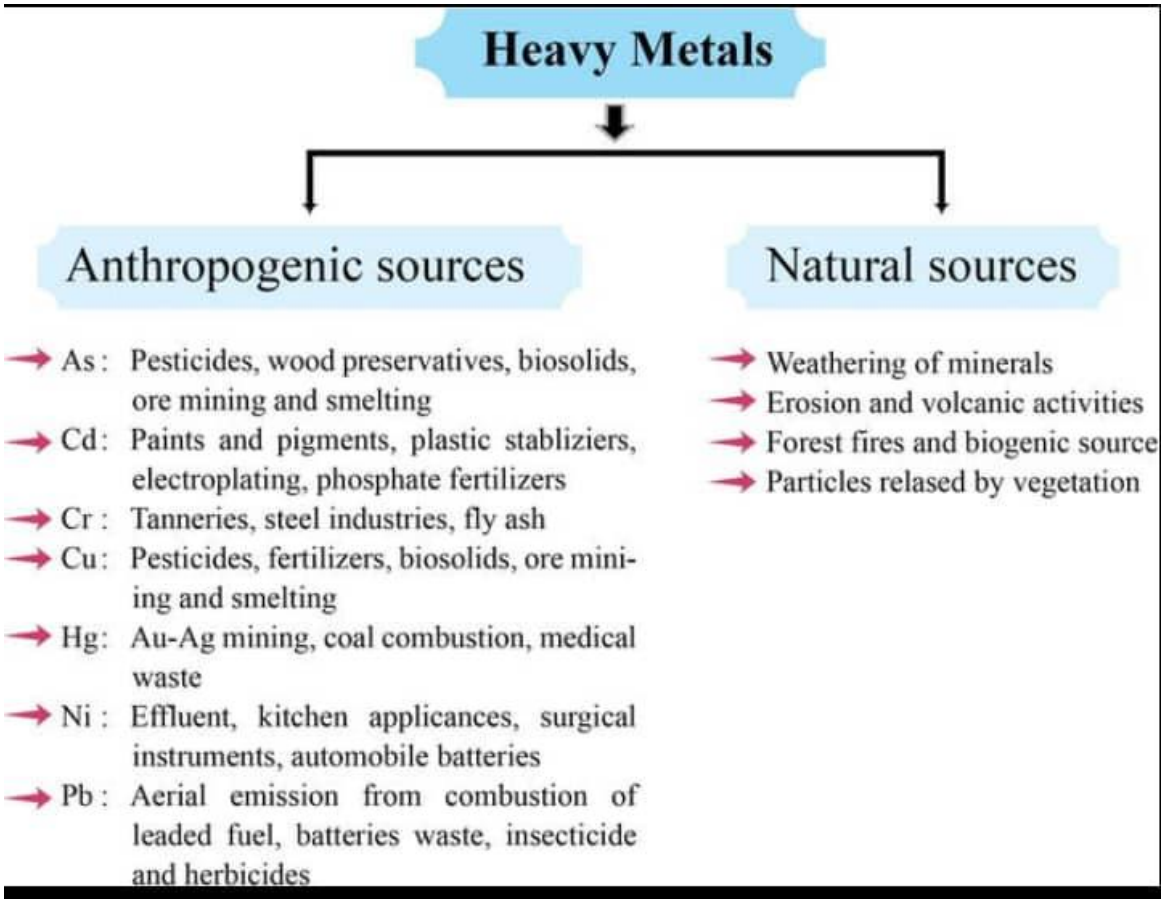

Figure 2.2: Sources of heavy metals in the environment.(Mohammad*etal.*,2021)

2.5 Metal uptake by Plants

Plant contamination with heavy metals may occur through water-plant, soil-plant, and air-plant interfaces; however, soil-plant interface is the major source of plant metal accumulation. It has been recorded in several studies that there is a strong relationship between heavy metals in soil and food crops (Bini *et al.*, 2012; Khan *et al.* 2015). In general, the bioavailability of heavy metals depends on the amount of exchangeable metals in soil. Carbonate-bound and exchangeable metals are more bioavailable than other fractions (Wong *et al.*, 2002). The bioavailability of heavy metals in plant varies for different plant organs, and the absorption and bioaccumulation rate is highest for roots as compared to other parts (Verma *et al.*, 2003). Similarly, solubility and soil type also affect the metal uptake by plants (Castro *et al.*, 2009). The mean heavy metal uptake by plants increases as the contents of these metals increase in the soil environment (Chaves *et al.*, 2011).

2.6: Metals and Their Health Effects

2.6.6: Zinc

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilises the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. Other than iron, zinc is in abundant trace chemical in the body and is a constituent of every cell and an essential food element and a coenzyme in cells, required in small amounts, for example, in men, 15 - 20 mg/day (WHO, 2008).

Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms.

Zinc plays a central role in the immune system, affecting a number of aspects of cellular and Humoral immunity (Shankar *et al.*,1998).

Dietary sources of zinc include; lean red meat, whole-grain cereals, pulses, processed cereals with low extraction rates, polished rice, fish, roots and tubers, green leafy vegetables and legumes.

Effects of Zinc Toxicity on Humans

Zinc deficiency has far-reaching implications, ranging from damaged neuropsychological functions, retarded or stunted development, hindered reproduction, immune diseases, dermatitis, poor wound healing, fatigue, anorexia and baldness (FAO, 1998). The clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioural changes (Hambidge, 1987).

Adverse effects of long-term high-dose zinc use include suppressed immunity, decreased high-density lipoprotein cholesterol levels, anaemia, copper deficiency, and possible genitourinary complications.

Zn toxicity can also induce a deficiency in Cu or iron owing to a competitive interaction among these elements for intestinal transport systems.

2.6.2: Mercury

During the last two centuries, emissions of toxic heavy metals have risen tremendously, significantly exceeded emissions from natural sources such as volcanic eruptions and from earth movements for practically all metals. In the case of Hg, it is mainly released by chloro-alkali plants (Biester *et al.*, 2002) and coal-fired power plants (Novoa-Munoz *et al.*, 2008).Even in the absence of direct exposure, toxic elements represent a hazard to human populace, because the food chain connects the elements of soil and air with humans. Uptake and accumulation by crop plants represents the main entry pathway for potentially health-threatening toxic metals into human and animal food. Over-seeing agencies (both regulatory and health) have placed high priorities on monitoring, evaluating and reducing risk to humans and wildlife from exposure to toxic chemicals including Hg (Rada *et al.*, 1990). Mercury ranks third on the CER-CLA list of substances in terms of the risk that it poses to human morbidity and mortality (ATSDR, 1999). The exposure to Hg both directly and through the food chain is of significant concern and has on more than one occasion resulted in remedial response activation in regions of the US and in other parts of the world (Driscoll *et al.*, 1994; Hanisch, 1998)

Major sources of mercury pollution include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater (Chen *et al.*, 2012).

Mercury exists mainly in three forms: metallic elements, inorganic salts and organic compounds, each of which possesses different toxicity and bioavailability. The metallic mercury is a naturally occurring metal which is a shiny silver-white, odourless liquid and becomes colourless and odourless gas when heated. Mercury is very toxic and exceedingly bio-accumulative. These forms of mercury are present widely in water resources such as lakes, rivers and oceans where they are taken up by the microorganisms and get transformed into methyl mercury within the microorganism, eventually undergoing bio-magnification causing significant disturbance to aquatic lives. Consumption of this contaminated aquatic animal is the major route of human exposure to methyl mercury (Trasande *et al.*, 2005).

Emissions of both elemental and inorganic mercury can occur from coal-fired power plants, burning of municipal and medical waste, and from factories that use mercury. Inorganic mercury can also enter water or soil from the weathering of rocks that contain inorganic mercury salts, and from factories or water treatment facilities that release water contaminated with mercury. This toxic metal is absorbed by the plant when it gets to the agricultural land.

Effects of Mercury Toxicity on Humans

Mercury is considered the most toxic heavy metal in the environment. Mercury poisoning is referred to as acrodynia or pink disease. Mercury is released into the environment by the activities of various industries such as pharmaceuticals, paper and pulp preservatives, agriculture industry, and chlorine and caustic soda production industry (Morais *et al.*, 2012). Mercury has the ability to combine with other elements and form organic and inorganic mercury. Exposure to elevated levels of metallic, organic and inorganic mercury can damage the brain, kidneys and the developing foetus (Alina *et al.*, 2012). Mercury is present in most foods and beverages in the range <1 to 50 μg/kg. In marine foods it is often seen at higher levels. Organic mercury can easily permeate across the biomembranes and since they are lipophilic in nature, mercury is present in higher concentrations in most species of fatty fish and in the liver of lean fish (Reilly, 2007). Micro-organisms convert the mercury present in soil and water into methyl mercury, a toxin which can accumulate with fish age and with increasing trophic levels.

Increased exposure of mercury can alter brain functions and lead to shyness, tremors, memory problems, irritability, and changes in vision or hearing. Exposure to metallic mercury vapours at higher levels for shorter periods of time can lead to lung damage, vomiting, diarrhoea, nausea, skin rashes, increased heart rate or blood pressure. Symptoms of organic mercury poisoning include depression, memory problems, tremors, fatigue, headache, hair loss, etc. Since these symptoms are common also in other conditions, it may be difficult to diagnose such cases (Martin *et al.*, 2009).

2.6.3: Lead

Globally it is an abundantly distributed, important yet dangerous environmental chemical (Mahaffey, 1990a). Its important properties like softness, malleability, ductility, poor conductibility and resistance to corrosion seem to make difficult to give up its use. Due to its non-biodegradable nature and continuous use, its concentration accumulates in the environment with increasing hazards.

Human exposure to lead and its compounds occurs mostly in lead related occupations with various sources like leaded gasoline, industrial processes such as smelting of lead and its combustion, pottery, boat building, lead based painting, lead containing pipes, battery recycling, grids, arm industry, pigments, printing of books, etc.

Though its widespread use has discontinued in many countries of the world, it is still used in many industries like car repair, battery manufacturing and recycling, refining, smelting, etc. Lead is a highly poisonous metal affecting almost every organ in the body. Of all the organs, the nervous system is the mostly affected target in lead toxicity, both in children and adults. The toxicity in children is however of a greater impact than in adults. This is because their tissues, internal as well as external, are softer than in adults.

The major source of environmental lead is metal smelting (Caussy *et al.*, 2003), but agriculture, industry, and urban activities are also important sources of Pb pollution (Marchiol *et al.*, 2004)

Effects of Lead Toxicity on animals

Once lead enters the body, it is distributed to organs such as the brain, kidneys, liver and bones. The body stores lead in the teeth and bones, where it accumulates over time. Lead stored in bone may be released into the blood during pregnancy, thus exposing the foetus.

Infants and young children are especially sensitive to even low levels of lead, which may contribute to behavioural problems, learning deficits and lowered IQ (Rubin *et al.*, 2008). Long-time exposure to lead has been reported to cause anaemia, along with an increase in blood pressure, and that mainly in old and middle aged people. Severe damage to the brain and kidneys, both in adults and children, were found to be linked to exposure to heavy lead levels resulting in death. In pregnant women, high exposure to lead may cause miscarriage. Chronic lead exposure was found to reduce fertility in males (Sokol *et al.*, 1991). Blood disorders and damage to the nervous system have a high occurrence in lead toxicity.

2.6.4: Arsenic

Arsenic is a metalloid, ubiquitously available in the earth's environment and considered to be a global health risk factor. Essentially, arsenic concentrates in earth's crust, bedrocks and leaches gradually into the drinking water (Vahter, 2008). One of the most stable forms of arsenic is 75As isotope and −3, 0, +3 and +5 are some of the common valence states of arsenic. Being a metalloid, arsenic exists in various allotropic forms such as elemental, sulphide and carbonate form (Henke, 2009). Exposure to inorganic arsenic through consumption of contaminated food, water, air and occupational exposure but not organic arsenic (majorly seafood such as fish, oysters, prawns, mussels, etc.) leads to serious effects on human health. Low doses and long term exposures of arsenic leads to a range of medical complications termed as "Arsenicosis" (McCarty *et al.*, 2011).

Arsenic is highly toxic in its inorganic form. People are exposed to elevated levels of inorganic arsenic through drinking contaminated water, using contaminated water in food preparation and irrigation of food crops, industrial processes, eating contaminated food and smoking tobacco.

Arsenic in soil results from human activities including pesticide use, mining and ore processing operations, operating coal burning power plants, and waste disposal. Sites of former tanneries, which make leather from animal hides, have large amounts of arsenic in the soil.

Effects of Arsenic Toxicity in Humans

Long-term exposure to inorganic arsenic, mainly through drinking-water and food, can lead to chronic arsenic poisoning. Skin lesions and skin cancer are the most characteristic effects. It can also lead to cancer of the bladder, lung, and prostate.

2.6.5: Cadmium

Naturally occurring Cd levels are extremely low. Reports indicate that Cd concentrations in non-contaminated soil vary from 0.01 to 5 mg kg^{-1} of soil (Kabata-Pendias, 2004); however, fertilizers produced from phosphate ores constitute a major source of spread cadmium pollution (Chen *et al.*, 2007). In addition, the inappropriate disposal of Cd containing wastes has increased its emission in populated areas around the world (Jarup, 2003). Although Cd is used in a number of industrial applications, the main source of Cd intake is through smoking and food (Jarup, 2003). Cadmium is an element that represents serious environmental hazards because it can be absorbed via the alimentary tract, penetrates through placenta during pregnancy, and damages membranes and DNA (Kabata-Pendias, 2004)

Effects of Cadmium Toxicity in Human

Chronic cadmium exposure produces a wide variety of acute and chronic effects in humans. Cadmium accumulates in the human body and especially in the kidneys.

Acute: pneumonitis (oxide fumes).

Chronic: proteinuria, lung cancer, osteomalacia.

Other effects: kidney and bone damage. Inhibition of progesterone and estradiol. Alterations in uterus, ovaries and oviduct (Massanyi *et al.*, 2007). Progesterone synthesis of ovaries (Zhang *et al.*, 2007). Endocrine disruption Henson and Chedrese (2004). Act as estrogen in breast cancer (Brama *et al.*, 2007). Excess risk of cardiovascular mortality (Jarup, 2003). It can also lead to hypercalciuria, formation of stones in the kidney, lung cancer and prostate cancer.

2.6.6: Chromium

Chromium (Cr) is considered to be one of the most detrimental elements to the environment. It occurs naturally as chromite ($FeCr_2O_4$) in ultramafic and serpentine rocks and in complexes with other heavy metals in minerals like crocoite (PbCrO4), bentorite $Ca_6\ (Cr,Al)_2(SO_4)_3$ and tarapacaite (K_2CrO_4), among others (Babula *et al.*, 2008). Besides its natural occurrence, Cr is also released to the environment by several industrial processes including electroplating, tanning, polishing, painting, pigment manufacture, and wood preservation (Bluskov *et al.*, 2005). The most stable and common

oxidation states of Cr are trivalent (Cr (III)) and hexavalent (Cr(VI)). Hexavalent Cr is the most toxic species because it has a high oxidizing potential, solubility, and mobility across the membranes in living organisms and through the environment (Marqués *et al.*, 1998). Trivalent Cr is relatively insoluble in water and tends to form hydroxide precipitates with Fe at typical ground water pH values. However, at high concentrations of oxygen or Mn oxides, Cr (III) can be oxidized to Cr(VI) (Bluskov *et al.*, 2005).

Chromium enters plants by reduction and/or complexation with root exudates, such as organic acids, which increase the solubility and mobility of Cr through the root xylem (Bluskov *et al.*, 2005).

Effects of Chromium Toxicity in Humans

Chronic cadmium exposure produces a wide variety of acute and chronic effects in humans. These effects include:

Acute: gastrointestinal hemorrhage, hemolysis, acute renal failure (Cr6+ ingestion).

Chronic: pulmonary fibrosis, lung cancer (inhalation), liver, and kidney cancer producer.

2.6.7: Selenium

Selenium has been implicated in the protection of body tissues against oxidative stress, maintenance of defences against infection, and modulation of growth and development. Approximately 30 percent of tissue selenium is contained in the liver, 15 percent in kidney, 30 percent in muscle, and 10 percent in blood plasma.

Selenium (Se) is an essential micronutrient for humans and animals, but lead to toxicity when taken in excessive amounts.

Selenium occurs naturally in sedimentary rocks formed during the carboniferous to quaternary period (White *et al.*, 2004). Worldwide, average Selenium concentration in soils is 0.4 mg/Kg however, in seleniferous soils elevated levels of Se (>2–5000 mg/Kg) are found (Hartikainen, 2005). The occurrence of Selenium in soil depends upon type of soil, organic matter and rainfall (Sors *et al.*, 2005).

Selenium exists as inorganic and organic forms in nature. Inorganic forms are selenate ($SeO^{2-}4SeO_4^{2-}$), selenite ($SeO^{2-}3SeO_3^{2-}$), selenide (Se^{2-}), elemental Se, and the major organic forms are SeCys and SeMet (Sors *et al.*, 2005; Bodnar *et al.*, 2012; Wu *et al.*, 2015).

In animals, Selenium acts as an antioxidant and helps in reproduction, immune responses, thyroid hormone metabolism. About 30 selenoproteins have been identified in animals, which play important roles in antioxidant defence, DNA synthesis, reproduction, immune response, formation of thyroid hormones.

Although, Se performs in variety of functions, its antioxidant and anti-cancerous properties are of primary concern for mankind (Reid *et al.*, 2008; Wallace *et al.*, 2009; Hatfield *et al.*, 2014).

Selenium acts as the catalytic centre of several selenoproteins, such as glutathione peroxidase (GSHPx), thioredoxinreductase, and iodothyronine-deiodinases, hence, it is important in the scavenging of free radicals, protection against oxidative stress, strengthening of immune system etc. (Méplan, 2011; Kaur *et al.*, 2014).

WHO has recommended 50–55 μg/day Se in human diet all over the world (WHO, 2009; Malagoli *et al.*, 2015; Wu *et al.*, 2015).In humans, Se deficiency occurs when dietary intake of Se is (<40 μg/day) and chronic toxicity is observed above levels of (>400 μg/day) (Winkel *et al.*, 2012).

Effects of Selenium Toxicity in Human

Selenium toxicity lead to a condition called selenosis i.e., garlic odour of the breath, gastrointestinal disorders, hair loss, sloughing of nails, and neurological damage. In extreme selenosis cirrhosis of the liver, pulmonary oedema, or even death can occur.

2.6.8: Iron

The average quantity of iron in the human body is about 4.5 grams (about 0.004 percent), of which approximately 65 percent is in the form of haemoglobin, which transports molecular oxygen from the lungs throughout the body; 1 percent in the various enzymes that control intracellular oxidation; and most of the rest stored in the body (liver, spleen, bone marrow) for future conversion to haemoglobin. Red meat, egg yolk, carrots, fruit, whole wheat, and green vegetables contribute most of the 10–20 milligrams of iron required each day by the average adult.

Iron is a mineral that is naturally present in many foods, added to some food products, and available as a dietary supplement. Iron is an essential component of haemoglobin, an erythrocyte (red blood cell) protein that transfers oxygen from the lungs to the tissues (Wessling-Resnick *et al.*, 2014). As a

component of myoglobin, another protein that provides oxygen, iron supports muscle metabolism and healthy connective tissue (Aggett *et al.*, 2012). Iron is also necessary for physical growth, neurological development, cellular functioning, and synthesis of some hormones (Murray-Kolbe *et al.,* 2010).

Most of the 3 to 4 grams of elemental iron in adults is in haemoglobin. Much of the remaining iron is stored in the form of ferritin or hemosiderin (a degradation product of ferritin) in the liver, spleen, and bone marrow or is located in myoglobin in muscle tissue (Wessling-Resnick *et al.,* 2014; Institute of Medicine, 2001). Humans typically lose only small amounts of iron in urine, faeces, the gastrointestinal tract, and skin. Losses are greater in menstruating women because of blood loss.

The richest sources of hemi iron in the diet include lean meat and seafood (Dietary Guidelines for Americans, 2015). Dietary sources of non-hemi iron include nuts, beans, vegetables, and fortified grain products. The Recommended Dietary Allowances (RDAs) for Iron for adult male is 8mg/kg and 18mg/kg for adult female (Institute of Medicine, 2001)

Effects of Iron Toxicity in Human

Iron toxicity lead to dizziness, low blood pressure and a fast or weak pulse, headache, fever, shortness of breath and fluid in the lungs, a greyish or bluish colour in the skin, jaundice (yellowing of the skin due to liver damage), seizures, liver failure and death if the individual is not treated.

2.6.9: Copper

Copper, an essential mineral, is naturally present in some foods and is available as a dietary supplement. It is a cofactor for several enzymes (known as “cuproenzymes”) involved in energy production, iron metabolism, neuropeptide activation, connective tissue synthesis, and neurotransmitter synthesis (Collins *et al.*, 2014; Prohaska *et al.*, 2012; Institute of Medicine, 2001).It is found in all body tissues and plays a role in making red blood cells and maintaining nerve cells and the immune system.

Copper is also involved in many physiologic processes, such as angiogenesis; neuron-hormone homeostasis; and regulation of gene expression, brain development, pigmentation, and immune system functioning (Collins *et al.*, 2014).

Most copper in the body is found in the liver, brain, heart, kidneys, and skeletal muscle. Sufficient copper in the diet may help prevent cardiovascular disease and osteoporosis, too.

Copper is found in a wide variety of foods. Good sources include: oysters and other shellfish, whole grains, beans, potatoes, yeast, dark leafy greens, cocoa, dried fruits, black pepper, kidneys, liver, cashews and almonds

The Recommended Dietary Allowances (RDAs) for Copper (Institute of Medicine, 2001) is 900mcg/kg for adults.

Effects of Copper Toxicity in Human

Copper toxicity produces a wide variety of effects in humans. Signs of copper toxicity include: nausea, vomiting, diarrhoea, and stomach pain, headache, dizziness, weakness.

More serious effects are rare, but they include: cirrhosis and jaundice abnormalities in red blood cells and heart problems. Increased serum copper levels have been linked with a higher risk of cardiovascular disease.

Chronic exposure to high levels of copper can result in liver damage and gastrointestinal symptoms (e.g., abdominal pain, cramps, nausea, diarrhoea, and vomiting) (Institute of Medicine, 1998; National Research Council Committee on Copper in Drinking Water, 2000).

2.6.10: Calcium

Calcium as a nutrient is most commonly associated with the formation and metabolism of bone. Over 99 percent of total body calcium is found as calcium hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) in bones and teeth, where it provides hard tissue with its strength. Calcium in the circulatory system, extracellular fluid, muscle, and other tissues is critical for mediating vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signalling, and hormonal secretion. Bone tissue serves as a reservoir for and source of calcium for these critical metabolic needs through the process of bone remodelling.

Almost all calcium in the body is stored in bones and teeth, giving them structure and hardness. The body needs calcium for muscles to move and for nerves to carry messages between your brain and

every part of your body. Calcium also helps blood vessels move blood throughout your body and helps release hormones that affect many functions in your body.

Sources of calcium include: milk, cheese and other dairy foods, green leafy vegetables - such as curly kale, okra but not spinach (spinach does contain high levels of calcium but the body cannot digest it all), soya drinks with added calcium, bread and anything made with fortified flour, fish where you eat the bones - such as sardines and pilchards

The Recommended Dietary Allowances (RDAs) for calcium for adults is 1000 mg/kg

Effects of Calcium Toxicity in Humans

Although excess intake of calcium is almost never due to calcium intake from foods, the use of calcium supplements (including the voluntary fortification of a range of foods that are not naturally sources of calcium) has increased (Ricci *et al.,* 1998; Riedt *et al.*, 2005), and excess calcium intake may occur as a result of high intake from calcium supplements. Excessive calcium intake can lead to the following anorexia, weight loss, polyuria, heart arrhythmias, fatigue, and soft tissue calcifications (Jones, 2008). It can also cause renal insufficiency, vascular and soft tissue calcification including calcinosis leading to nephrocalcinosis, and nephrolithiasis.

High levels of calcium in the blood and urine can cause poor muscle tone, poor kidney function, low phosphate levels, constipation, nausea, weight loss, extreme tiredness, frequent need to urinate, abnormal heart rhythms, and a high risk of death from heart disease. However, high levels of calcium in the blood and urine are usually caused by a health condition such as high levels of parathyroid hormone or cancer, not by high calcium intakes.

2.6.11: Magnesium

Magnesium (Mg^{2+}) has several functions in the human body. It acts as a cofactor for more than 300 enzymes, regulating a number of fundamental functions such as muscle contraction, neuromuscular conduction, glycemic control, myocardial contraction, and blood pressure (Bertinato *et al.,* 2015; Grober *et al.*, 2015).

Moreover, magnesium also plays a vital role in energy production, active trans membrane transport for other ions, synthesis of nuclear materials, and bone development (Grober *et al.*, 2015).Magnesium antagonizes calcium and functions as a signal transducer as well.

Recommended Daily Allowance for magnesium is 420 mg for adult males and 320 mg for adult females, respectively (Baaij *et al.*, 2015).

Dietary sources of magnesium include legumes, whole grains, vegetables (especially broccoli, squash, and green leafy vegetables), seeds, and nuts (especially almonds). Other sources include dairy products, meats, chocolate, and coffee. Water with a high mineral content, or "hard" water, is also a source of magnesium. Magnesium is naturally present in many foods, including green leafy vegetables, whole grains, beans, nuts, and milk.

Effects of Magnesium Toxicity in Humans

Hypermagnesemia is a rare but serious electrolytic disorder, which can be fatal if not recognized and treated promptly. Hypermagnesemia occurs primarily in patients with acute or chronic kidney disease, but other pathological conditions, such as hypothyroidism and especially cortico-adrenal insufficiency, and the effect of various drugs, especially in already compromised patients, can allow the establishment of this condition.

2.6.12: Manganese

Manganese (Mn) is an essential element in the human body that is mainly obtained from food and water. Manganese is absorbed through the gastrointestinal tract and then transported to organs enriched in the mitochondria (in particular the liver, pancreas, and pituitary) where it is rapidly concentrated (Deng *et al.*, 2013). Furthermore, Manganese is involved in the synthesis and activation of many enzymes (e.g., oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases); metabolism of glucose and lipids; acceleration in the synthesis of protein, vitamin C, and vitamin B; catalysis of hematopoiesis; regulation of the endocrine; and improvement in immune function (Aschner *et al.*, 2005). Moreover, Manganese metalloenzymes including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and Manganese superoxide dismutase (MnSOD) also contribute to the metabolism processes listed above and reduce oxidative stress against free radicals.

Manganese is a component or activator of some enzymes, mostly antioxidants, and plays an important role in metabolisms of carbohydrates and lipids, even in maintaining the normalization of the synthesis and secretion of insulin as well.

Dietary sources of manganese include: Nuts; such as almonds and pecans, beans and legumes, such as lima and pinto beans, oatmeal and bran cereals, whole wheat bread, brown rice, leafy green vegetables; such as spinach; fruits, such as pineapple and acai, dark chocolate

Effects of Toxicity of Manganese in Human

Manganese toxicity can result in a permanent neurological disorder known as manganism with symptoms that include tremors, difficulty walking, and facial muscle spasms. These symptoms are often preceded by other lesser symptoms, including irritability, aggressiveness, and hallucinations. In addition to neural damage, reproductive and immune system dysfunction, nephritis, testicular damage, pancreatitis, lung disease, and hepatic damage can occur with manganese toxicity

2.6.13: Sodium

Sodium is an essential nutrient involved in the maintenance of normal cellular homeostasis and in the regulation of fluid and electrolyte balance and blood pressure (BP). Its role is crucial for maintaining ECF volume because of its important osmotic action and is equally important for the excitability of muscle and nerve cells and for the transport of nutrients and substrates through plasma membranes.

Sodium absorption occurs almost quantitatively in the distal small bowel and the colon. Sodium balance in the body is closely linked to that of water and is finely maintained by the kidneys.

Sources of Sodium include: breads and rolls, pizza, sandwiches, cold cuts and cured meats, soups, burritos and tacos, savoury snacks, chicken, cheese, eggs and omelettes, chips, popcorn, pretzels, snack mixes, and crackers (U.S. Department of Health and Human Services, U.S. Department of Agriculture, 2013).

Effects of Sodium Toxicity in Human

High sodium consumption can raise blood pressure, and high blood pressure is a major risk factor for heart disease and stroke (National Academies of Sciences, Engineering, and Medicine 2019). Most of the sodium we consume is in the form of salt.

In certain pathologic conditions (e.g., heart failure, decompensated liver cirrhosis, and renal failure), severe sweating or fever; vomiting and diarrhoea with markedly elevated sodium levels,

2.6.14: Potassium

Potassium, the most abundant intracellular cation, is an essential nutrient that is naturally present in many foods and available as a dietary supplement. Potassium is present in all body tissues and is required for normal cell function because of its role in maintaining intracellular fluid volume and trans-membrane electrochemical gradients (Institute of Medicine. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate, 2005; Stone *et al.*, 2016).

Potassium has a strong relationship with sodium, the main regulator of extracellular fluid volume, including plasma volume.

The total amount of potassium in the adult body is about 45 millimole (mmol)/kg body weight (about 140 g for a 175 pound adult; 1 mmol = 1 milliequivalent [mEq] or 39.1 mg potassium) (Preuss *et al.*, 2012). Most potassium resides intracellular, and a small amount is in extracellular fluid (Stone *et al.*, 2016; Preuss *et al.*, 2012). The intracellular concentration of potassium is about 30 times higher than the extracellular concentration, and this difference forms a transmembrane electrochemical gradient that is maintained via the sodium-potassium (Na+/K+) ATPase transporter. In addition to maintaining cellular tonicity, this gradient is required for proper nerve transmission, muscle contraction, and kidney function.

Sources of potassium in the diet include: Fruits, such as apricots, bananas, kiwi, oranges, and pineapples; vegetables, such as leafy greens, carrots, and potatoes; lean meats; whole grains; beans and nuts

Effects of Potassium Toxicity in Humans

There is no evidence that high intakes of potassium cause hyperkalemia in adults with normal kidney function or other adverse effects.

The most obvious symptom of too much potassium is an abnormal heartbeat (arrhythmia). Severe cases can lead to death.

Also, in extreme cases, high potassium can cause paralysis or heart failure.

2.6.15: Phosphorus

Phosphorus is an essential nutrient for the body and is routinely consumed through food. After consumption, phosphorus is usually bound with oxygen and exists as phosphate in the body. Both organic and inorganic forms of phosphate are present in regularly consumed foods. The amount of total phosphate ingestion can be significantly influenced by processed food. Following a meal, inorganic phosphate can be rapidly absorbed across the small intestine and enter the bloodstream causing an elevation in serum phosphate levels. An increase in serum levels of inorganic phosphate usually reduces serum levels of ionic calcium by forming a calcium phosphate complex.

The main function of phosphorus is in the formation of bones and teeth. It plays an important role in how the body uses carbohydrates and fats. It is also needed for the body to make protein for the growth, maintenance, and repair of cells and tissues. Phosphorus also helps the body make ATP, a molecule the body uses to store energy.

Phosphorus works with the B vitamins. It also helps with the following:

Kidney function, muscle contractions, normal heartbeat, nerve signalling.

Sources of phosphorus in the diet include: Pork, cod, salmon, and tuna are all high in phosphorus; Good dairy products like, milk, chocolate, yogurt, eggnog, ricotta and American cheese instant pudding. Whole grains, eggs and lentils are also high in phosphorus. While phosphorus is naturally present in many foods, processed foods also contain large amounts from additives.

Effects of Phosphorus Toxicity in Human

Common toxicity of phosphate in humans includes impaired renal function, rhabdomyolysis, and tumourlysis syndrome.

2.7: Pesticides

Pesticides are widely used in agricultural production to prevent or control pests, diseases, weeds and other plant pathogens in an effort or reduce yield losses and maintain high product quality.

They are generally called insecticides, fungicides, bactericides, herbicides or rodenticides. Most of the pesticides have the ability to destroy a wide variety of pests or weeds, but some are developed against

specific pests or pathogens. Most of these chemicals are designed in such a way as to disturb the physiological activities of the target organism, leading to dysfunction and reduced vitality. Pesticide residues may constitute a significant source of contamination of environmental factors such as air, water and soil.

Although pesticides are developed through very strict regulation processes, pesticides use has raised serious concern not only of potential effect on human health, but also about impact on wildlife and sensitive ecosystem (Kamrin, 1997).

The World Health Organization estimates that there are three million severe acute poisoning world wise each year and out of this, approximately 2,20,000 death are attributable pesticides, out of which, 1% of these death occurs in industrialized countries (Sataka *et al.*, 1997).

Pesticides that are being used in agriculture fields disseminate into the environment and come in human contact directly or indirectly. Human are exposed to pesticides that are found in the environmental by different routes of exposures like inhalation, ingestion and dermal contacts, keeping these in the backdrop among the farmers.

2.8: Classification of Pesticides

Classification of pesticides is mainly based on:

- Chemical nature (organochlorines, organophosphates, etc).
- Application requirement (agriculture, public health, domestic).
- Target organism or targeted use (insecticide, herbicide, fungicide, etc).

Classification of pesticides based on chemical nature is given in Table 2.2.

2.8.1: Organochlorines

Organochlorines (OC) are a group of chlorinated compounds widely used as pesticides. These chemicals belong to the class of persistent organic pollutants (POPs) with high persistence in the environment. OC insecticides were earlier successfully used in control of malaria and typhus, yet they

are banned in most of the advanced countries (Aktar *et al.*, 2009). The review statistics on the use of different pesticides shows that 40% of all pesticides used belong to the organochlorine class of chemicals (Gupta, 2004; FAO, 2005). Due to their low cost and the need against various pests, organochlorine insecticides such as DDT, hexachlorocyclohexane (HCH), endosulphan, dicofol, methoxychlor and dieldrin are among the most widely used pesticides in developing countries of Asia (FAO, 2005; Gupta, 2004; Lallas, 2001).

The most widely known organochlorine pesticide is dichlorodiphenyltrichloroethane, i.e., the insecticide DDT, the uncontrolled use of which raised many environmental and human health issues (Alewu *et al.*, 2011).

Table 2.2. Classification of pesticides based on their chemical nature.

No.	Chemical Group	Chemical names
1	Organochlorines	DDT,DDD, Dicofol, Eldrin, Dieldrin, Chlorobenziate, Lindane, BHC, MethoxychloroAldrin, Chlordane, Heptaclor, Endosufan, Isodrin, Isobenzan, Toxaphene, Chloropropylate
2	Organophosphates	Dimefox, Mipafox, Methyl Parathion, Ronnel, enitrothion, Bidrin,Phorate, Fenthion, caumphos, Abate, Dichlorovas, Diptrex, Phosphomidon, Demetox, Oxydemeton-methyl, Malathion, Dimethoate, Trichlorofan
3	Carbamates	**Methyl** Carbaryl, Carbanolate, Prupoxur, Dimethan, Dimetilan, Isolan, Carbofuran, Pyrolan, Aminocarb, Aldicarb **Thio** Vernolate, Pebulate, Diallate, Monilate, Butylate, Cycloate, Trillate, Thiourea **Dithio** Methan, Thiram, Ferban, Amoban, Naban, Zineb, Maneb, ZiramPolyran, Dithane M- 45
4	Pyrethroids	Allethrin, Bonthrin, Dimethrin, Tetramethrin, Ptrethrin, Cyclethrin, Furethrin, Fenevelerate, Alphamethrin, Decamethrin, Cypermethrin
5	Phenyl amides	**Carbanilates**

		Barban, Carbetamide, Chlororprofan, Prophan, Phenyl Urea, Fenuron, Monuron, Diuron,Flumeturon, Chloroxuron, Neburon, Bromuron
		Acylanalide
		Propanil, Solan, Dicryl, Karsil, Propachlor, Alachlor, Butachlor
		Toluidines
		Trifluralin, Dipropanil, Benefin, Oryzalin, Isopropanil, Nitralin
		Acetamide
		Diphenamid
6	Phenoxyalkonates	2,4-D(2,4 Dichlorophenoxy acetic acid) 2,4 5 T(2,4 5 TrichloroPhenoxy acetic acid) Dichloroprop, Mecoprop, Erbin, Sesone
7	Trazines	Atrazine, Simazine, Ametryn, Atratone, Chlorazine, Cynazine, Cyprazine, Metribuzin, Propazine, Turbutryn, Simetryn
8	Benzoic acid	Dicamba, Dichlorobenil, Chloroambin, Tricamba, Neptalan, Bromoxynil
9	Phtalimides	Captan, Diflotan, Folpet
10	Dipyrids	Paraquat, Diaquat
11	Others	Pentachlorophenol, Floroacetate, Phenyl mercuric acetate, Ethyl mercuric Phosphate,Methyl mercuric chloride, Sodium arsenate, Calcium arsenate, Lead arsenate, Cacodylic acid, Aluminium phosphide, Zinc phosphide

2.8.2: Organophosphates

Organophosphates (OP) are esters of phosphoric acid. The OP group of pesticides asserts its effects through irreversible inactivation of the enzyme acteylcholinesterase, which is essential for nerve function in humans, insects and many other animals. OP samples degrade rapidly by hydrolysis on exposure to light, air and soil, however small amounts are detected in food and drinking water. Most organophosphates are insecticides. They were developed during the early 19th century, but their effect on insect, which are similar to their effects on human, were discovered in 1932. Some are very poisonous and they usually are not persistent in the environment.

2.8.3: Carbamates

Carbamates are organic compounds derived from carbamic acid (NH_2COOH). Carbamates are similar to organophosphates. However, they differ in their origin. Organophosphates are derivatives of phosphoric acid, while carbamates derived from carbamic acid. The functional group present in carbamate insecticides are carbamate esters. Their mechanism of action is similar to organophosphate pesticides which are by reversible inactivation of the enzyme acetyl cholinesterase which affects the transmission of nerve signals resulting in the death of the pest by poisoning.

They can be easily degraded under natural environment with minimum environmental pollution (Goel *et al.*, 2007). Some of the widely used insecticides under this group include carbaryl, carbofuran, and propoxur.

2.8.4: Pyrethroides

Pyrethroides and pyrethrins are similar organic compounds isolated from the flowers of pyrethrums (Chrysanthemum Coccineum and C. cinerariaefolium). The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids. Pyrethroides affect the sodium channels and lead to paralysis of the organism. Pyrethroides have a comparatively slight level of mammalian toxicity and have a fast biodegradation capacity. Exposure to very high levels of the compounds in air, food or water may cause giddiness, headache, vomiting, muscle twitching, low energy, convulsions and loss of consciousness (Goel *et al.*, 2007).

2.8.5: Phenylamides

Phenylamide fungicides are systemic compounds that show potent eradicative anti-fungal activity. When added to the soil, they enhance plant growth and yield; in addition, these fungicides affect the homeostastis of the soil system (Monkiedje *et al.*, 2002). These chemicals affect nutrient cycling and enter the food chain, and have thus been reported to affect higher organisms including humans. They affect nucleic acids by inhibiting the activity of RNA polymerase I system. They are known to impact mitosis and cell division in target fungi (Chao *et al.*, 2011).

2.8.6: Phenoxyalkonates

Phenoxyalkonates are a widely used family of herbicides. These pesticides are mainly used to control weeds in agriculture. Nearly all compounds of this group are degraded by microorganisms (Viltos, 1952).

2.8.7: Triazines

The compounds that fall under this category are herbicidal pesticides. They include desmetryne, chlorazine, atriazine, propazine, etc. These compounds are known to have potential use as insect chemosterilants. Higher concentrations of these herbicides were found to inhibit plant catabolism pathway (Evan *et al.*, 2007).

2.8.8: Benzoic acid

Benzoic acid herbicides include dicamba, dichlobenil, chlorambin, bromoxynil, ioxynil and naptalam. Little information is available regarding their degradation by soil microbes. Ioxynil is found to precipitate in acid soils.

2.8.9: Phthalimide

Phthalimides include three fungicides, captan, folpet and captafol which together represent the second most important group of organic fungicides used in American agriculture. They represent about half the usage of the dithiocarbamates (NAS, 1975). The fungicides difolatan, captan and folpet react with thiols such as cysteine and glutathione at acidic pH levels of 4.0 to 5.0.

DipyridsThedipyridyl herbicides include paraquat and diquat. They are strongly adsorbed as organic cations in the soil (Funderburk, 1969). Microorganisms metabolize paraquat as the main source of nitrogen (Baldwin *et al.*, 1966).

2.8.10: Others

There are many more pesticides used in agricultural practice and heavy metals have found vast use in the preparation and production of pesticide. Elements like iron, lead, sulphur, arsenic, mercury, zinc, tin, etc. have been used in inorganic or organic metal form.

Among the various classes of pesticides, organochlorines and organophosphates are widely used. Organochlorines are known for their high persistence and toxicity characteristics. These pesticides cause neurological damage, endocrine disorders, and have acute and chronic health effects. Hence contamination of the environment with organochlorine pesticides drastically affects the ecosystem.

2.9: Pesticides Toxicity in Humans

2.9.1 Acute (Immediate) Health Effects

Immediate health effects from pesticide exposure includes irritation of the nose, throat, and skin causing burning, stinging and itching as well as rashes and blisters. Nausea, dizziness and diarrhoea are also common. People with asthma may have very severe reactions to some pesticides, particularly pyrethrin/pyrethroid, organophosphate and carbamate pesticides.

In many cases, symptoms of pesticide poisoning mimic symptoms of colds or the flu. Since pesticide-related illnesses appear similar or identical to other illnesses, pesticide poisonings are often misdiagnosed and under-reported. Immediate symptoms may not be severe enough to prompt an individual to seek medical attention, or a doctor might not even think to ask about pesticide exposure. It is important that exposed individuals seek medical attention on exposure or on suspicion of been poisoned by pesticides.

2.9.2 Chronic (Long-term) Health Effects

Chronic health effects include cancer and other tumours; brain and nervous system damage; birth defects; infertility and other reproductive problems; and damage to the liver, kidneys, lungs and other

body organs. Chronic effects may not appear for weeks, months or even years after exposure, making it difficult to link health impacts to pesticides.

Pesticides have been implicated in human studies of leukaemia, lymphoma and cancers of the brain, breasts, prostate, testes and ovaries. Reproductive harm from pesticides includes birth defects, still birth, spontaneous abortion, sterility and infertility.

2.10: Regulatory Control of Pesticides Use

The use of pesticides is control all over the world because of the huge number of compounds in use as pesticides and their potential to cause harm to people and the environment. A pesticide is registered in any country of comprehensive tests reveal that it is safe and that benefits from it's use out-weigh the risks. Many pesticides are registered for agricultural use while others are registered only for public health and other uses. In addition, a registered compound may become banned or its use restricted if a review of its use finds that it poses unreasonable risks to human health and environment.

Decisions to banned or severely restrict the importation and use of any compound are usually taken by national regulatory authorities, guided by information from internal advisory/reference authorities. Two of such bodies are Codex Alimentarius Commission which deals mainly with the control of chemicals used in food and the Prior Informed Consent (PIC) Procedure which controls the import and export of pesticides and industrial chemicals.

In the United States of America, the Environmental Protection Agency (EPA) issues registration for marketing of pesticides products. EPA also conducts enforcement activities in conjunction with the Food and Drug Administration (FDA) and the Department of Agriculture, to ensure compliance with registration conditions or decisions.

In Nigeria, pesticides products are registered by NAFDAC which is the body given the mandate to regulate and control the importation, manufacture, exporting, distribution, advertisment, sales and use of chemicals including pesticides (Ugbeye, 2004). In order to ensure that pesticides residue in food do not pose a high risk to human health, safety levels have been established for individual pesticide active ingredient. Such safety levels include Maximum Residual Level (MRL) and Acceptable Daily Intakes (ADIs).

2.11: Maximum Residue Limits (MRLs) - These are statutory limits set on active ingredient and commodity combinations. An MRL is the maximum acceptable concentration of a pesticide residual likely to occur in or on a crop or food commodity resulting from approved use of the pesticide or after the pesticide has been used according to Good Agricultural Practice (GAP) (Handa *et al.,* 1999). It is expressed in milligram of pesticide residue per kilogram of commodity.

MRLs provides a quantifiable means of ensuring the pesticides are not mis-used and may be used for enforcement purposes to ensure that the pesticide is only being used in accordance with GAP. It is therefore an offense to trade any agricultural commodity with residue levels that exceed the relevant MRL.

2.12: Acceptable Daily Intakes (ADI) - The ADI of a pesticide is the daily intake of the pesticide which during a life time appears to be without appreciable risk to the health of the consumer (Handa *et al.,* 1999). It is expressed in milligrams of pesticide per kilogram of body weight. Maximum Residual Level and Acceptable Daily Intake of some pesticides are presented in Table 2.3.

Table 2.3: The maximum residue Limits (MRLs) of pesticides

Component	MRL (mg/kg)	Reputable International Regulators	References
Isopropylamine	0.1	United States Environmental Protection Agency (EPA)	U.S. Environmental Protection Agency (EPA), 1989
DichloroBiphenyl	0.2	Commission Regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs"	Commission Regulation (EC), 2006.
HCB (Hexachlorobenzene)	0.5	United States Environmental Protection Agency (EPA)	Booth *et al.*, 1975.
Endosulfan	0.05	FAO Working Party of Experts and the WHO Expert Committee on Pesticide Residues	Food and Agriculture Organization (FAO), 1967.
Aldrin	0.2	FAO/WHO Joint Meeting on Pesticide Residues	FAO/WHO, 1989.
Profenofos	0.05	International Food Standards/Codex Alimentarius FAO/WHO	FAO/WHO, 2007
DDT	0.01	Joint FAO/WHO Meeting on Pesticide Residues (JMPR)	FAO/WHO, 2002
Lindane	0.02	Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (b)	FAO/WHO, 1998.
g-chlordane	0.01	International Programme on Chemical Safety (IPCS)	International Programme on Chemical Safety (IPCS), 1984.
Dichlorvos (DDVP)	0.01	International Food Standards/Codex Alimentarius FAO/WHO (b)	FAO/WHO 2004
Heptachlor	0.01	International Food Standards/Codex Alimentarius FAO/WHO (c)	FAO/WHO 2004
t-nonachlor	0.04	Canadian Health Measures Survey	Singh, 2018.
DichloroBiphnyl	0.2		Odewale *et al.*, 2021

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and Reagents

The following chemicals and reagents were used in this study.

Sulphuric acid solution, Sodium hydroxide, Hydrochloric acid, Methanol, Petroleum ether, Copper sulphate, Sodium sulphate, Boric acid, Ammonium chloride, Perchloric acid, Nitric acid, Distilled water, Metal (Spectra AA Ltd South African), n-hexane, Acetonitrile, Anhydrous magnesium sulphate, Chloroform, Benzene, Helium.

3.1.2 Equipment

Desiccators, Oven, Muffle furnace, Soxhlet extractor, Atomic Absorption Spectrophotometer (AAS), Gas Chromatography (GC), Centrifuge.

3.1.3 Consumables

Thermometer, Crucibles, Beaker, Buckner funnel, Muslin cloth, Elastic band, Kjeldahl flask, Spatula, Test tube, Pipette, Conical flasks, Ceramic mortar and pestle, Plain tubes, Filter paper.

3.1.4 The study area

The sample source of this study was Port Harcourt, Obio-Akpor Local Government area of Rivers State, Nigeria. Port Harcourt is known as port town and capital of Rivers State, Niger Delta, Southern Nigeria (Figure 3.1). It lies along the Bonny River (eastern tributary of the Niger River) 41 miles (66 km) upstream from the Gulf of Guinea. Port Harcourt was founded in 1912 in an area traditionally occupied by the Ijo (Ijaw), Okrikans, and Ikwere people.

Today, Port Harcourt is made up of two large local governments of Port Harcourt City and Obio-Akpor to which the study location is sited (Mile 3 market).

The map of Port Harcourt showing the area of study is presented in Figure 3.2. The coordinate of the study station is as follows; Mile 3 market (RSU Round About, 40 48’19” N, 60 59’16”E; Mile 3 Park, 40 48’13”N, 6o59’22”E).

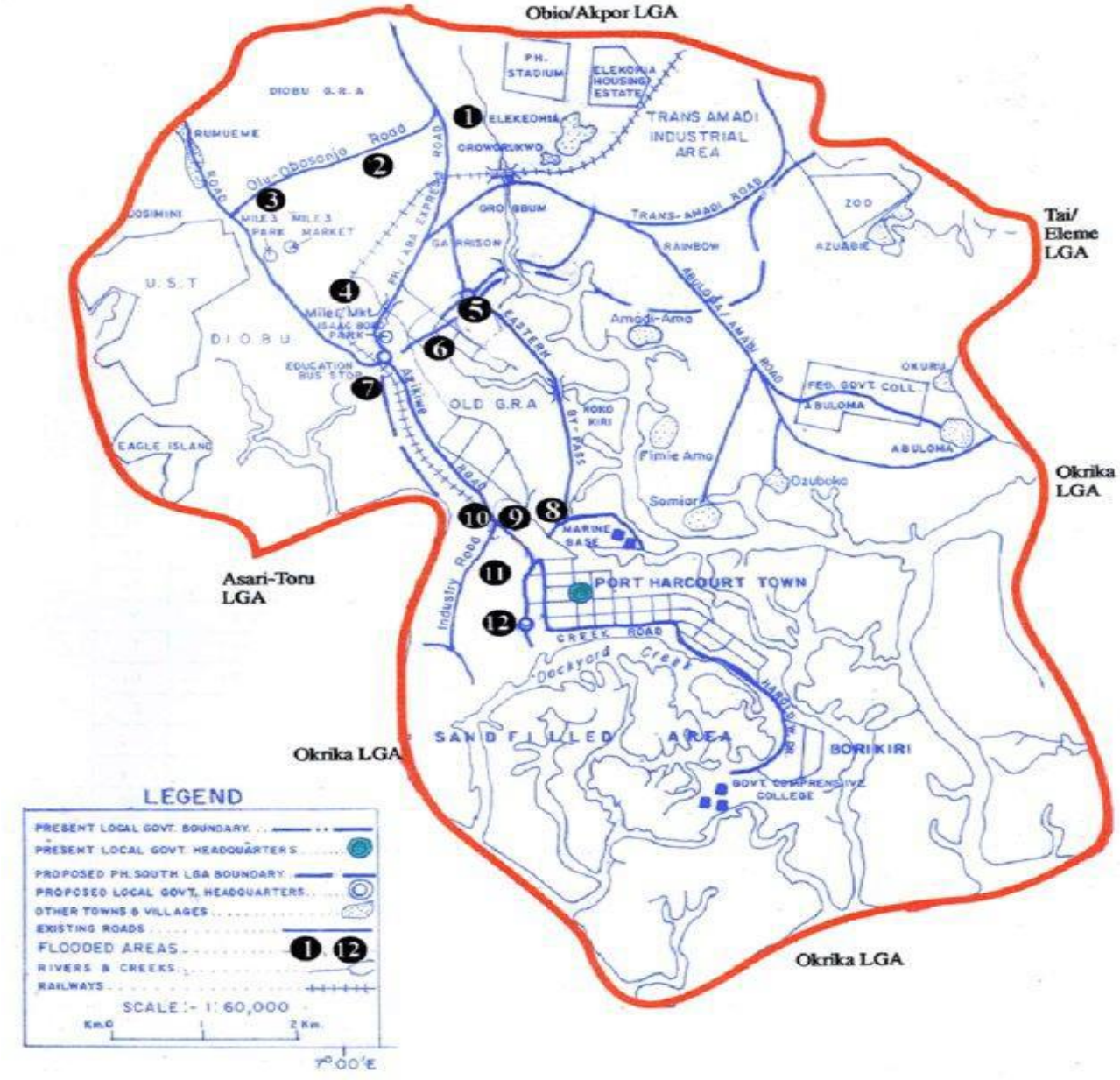

Figure 3.1. Map of Port HarcourtNwankwoala *et al.* (2014).

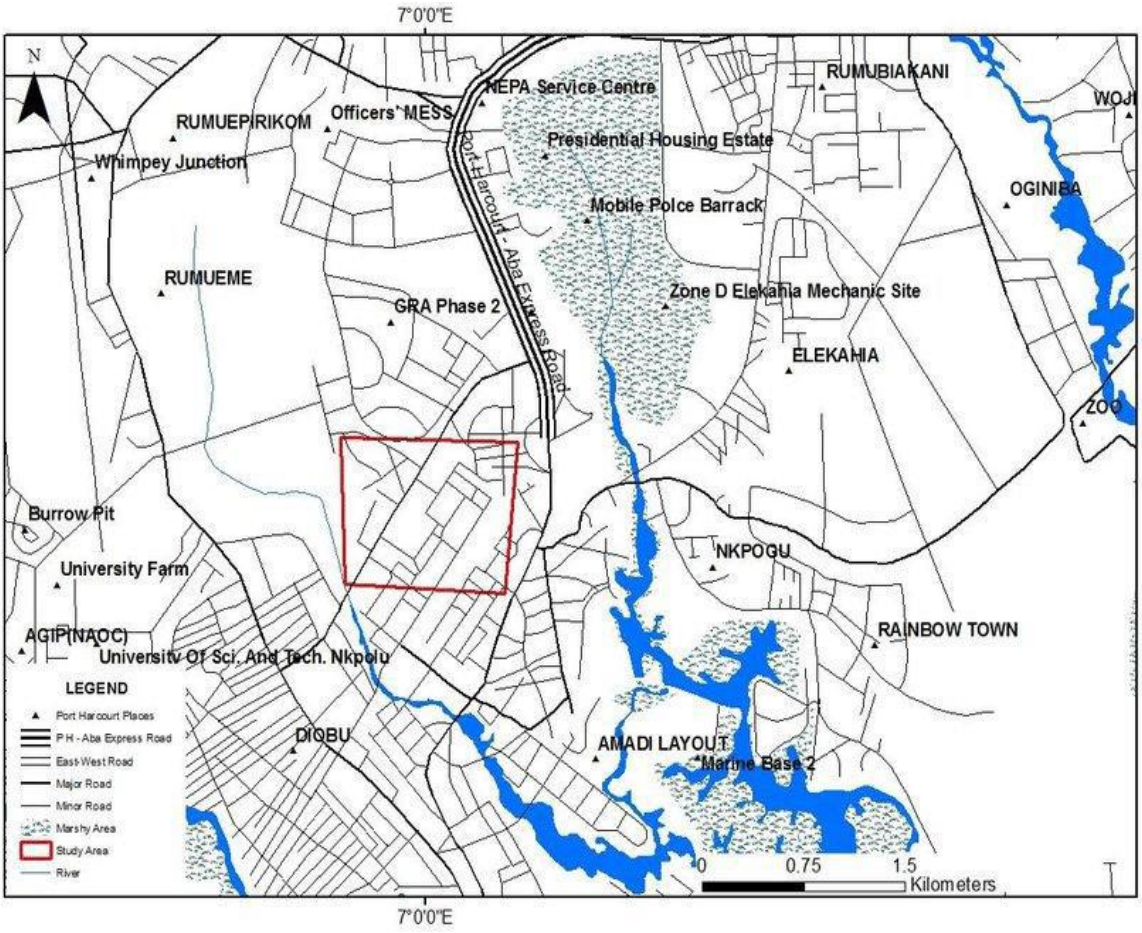

Figure 3.2. Image Uploaded by Nwankwoala *et al.*, (2014).

3.1.5 Description of the Sampling Sites

The sampling site was Mile 3 market (M3). These areas are purely residential and business districts.

3.1.6 Sample Collection

Four beans varieties (*Phaselous vulgaris L.*) with local names: Patisco, Iron, Brown, Local (Figures 3.3 to 3.6) were purchased from the sample area, Mile 3 market, Port Harcourt, Rivers State Nigeria, using systematic random sampling. The samples were conveyed to the laboratory in four separate clean plastic containers. The beans samples were identified by Mr. Olaniyi Yinka a taxonomist at the International Herbarium of the department of Botany, Obafemi Awolowo University (OAU) Ile-Ife with voucher numbers: Patisco beans (IFE, 16974), Iron beans (IFE, 16975), Brown beans (IFE, 16978) and Local beans (IFE, 16977).

Figure 3.3. Patisco beans

Figure 3.4. Iron beans

Figure 3.5. Brown beans

Figure 3.6. Local beans

3.2 METHODS

3.2.1: Preparation of samples

The foreign particles present in the beans were carefully removed by hand picking. Each of the samples were divided, into two and labeled: Raw samples and Parboiled samples. The Parboiled samples were processed by parboiling using the local method beans preparation before cooking. The Parboiled beans samples were placed in a pot containing distilled water and cooked for 8 minutes and allowed to dry at room temperature. Afterwards, the Raw and Parboiled samples were pulverized using agate mortar.

3.2.2: Determination of Proximate Composition

Proximate analysis of the bean varieties was carried out to determine crude protein, crude fibre, total ash, total carbohydrate, crude lipid and moisture content using the methods of AOAC (2005).

3.2.3 Methods of Proximate Analysis

1. Moisture Content

The container for use was washed and dried in the oven. Then it was transferred to the desiccator, and it was weighed. Two grams (2g) of the sample was weighed, and dried in the oven at a temperature of 105°C for ten minutes. The container and sample was reweighed, and taken back to the oven and dried. This process continued until a consistent result was obtained.

Calculation:

$$\% \text{ Moisture Content} = \frac{W_1 - W_2}{W_1 - W} \times \frac{100}{1}$$

Where:

W_1 = Mass of container + sample before drying

W_2 = Mass of container + sample after drying

W= Mass of container

2. Ash Content

The ash content of a material is the residue remaining after ignition at 575 ± 25°C for 3 hr or longer to burn off all the organic matter or carbon.

Procedure

The crucible for use was washed, dried and cooled in a desiccator. The weight of the crucible was measured and 2 g of the sample was weighed into the crucible. The crucible and contents was placed in the muffle furnace, and the temperature regulated at 575 ± 25°C until it carbonized. Then the furnace was switched off and allowed to cool for fifteen minutes before the crucible and its content was weighed.

Calculation:

$$\% \text{ Ash Content} = \frac{W3 - W1}{W2 - W1} \times \frac{100}{1}$$

Where:

W_1	=	Mass of crucible
W_2	=	Mass of crucible + sample before ignition
W_3	=	Mass of crucible + Ash after ignition
$W_2 - W_1$	=	Mass of sample taken for ignition.

3. Crude Fibre

Crude fibre is the combustible organic residue that is left after other biomolecules like proteins have been removed by successive treatments with boiling, acid and alkalis, alcohols and ether. This empirical treatment provides a crude fibre consisting largely of the cellulose content together with a proportion of the lignin and hemicelluloses content of the sample.

Procedure

Two grams (2 g) of the sample was weighed into a beaker and placed in a hot 200 ml of 1.25% $H_2 SO_4$ and boiled for 30 mins. It was filtered through a buckner funnel equipped with muslin cloth and held firm with elastic band. The funnel was made hot by pouring boiling water on to it. The hot acid sample solution was filtered. The residue was washed with boiling water to remove acid from it. Then the residue was returned to 200 ml boiling 1.25% NaOH and boiled for 30 mins. It was filtered and progressively washed with boiling water, 1% HCl and boiling water to remove acid from it. The residue was washed twice with alcohol and thrice with petroleum ether using small quantities. Then the residue was drained and transferred completely to a porcelain crucible and dried in the oven to a constant mass. It was cooled and weighed. Then it was incinerated at 600°C for 2 hrs in muffle furnace. The crucible and content were weighed after cooling in a desiccator. The loss on incineration was the mass of crude fibre.

Calculation:

$$\% \text{ Crude Fibre} = \frac{W_3 - W_4}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = Mass of beaker

W_2 = Mass of beaker + sample

W_3 = Mass of crucible + residue after drying

W_4 = Mass of crucible + ash after incineration.

4. Crude Fat

The fatty constituents of food sample consist of a number of lipid substances. The fat content (sometimes called the ether extract or crude fat), which may be considered as consisting of the 'free' lipid constituents is that which can be extracted by the less polar solvents such as light petroleum fractions, diethyl ether, whereas the 'bound' lipid constituents require more polar solvents such as alcohols for their extraction.

Procedure

Two grams (2 g) of the sample was weighed into a thimble and carefully wrapped and tied with thread. The flask for use was washed, dried and then weighed. The thimble and content were placed in the soxhlet extractor column and extracted for about 6 hrs. The solvent cleared from the column, meaning the fat has been extracted. The defatted sample was carefully removed and the solvent recovered. The flask and oil was oven dried until all the solvent was gone. Then the flask and its content were reweighed.

Calculation:

$$\% \text{ Crude fat} = \frac{M_2 - M_1}{M_3} \times \frac{100}{1} \qquad \frac{(\text{Mass of oil extracted})}{\text{Mass of sample}} \times \frac{100}{1}$$

Where:

M_1 = Mass of the flask

M_2 = Mass of flask + fat

M_3 = Mass of the sample

5. Crude Protein (The Kjeldahl Method)

The Kjeldahl method is based on the wet combustion of the sample by heating with conc. H_2SO_4 in the presence of metallic and other catalysts to effect the reduction of organic nitrogen in the sample to ammonia, which is retained in solution as ammonium sulphate.

Procedure8

Two hundred milligram (0.20 g) of the sample was weighed and carefully transferred to a Kjeldahl flask containing boiling chips. Using spatula, copper and sodium sulphate were added. This raised the temperature of boiling. Twenty millilitres (20 ml) of concentrated H_2SO_4 was added to the flask to assist the oxidation. The mixture was heated until it became clear. It was then cooled to room temperature and transferred quantitatively to a 100 ml volumetric flask and the flask was rinsed with water. The same procedure was carried out for a blank experiment.

Twenty millilitres (20 ml) of the digest was transferred using a pipette into the distillation flask. Ten millilitres (10 ml) of 2% Boric acid was measured into a receiver (small beaker) and two drops of methyl red indicator was added. Thirty five millilitres (35 ml) of 40% NaOH was added to the distillation flask and the plug was quickly replaced. The mixture was distilled until 30ml of the distillate was collected. The same procedure was carried out for the blank experiment. It was titrated against standard 0.1 NHCl.

Calculation:

% Crude Protein

$$= \frac{(T\text{-}B) \times NHCl \times 6.25 \times volume \times 0.00014}{Aliquot \times Mass\ of\ sample\ used} \times \frac{100}{1}$$

Where:

T	=	Titre value of the sample
B	=	Blank titre value
NHCl	=	Normality of HCl used
Aliquot	=	Sample aliquot (volume) taken

The volume it was made up to = $100cm^3$

3.3.3: Methods for the Elemental Analysis

Elemental analysis was conducted using Varian AA240 Atomic Absorption Spectrophometer according to the method of American Public Health Association, (1995) (APHA, 1995). The operating manual was used to give guidance setting up and optimization of the instrument and air-acetylene mixture was used as source of flame.

Sample Digestion

The beans samples were digested according to the procedure recommended by Adrian (1973).

From the dried sample, two grams (2 g) were weighed into a digestion flask and 20ml of the acetic acid mixture (650 ml conc. HNO_3; 80 ml perchloric acid; 20ml conc. H_2SO_4) were added. The flask was heated until a clear digest was obtained. Then the digest was diluted with distilled water to the 100 ml mark. The solution was carefully and properly shaken, transferred into a clean sampling bottle and awaited analysis by Atomic Absorption Spectroscopy.

3.3.4: Preparation of metal standard stock solutions

The following standard stock solutions were prepared in readiness for the metal analysis.

1. Cadmium(Cd) stock solution

Cadmium (Cd) standard stock solution of 1,000 mg/l was prepared by dissolving 1,000g of cadmium metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

2. Chromium (Cr) stock solution

Chromium (Cr) standard stock solution of 1,000 mg/l was prepared by dissolving 1,000g of chromium metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

3. Selenium(Se) stock solution

Selenium (Se) standard stock solution of 1,000 mg/l was prepared by dissolving 1,000g of vanadium metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

4. Lead (Pb) stock solution

Lead (Pb) standard stock solution of 1,000 mg/l of lead (Pb) was prepared by dissolving 1,000g of lead metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

5. Copper (Cu) stock solution

Copper (Cu) standard stock solution of 1,000 mg/l was prepared by dissolving 1,000g of copper metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

6. Iron(Fe) stock solution

Iron (Fe) standard stock solution of 1,000 mg/l was prepared by dissolving 1,000g of iron metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

7. Mercury(Fe) stock solution

Mercury (Hg) standard stock solution of 1,000 mg/l was prepared by dissolving 1,000g of mercury metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

8. Arsenic(As) stock solution

Arsenic (As) standard stock solution of 1,000 mg/l of Arsenic (As) was prepared by dissolving 1,000g of arsenic metal (Spectra AA Ltd South African) in a minimum volume of (1+1) HNO_3. Then the solution was diluted to 1 L with 1% (v/v) HCl.

3.3.5: Preparation of reference solutions

Quality assurance was ascertained by analysis of reference (blank) solutions. A calibration blank was prepared using all the reagents except for the metal stock solutions.

3.3.6: Analysis of the metals with Atomic Absorption Spectrometry (AAS)

Elemental analysis was conducted using Varian AA240 Atomic Absorption Spectrophometer according to the method of APHA (1995). The Atomic Absorption spectrophotometer was warmed up and the recommended wavelengths and flame/gas types set for the various metals as shown in Table 3.1 below. The samples were analyzed in triplicates to minimize errors.

Table 3.1 Atomic Absorption Spectrometry wavelengths and flame gas used for metal analysis

Element	Wavelength (nm)	Flame/gases
Cadmium	226.5	Air-acetylene
Chromium	267.7	Air-acetylene
Selenium	292.2	Nitrous oxide-acetylene
Lead	220.3	Air-acetylene
Copper	324.8	Air-acetylene
Iron	231.6	Air-acetylene
Mercury	405	Air-acetylene
Arsenic	193.7	Nitrous oxide-acetylene

3.4: Determination of Pesticides in Food

Pesticides content of beans samples were conducted using Gas Chromatography (GC) according to the method of AOAC (1990).

3.4.1: Extraction of Pesticides from Beans Samples

Ten grams (10 g) of the crushed samples were weighed and placed into a 500 ml beaker. Then six grams (6 g) of sodium sulphate was mixed with the sample and the mixture extracted using 300 ml n-hexane. Ten milliliter (10 ml) of acetonitrile was added to the homogenized sample and the mixture agitated firmly for 2 min. Extra 10 ml of acetonitrile was added, and the separating funnel closed and put on a horizontal shaker. It was then arranged to shake vigorously for 30 min at 300 rpm/min and finally allowed to stand for 5 min to sufficiently separate the phases. Furthermore, 2g anhydrous magnesium sulphate was applied in drying 10ml of the supernatant. Using filter paper, the mixture was filtered into a 50 ml round bottom flask and with the aid of rotary evaporator,it was concentrated to 1ml and made available for silica clean up step.

3.4.2 Clean-Up of Extract Clean-Up (purification using Silica SPE cartridge)

One millilitre (1 ml) of filtered residue was dissolved in 50 ml of chloroform, transferred to a 100 ml volumetric flask and then diluted to the mark. At room temperature, most of the chloroform was diluted after which a mixture of 1 ml of the reagent which is 20 vol% benzene and 55 vol% methanol was added. The setup was firmly closed and heated at 40^0C in water bath for 10 min. After heating, the organic phase was extracted with hexane and water, to achieve a final mixture of the reagent, hexane and water, in proportion of 1:1:1. The mixture was vigorously agitated by hand for 2 min and emulsion reduced by centrifugation. Finally, half of the top hexane phase was transferred to a small test tube for injection into GC column.

3.4.3 Gas Chromatographic conditions for Pesticide Determination

Finally, the extracts were analyzed using Buck M910 scientific gas chromatography equipped with Flame ionization detector (GC-FID). The temperature of the detector and injector were set at 280 ºC and 250ºC respectively. The temperature of the oven was set as follows: 120 ºC held for 4 min, ramp at 10 ºC/ min to 180 ºC, held for 2 min, and finally ramp at 5 ºC/ min to 300 ºC. Helium served as

carrier gas and at a flow rate of 1.0 ml/ min and detector make-up gas of 29 Ml min^{-1}. The volume of injection of the GC was 10.0 µl. Finally, the total run time for a sample was 43 min.

3.4.4: Quantification of Pesticides Residues.

The residue levels of pesticides were quantitatively determined by the external standard method using peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times coincided with the standards were extrapolated on their corresponding calibration curves to obtain the concentration.

3.4.5: Preparation of Standard

Ten microliter (10µl) of accu standard was injected in the chromatography and the retention time compared with retention time of standard.

3.5:Estimation of Daily Intake of Metals(EDI)

The EDI of the various residues in beans were determined as described by the methods of Handa *et al.* (1999).Calculation of the EDIs were based on an average daily consumption rate (Cr) of 70g of beans per person and a correction factor of 0.5.

The EDIs were calculated as follows:

EDI = Rm x Cr x 0.5

Rm = Mean residue concentration in beans samples

Cr = Consumption rate

3.6: Estimation of Health Risk from Pesticides

The risk of exposure to a pesticide residue by an individual (with an average body weight of 70kg) was calculated using the formula of Akoto *et al.* (2015)

$$\text{HRI} = \frac{EDI}{ADI}$$

HRI = means health risk index,

EDI = means estimated daily intake,

ADI = means acceptable daily intake.

According to the method of Akoto *et al.* (2015), when the HRI of the food containing the pesticides exceeds one (1), lifetime consumption could pose health risks.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Result of Proximate Analysis

The result of the proximate analysis carried out on the beans samples are shown in Table 4.1

Table 4.1: The levels of nutrients in the four beans varieties

Parameters	**% Moisture**	**% Ash**	**% Protein**	**% Fat**	**% Fibre**	**%Carbohydrate**
Raw Iron	9.59±0.41^{b}	2.62± 0.95^{c}	34.70±2.07^{f}	1.95±0.05^{e}	8.95±0.04^{e}	42.19±1.97^{c}
Raw Brown	7.15±0.17^{ab}	2.49±0.04^{c}	29.59±1.69^{e}	1.82±0.04^{de}	8.65±0.05^{de}	50.30±1.95^{d}
Raw Patasco	10.35±0.15^{b}	3.90±0.17^{e}	26.08±0.14^{d}	1.76±0.01^{d}	8.55±0.15^{d}	49.36±2.20^{e}
Raw Local	5.42±0.30^{a}	3.68±0.23^{d}	23.49±2.17^{c}	2.10±0.20^{f}	8.45±0.18^{d}	56.86±1.62^{f}
Parboiled Iron	56.00±2.00^{c}	1.20±0.10^{a}	11.94±0.11^{a}	0.69±0.04^{ab}	3.80±0.20^{b}	26.37±1.75^{b}
Parboiled Brown	52.50±3.38^{c}	1.52±0.02^{b}	10.33±0.26^{a}	0.64±0.03^{a}	2.70±0.13^{a}	32.31±2.00^{c}
Parboiled Patisco	53.61±3.07^{c}	1.51±0.02^{b}	12.02±0.03^{a}	0.94±0.06^{c}	2.40±0.20^{a}	29.51±1.09^{c}
Parboiled Local	52.01±3.44^{c}	1.47±0.02^{b}	20.16±0.81^{b}	0.79±0.03^{b}	4.40±0.35^{c}	21.21±1.06^{a}

Values are Mean±SD of triplicate determinations. Columns with different superscripts are statically significant at $p< 0.05$. Columns with different superscripts are not statically different from the rest.

The proximate content of the four varieties of beans are presented in Table 4.1. Results obtained shows that the moisture content was significantly ($p<0.05$) higher in the parboiled samples than in the raw samples. Also the ash, protein, fat, fibre and carbohydrate contents were significantly higher in the raw samples than in the parboiled samples.

The crude protein ranged from 23.49±2.17 to 34.70±2.07 with Raw Local Beans recording the least concentration and Raw Iron Beans recording the highest.While in the parboiled samples, crude protein ranged from 10.33±0.26 to 20.16±0.81 with Parboiled Brown Beans recording the least concentration and Parboiled Local Beans recording the highest.

The moisture contents of the raw samples ranged from 5.42±0.30 to 10.35±0.15 with Raw Local Beans recording the least concentration and Raw Patisco Beans recording the highest. While in the parboiled samples, moisture concentration ranged from 52.01±3.44 with Parboiled Local Beans recording the least concentration and Parboiled iron Beans recording the highest.

The fiber content recorded in the study ranged from 8.45±0.18 to 8.95±0.04 in the raw beans samples with Raw Local Beans recording the least concentration and Raw Iron Beans recording the highest concentration. While in the parboiled samples, fiber content ranged from 2.40±0.20 to 4.40±0.35 with Parboiled Patisco Beans recording the least concentration and Parboiled Local Beans recording the highest.

The ash content for the raw beans samples ranged from 2.49±0.04 to 3.90±0.17 with Raw Brown Beans having the least concentration and Raw Patisco Beans the highest. The parboiled section, ash content ranged from 1.20±0.10 to 1.52±0.02 with Parboiled Iron Beans recording the least and Parboiled Brown Beans recording the highest ash content.

The fat content for the raw beans samples ranged from 1.76±0.01 to 2.10±0.20with Raw Patisco Beans recording the least and Raw Local Beans the highest fat content. In the parboiled section, the fat content ranged from 0.64±0.03 to 0.94±0.06 with Parboiled Brown Beans recording the least and Parboiled Patisco the highest fat content.

The carbohydrate concentration for the raw beans samples ranged from 42.19±1.97 to 56.86±1.62with Raw Iron Beans recording the least and Raw Local Beansthe highest concentration at the raw samples. In the parboiled section, the carbohydrate content ranged from 21.21±1.06 to 32.31±2.00 with Parboiled Local Beans recording the least carbohydrate content and Parboiled Brown the highest carbohydrate content.

4.1.2 Levels of Toxic Metals in the Samples

Table 4.2: The levels of toxic metals in the four beans varieties and WHO recommended value in food.

Parameters	**Lead (mg/kg)**	**Cadmium (mg/kg)**	**Mercury (mg/kg)**	**Arsenic (mg/kg)**	**Chromium mg/kg**
Raw Iron	0.01 ± 0.00^{ab}	ND	0.04 ± 0.00^{b}	0.01 ± 0.00^{b}	0.01 ± 0.00^{c}
Raw Brown	0.01 ± 0.00^{ab}	ND	0.03 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.02 ± 0.00^{d}
Raw Patisco	0.04 ± 0.00^{d}	0.02 ± 0.00^{b}	0.42 ± 0.03^{c}	0.01 ± 0.00^{b}	0.01 ± 0.00^{ab}
Raw Local	0.03 ± 0.00^{c}	0.04 ± 0.00^{c}	0.01 ± 0.00^{a}	0.01 ± 0.00^{b}	ND
Parboiled Iron	0.001 ± 0.00^{a}	ND	0.02 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Parboiled Brown	0.001 ± 0.00^{a}	ND	0.03 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.01 ± 0.00^{b}
Parboiled Patisco	0.04 ± 0.01^{d}	ND	0.03 ± 0.00^{ab}	0.00 ± 0.00^{a}	ND
Parboiled Local	0.01 ± 0.01^{b}	ND	0.01 ± 0.00^{a}	0.00 ± 0.0007^{a}	ND
Maximum permissible Limit					
WHO	0.5 mg/kg	0.02 mg/kg	0.0005 mg/kg	0.25 mg/kg	0.04 mg/D

Values are Mean±SD of triplicate determinations. Columns with different superscripts are statically significant at $p< 0.05$. Columns with different alphabets as superscripts are not statically different from the rest.

The toxic metal concentration of the four varieties of beans is presented in Table4.2. Results obtained shows that the concentration of lead decreased significantly ($p<0.05$) in parboiled Local beans when compared to the raw Local beans. The lead (Pb) concentrations were greater in the raw beans sample and Pb concentration ranged from 0.00 ± 0.00mg/kg to 0.04 ± 0.00mg/kg in the raw samples. The Raw Brown Beans presented the least Pb concentration was recorded, whereas in Raw Patisco Beans the

highest Pb concentration was recorded. Also, in the parboiled beans samples, Pb concentration ranged from 0.00 ± 0.00mg/kg to 0.04 ± 0.01mg/kg with Parboiled Brown Beans and Parboiled Iron Beans presenting the least Pb concentration and Parboiled Patisco Beans presented the highest Pb concentration.The result of this study showed no significant difference between in Pb concentration of raw and parboiled beans samples. The concentrations of Pb in the raw and parboiled beans samples were below WHO established permissible limit of 0.5 mg/kg. WHO(2001).

Cadmium was not detected in raw iron and brown beans but was discovered in raw patisco and local. Cadmium (Cd) ranged from 0 to 0.04 ± 0.00mg/kg in the raw samples with Raw Local Beans having the highest cadmium concentration. Cadmium was not detected in any of the parboiled samples. In all the samples analyzed the concentrations of cadmium in the raw local beans samples were observed to be above the permissible limit of 0.02 mg/kg as reported by WHO (2001).The concentration of cadmium in raw patisco and local beans decreased significantly in the parboiled samples.

Mercury ranged from 0.01 ± 0.00mg/kg to 0.42 ± 0.03mg/kg in the raw samples. Raw Local Beans recording the least Hg concentration and Raw Patisco Beans recording the highest. While in the parboiled beans section, Hg ranged from 0.01 ± 0.00mg/kg to 0.03 ± 0.00mg/kg with Parboiled Local having the least concentration and Parboiled Brown Beans the highest.The concentration of mercury exceeded WHO established permissible limit of 0.0005 mg/kg WHO (2001). The result shows there was no statistical difference in the concentration of the samples.

Arsenic ranged from0.01 ± 0.00mg/kg to 0.01 ± 0.00mg/kg in the raw samples. Raw Patisco and Raw Local Beanshad the least concentration and Raw Brown Beans the highest. While in the parboiled samples, arsenic ranged from0.00 ± 0.00mg/kg to 0.00 ± 0.00mg/kg and Parboiled Brown Beans had the least concentration while Parboiled Iron Beans had the highest.It was below the WHO established maximum allowable daily level of arsenic in foodstuff is taken as 0.22 mg WHO (2001).The result from Table 4.2 shows there is statistical difference in the arsenic content of all the Raw and Parboiled samples. It was observed that parboiling decreased the arsenic content of all the samples.

Chromium ranged from 0 to 0.02±0.00mg/kg in the raw samples. Raw Local Beans had no detectable chromium concentration and Raw Brown Beans had the highest. While in the parboiled section, chromium ranged from 0 to 0.01±0.00mg/kg with Parboiled Brown Beans having the highest concentration.The concentration of chromium decreased significantly ($p<0.05$) across the samples.

4.1.3 Result of Micro Elements of the Samples

Table 4.3: The levels of micro metals in the four beans varieties and WHO recommended value in food.

Parameters	**Zinc (mg/kg)**	**Manganese (mg/kg)**	**Copper (mg/kg)**	**Selenium (mg/kg)**	**Iron (mg/kg)**
Raw Iron	1.03 ± 0.01^{c}	0.01 ± 0.00^{a}	0.25 ± 0.05^{d}	0.04 ± 0.00^{ab}	1.98 ± 0.051^{bc}
Raw Brown	1.13 ± 0.01^{c}	0.02 ± 0.00^{b}	0.44 ± 0.05^{f}	0.06 ± 0.01^{c}	1.46 ± 0.069^{a}
Raw Patisco	1.38 ± 0.20^{d}	0.03 ± 0.00^{c}	0.51 ± 0.01^{g}	0.04 ± 0.00^{abc}	2.34 ± 0.16^{d}
Raw Local	0.65 ± 0.07^{a}	0.03 ± 0.00^{d}	0.18 ± 0.01^{c}	0.04 ± 0.01^{a}	1.98 ± 0.18^{bc}
Parboiled Iron	0.67 ± 0.11^{a}	0.01 ± 0.00^{a}	0.10 ± 0.01^{b}	0.04 ± 0.00^{ab}	2.27 ± 0.20^{cb}
Parboiled Brown	0.78 ± 0.02^{ab}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.05 ± 0.00^{bc}	1.85 ± 0.14^{b}
Parboiled Patisco	0.92 ± 0.14^{bc}	0.02 ± 0.00^{b}	0.36 ± 0.03^{e}	0.04 ± 0.00^{ab}	1.47 ± 0.03^{a}
Parboiled Local	0.75 ± 0.04^{ab}	0.02 ± 0.00^{b}	0.13 ± 0.00^{c}	0.05 ± 0.00^{abc}	1.79 ± 0.13^{bc}
Recommended values in food					
WHO	1.50 mg/kg	5.0 mg/kg	0.50 mg/kg	2 mg/kg	0.80 mg/kg

Values are Mean±SD of triplicate determinations. Columns with different superscripts are statically significant at $p< 0.05$. Columns with two different alphabets as superscripts are not statically different from the rest.

The microelement concentration of the four varieties of beans is presented in Table 4.3. Results obtained shows that the concentration of zinc decreased significantly ($p<0.05$) in iron, brown and patisco beans samples while there was no significant difference in the local beans sample. Manganese concentration decreased significantly ($p<0.05$) in Brown, patisco and local beans while there was no significant difference in iron beans. The concentration of copper decreased significantly in iron, brown and patisco beans samples but showed no significant difference in local beans. Selenium concentration showed no significant difference ($p<0.05$) in all the samples. Iron concentration was significantly ($p<0.05$) reduced in brown and patisco beans samples but showed no significant difference in iron and local beans samples.

Iron ranged from 1.46±0.07mg/kg to 2.34±0.16mg/kg in the raw beans section with Raw Brown Beans recording the least and Raw Patisco Beans the highest iron concentration. While in the parboiled beans section, iron ranged from1.47±0.03mg/kg to 2.27±0.20mg/kg with Parboiled Brown Patisco and presenting the least iron concentration and Parboiled Iron Beans the highest.

Selenium ranged from 0.04 ± 0.00mg/kg to 0.06±0.01mg/kg in the raw beans section with Raw Local Beans having the least selenium concentration and Raw Brown Beans the highest. While in the parboiled section, selenium ranged from 0.04±0.00mg/kgto 0.05±0.00mg/kgwith Parboiled Iron Beans having the least selenium concentration and Parboiled Brown Beans the highest.

Zinc ranged from 0.65 ± 0.07mg/kg to 1.38±0.20mg/kg in the raw beans section with Raw Local Beans having the least zinc concentration and Raw Patisco Beans the highest zinc concentration. While in the parboiled section, zinc ranged from 0.67 ± 0.11mg/kg to 0.92± 0.14mg/kg with Parboiled Iron Beans presenting the least zinc concentration and Parboiled Patisco Beans the highest.

Manganese ranged from 0.01 ± 0.00mg/kg to 0.03± 0.00mg/kg in the raw beans section with Raw Iron Beans having the least manganese concentration and Raw Local Beans the highest. While in the parboiled section, manganese ranged from 0.01 ± 0.00mg/kg to 0.02±0.00mg/kg with Parboiled Iron Beans presenting the least manganese concentration and Parboiled Local Beans the highest.

Copper ranged from 0.18 ± 0.01mg/kg to 0.51± 0.01mg/kg in the raw beans section. The least copper concentration was recorded for Raw Local Beans while Raw Patisco Beans had the highest. In the parboiled section, copper ranged from 0.02±0.00mg/kg to 0.36±0.03mg/kg with Parboiled Brown Beans having the least copper concentration and Parboiled Patisco Beans the highest.

4.1.4 Levels of Macro Elements in the Samples

Table 4.4: The levels of macro elements in the four beans varieties and WHO recommended value in food.

Parameter	Magnesium (mg/kg)	Sodium (mg/kg)	Calcium (mg/kg)	Potassium (mg/kg)	Phosphorus (mg/kg)
Raw Iron	1.82±0.04^{a}	3.10 ±0.05^{a}	5.99±0.055^{c}	5.30±0.27^{a}	4.09±0.03^{b}
Raw Brown	1.82±0.01^{a}	3.19±0.09^{a}	7.65±0.04^{e}	6.95±0.31^{d}	3.68±0.27^{a}
Raw Patisco	1.84±0.07^{a}	3.18±0.07^{a}	5.10±0.07^{a}	5.33±0.14^{a}	4.98±0.20^{c}
Raw Local	1.80±0.07^{a}	4.07±0.05^{c}	4.90±0.13^{a}	5.90±0.29^{bc}	4.99±0.06^{c}
Parboiled Iron	1.80±0.07^{a}	3.29±0.38^{ab}	5.90±0.09^{c}	5.57±0.11^{ab}	5.47±0.14^{d}
Parboiled Brown	1.77±0.031^{a}	3.58±0.14^{b}	4.99±0.06^{a}	6.18±0.01^{c}	5.10±0.01^{c}
Parboiled Patisco	1.84±0.071^{a}	3.18±0.05^{a}	6.79±0.03^{d}	6.29±0.05^{c}	4.78±0.14^{c}
Parboiled Local	1.80±0.017^{a}	3.06±0.07^{a}	5.68±0.14^{b}	6.79±0.13^{d}	5.67±0.17^{d}
Recommended values in food					
WHO	420 mg/D	2300 mg/D	1200 mg/D	3510 mg/D	4000 mg/D

Values are Mean±SD of triplicate determinations. Columns with different superscripts are statically significant at $p< 0.05$. Columns with different alphabets as superscripts are not statically different from the rest.

The macro elements concentrations of the four varieties of beans are presented in Table4.4. Results obtained shows that there was no significant difference in the magnesium concentration of the samples. Sodium concentration showed a significant difference in brown and local beans samples while iron and patisco beans samples showed no significant difference. The calcium concentration showed significant difference in brown, patisco and local beans samples but no significant difference in iron beans. Potassium concentration showed significant difference in brown, patisco and local beans samples while there was no significant difference in iron beans sample. Phosphorus concentration

showed significant difference in iron, brown and local beans samples but no significant difference in patisco beans sample.

Magnesium ranged from 1.80±0.07mg/kg to 1.84±0.07mg/kg in the raw beans section with Raw Local Beans having the least magnesium concentration and Raw Patisco Beans the highest. While in the parboiled beans section, magnesium ranged from 1.77±0.03mg/kg to 1.84±0.07mg/kg with Parboiled Brown Beans presenting the least magnesium concentration and Parboiled Patisco Beans the highest.

Calcium ranged from 4.90±0.13mg/kg to 7.65±0.04mg/kg in the raw beans section with Raw Local Beans having the least calcium content and Raw Brown Beans the highest. While in the parboiled beans section, calcium ranged from 4.99±0.06mg/kg to 6.79±0.03mg/kg with Parboiled Brown Beans presenting the least calcium concentration and Parboiled Patisco Beans the highest.

Sodium ranged from 3.10±0.05mg/kg to 4.07±0.05mg/kg in the raw beans section with Raw Iron Beans having the least sodium content and Raw Local Beans the highest sodium content. While in the parboiled beans section, sodium ranged from 3.06±0.07mg/kg to 3.58± 0.14mg/kg with Parboiled Local Beans presenting the least sodium concentration and Parboiled Brown Beans the highest.

Potassium ranged from 5.3±0.27mg/kg to 6.95±0.31mg/kg in the raw beans section. The least potassium concentration was recorded for Raw Iron Beans while Raw Brown Beans had the highest. In the parboiled section, potassium concentration ranged from 5.57±0.11mg/kg to 6.79±0.13mg/kg with Parboiled Iron Beans having the least potassium concentration and Parboiled Local Beans the highest.

Phosphorus ranged from 3.68±0.27mg/kg to 4.99±0.06mg/kg in the raw beans section. The least phosphorus concentration was recorded for Raw Brown Beans while Raw Local Beans hadthe highest. In the parboiled section, phosphorus content ranged from 4.78±0.14mg/kg to 5.67±0.17mg/kg with Parboiled Patisco Beans having the least phosphorus concentration and Parboiled Local Beans the highest.

4.1.5: Summary of the results of toxic metals

Table 4.5: Toxic metal that persisted after parboiling of samples.

Parameters	**Mercury (mg/kg)**
Raw Iron	* 0.04±0.00[b]
Raw Brown	*0.03±0.00[ab]
Raw Patasco	* 0.42±0.03[c]
Raw Local	* 0.01±0.00[a]
Parboiled Iron	* 0.02±0.00[ab]
Parboiled Brown	*0.03±0.00[ab]
Parboiled Patasco	*0.03±0.00[ab]
Parboiled Local	* 0.01±0.00[a]
Maximum Permissible Limit	
WHO	0.0005 mg/kg

Values having asterisk (*) are above the maximum permissible limit

4.1.6 Results of Pesticides of the Samples

Table 4.6: Residual Pesticides of Raw Iron Beans and Parboiled Iron Beans

Component	Raw Iron Beans (mg/kg)	Parboiled Iron Beans (mg/kg)
2_4_dichloro	0.05 ± 0.00[a]	0.05 ±0.00[a]
Aldrin	0.12±0.01	ND
Carbofuran	0.43 ± 0.03[b]	0.23±0.10[a]
Dichlorovos	0.18 ±0.05[a]	0.13±0.01[a]
Endosulphan	0.18±0.01[b]	0.09 ±0.01[a]
Glyphosate	0.08 ±0.01[a]	0.08 ±0.01[a]
HCB	0.62 ±0.10[b]	0.31 ±0.01[a]
Heptachlor	0.23 ±0.02	ND
P, p'-DDD	0.09±0.01[a]	0.09 ±0.01[a]
Profenos	0.55 ±0.04[b]	0.38±0.01[a]
t-nonachlor	0.32 ±0.00[b]	0.22±0.02[a]

Table 4.6 indicates that Aldrin, Carbofuran, Endosulphan, HCB, Heptachlor, Profenofos, t-nonachlorwerereduced significantly $p<0.005$ in Iron beans sample.Also observed was the non-significant difference ($p<0.05$)in 2_4-dichloro, Dichlorovos, Glyphosphate, and P'p-DDD. This table also shows that parboiling and decanting the water slightlyreduced the pesticide residues concentrations in the Iron beans sample.

The result in Table 4.6 shows the pesticide residue content in Raw Iron Beans and Parboiled Iron Beans range as follows:

2_4_dichloro (0.05 - 0.05 mg/kg), Aldrin (0.12- ND mg/kg) ,Carbofuran (0.43 - 0.23mg/kg), Dichlorovos (0.18 -0.13mg/kg), Endosulfan (0.182 -0.0852mg/kg), Glyphosphate (0.08-0.08mg/kg), HCB (0.62-0.31mg/kg), Heptachlor (0.23-NDmg/kg), Pp-DDD (0.09-0.09mg/kg), Profenofos (0.55-0.38mg/kg), t-nonachlor (0.32-0.22mg/kg).

Table 4.7: Residual Pesticides of Raw Patisco Beans and Parboiled Patisco Beans

Component	Raw Patisco (mg/kg)	Parboiled Patisco (mg/kg)
2_4_dichloro	0.42±0.01[b]	0.17±0.02[a]
Aldrin	0.30±0.01[b]	0.13±0.01[a]
Carbofuran	0.50±0.02[b]	0.25±0.01[a]
DDVP	0.24 ±0.02[b]	0.08 ±0.02[a]
Dichlorovos	0.16±0.01[b]	0.10 ± 0.00[a]
Endosulfan	0.11±0.01	ND
Heptachlor	0.99±0.00[b]	0.67 ±0.02[a]
P, p'-DDD	0.30 ±0.00[b]	0.17 ±0.01[a]
DichloroBiphnyl	0.24 ±0.01[a]	0.22±0.01[a]
g-chlordane	0.15±0.02[b]	0.11±0.00[a]
Lindane	0.24 ±0.01[b]	0.14 ±0.01[a]

Table 4.7 indicates that 2_4-dichloro, Aldrin, Carbofuran, DDVP, Dichlorovos, Endosulphan, Heptachlor, P'p-DDD, g-chlordane, and Lindane were reduced significantly $p<0.005$ in Patisco beans sample. Also observed was the non-significant difference ($p<0.05$) in DichloroBiphnyl. This result from this table also shows that parboiling and decanting the water slightly reduced the pesticide residues concentrations in the sample.

In Table 4.7, the residual pesticide residue content in Raw Patisco Beans and Parboiled Patisco Beans range as follows:

2_4_dichloro (0.42-0.17mg/kg), Aldrin (0.30-0.13mg/kg), Carbofuran (0.50-0.25mg/kg), DDVP (0.24-0.08mg/kg), DichloroBiphnyl (0.23-0.23mg/kg), Dichlorvos (0.15-0.10mg/kg), Endosulfan (0.11-NDmg/kg), g-chlordane (0.15-0.11mg/kg), Heptachlor (0.99-0.67mg/kg), Lindane (0.24-0.14mg/kg), P'p-DDD (0.30-0.17mg/kg).

Table 4.8: Residual Pesticides of Raw Brown Beans and Parboiled Brown Beans

Component	Raw Brown Beans	Parboiled Brown Beans
2_4-dichloro	0.20±0.00[b]	0.12 ±0.01[a]
Carbofuran	0.23±0.06[a]	0.21± 0.05[a]
DDVP	0.21±0.00[a]	0.21 ±0.00[a]
Dichlorvos	0.15±0.00[b]	0.12 ±0.00[a]
Endosulfan	0.11±0.02[a]	0.11±0.02[a]
Glyphosphate	0.13±0.01[b]	0.10 ±0.00[a]
Heptachlor	0.24±0.00[a]	0.24 ±0.00[a]
Profenos	0.20±0.00[b]	0.10±0.00[a]
t-nonachlor	0.13±0.00[a]	0.13 ±0.00[a]
DichhloroBiphnyl	0.39±0.01[a]	0.36 ±0.00[a]
g-chlordane	0.26±0.00[b]	0.12 ±0.00[a]
Biphenyl	0.15±0.01	ND

Table 4.8indicates that 2_4-dichloro, Dichlorovos, Glyphosphate, Profenofos, g-chlordane, and Biphenyl were reduced significantly $p<0.005$ in Brown beans sample. Also observed was the non-significant difference ($p<0.05$) in Carbofuran, DDVP, Endosulphan, Heptachlor, t-nonachlor, and DichloroBiphnyl. The result from this table also shows that parboiling and decanting the waterslightly reduced the concentration of pesticide residues.

The residual pesticide isomers level of Raw Brown Beans and Parboiled Brown Beans range as follows:

2_4-dichloro (0.20-0.12mg/kg), Biphenyl (0.15-0 mg/kg), Carbofuran (0.23-0.21mg/kg), DDVP (0.21-0.21mg/kg), DichloroBiphny (0.39-0.36mg/kg), Dichlorvos (0.15-0.12mg/kg), Endosulfan (0.11-0.11mg/kg), g-chlordane (0.26-0.12mg/kg), Glyphosphate (0.13-0.10mg/kg), Heptachlor (0.24-0.24mg/kg), Profenos (0.20-0.10mg/kg), t-nonachlor (0.13-0.13mg/kg).

Table 4.9: Residual Pesticides of Raw Local Beans and Parboiled Local Beans

Component	Raw Local Beans	Parboiled Local Beans
2_4-dichloro	0.16±0.01[b]	0.10± 0.00[a]
Carbofuran	0.43± 0.03[b]	0.21± 0.01[a]
DDVP	0.15± 0.00[b]	0.15± 0.00[a]
Dichlorvos	0.18± 0.02[a]	0.12± 0.00[a]
Glyphosate	0.19± 0.00[b]	0.14± 0.00[a]
HCB	0.15±0.00[b]	0.10± 0.00[a]
P, p'-DDD	0.11± 0.00[b]	0.07±0.00[a]
Profenofos	0.34± 0.00[a]	0.34± 0.00[a]
DichhloroBiphnyl	0.18± 0.01[a]	0.18± 0.00[a]
g-chlordane	0.10 ±0.00[a]	0.10± 0.00[a]
Lindane	0.15± 0.00[a]	0.15± 0.00[a]
Isopropylamine	0.02± 0.00[a]	0.02± 0.00[a]

Table 4.9 indicates that 2_4-dichloro, Carbofuran, DDVP,Glyphosphate, HCB, and P'p-DDD were reduced significantly $p<0.005$ in Local beans sample. Also observed was the non-significant difference ($p<0.05$) in Dichlorovos, Profenofos, DichloroBiphnyl, g-chlordane, Lindane, and Isopropylamine. This table also shows that parboiling and decanting the water reduced the concentration of pesticide residues.

The residual pesticide isomers level of Raw Local Beans and Parboiled Local Beans range as follows:

2_4-dichloro (0.16-0.97mg/kg), Carbofuran (0.43-0.21mg/kg), DDVP (0.15-0.14mg/kg), DichhloroBiphnyl (0.18-0.18mg/kg), Dichlorvos (0.18-0.12mg/kg), g-chlordane (0.10-0.10mg/kg), Glyphosphate (0.19-0.14mg/kg), HCB (0.15-0.10 mg/kg), Isopropylamine (ND-0.02mg/kg), Lindane (0.15-0.15mg/kg), P'p-DDD (0.11-0.07mg/kg), Profenofos (0.34-0.34mg/kg).

Table 4.10: Comparing Residual Pesticides of Raw Iron Beans, Parboiled Iron Beans and Maximum Residue Limits (MRL).

Component	Raw Iron Beans (mg/kg)	Parboiled Iron Beans (mg/kg)	MRL
2_4_dichloro	0.05	0.05	0.1
Aldrin	0.12	ND	0.2
Carbofuran	0.23	0.23	0.1
DDVP	0.00	ND	0.01
Dichlorovos	0.13	0.13	0.01
Endosulfan	0.09	0.18	0.05
Glyphosphate	0.08	0.08	0.05
HCB	0.62	0.31	0.5
Heptaclor	0.23	ND	0.01
Pp-DDD	0.09	0.09	0.01
Profenofos	0.55	0.38	0.05
t-nonachlor	0.32	0.27	0.04

Table 4.11: Comparing Residual Pesticides of Raw Patisco Beans, Patisco Beans and Maximum Residue Limits (MRL).

Component	Raw Patisco (mg/kg)	Parboiled Patisco (mg/kg)	MRL
2_4_dichloro	0.42	0.17	0.1
Aldrin	0	0.30	0.2
Carbofuran	0.50	0.25	0.1
DDVP	0.24	0.08	0.
DichloroBiphnyl	0.23	0.23	0.2
Dichlorvos	0.16	0.10	0.01
Endosulfan	0.11	ND	0.05
g-chlordane	0.1473	0.11	0.01
Glyphosphate	0.00	ND	0.05
HCB	0.00	ND	0.5
Heptachlor	0.99	0.67	0.01
Lindane	0.24	0.14	0.02
P'p'-DDD	0.30	0.17	0.24
t-nonachlor	0.00	ND	0.04

Table 4.12: Comparing Residual Pesticides of Raw Brown Beans, Parboiled Brown Beans and Maximum Residue Limits (MRL).

Component	**Raw Brown Beans**	**Parboiled Brown Beans**	**MRL**
2_4-dichloro	0.20	0.12	0.1
Biphenyl	0.15	ND	0.1
Carbofuran	0.15	0.27	0.1
DDVP	0.21	0.21	0.01
DichhloroBiphnyl	0.39	0.36	0.2
Dichlorvos	0.15	0.12	0.01
Endosulfan	ND	0.11	0.05
g-chlordane	0.26	0.12	0.01
Glyphosphate	0.13	0.10	0.05
Heptachlor	0.24	0.24	0.01
Profenos	0.20	0.10	0.05
t-nonachlor	0.13	0.13	0.04

Table 4.13: Comparing Residual Pesticides of Raw Local Beans and Parboiled Local Beans and Maximum Residue Limits (MRL).

Component	**Raw Local Beans**	**Parboiled Local Beans**	**MRL**
2_4-dichloro	0.16	0.10	0.1
Carbofuran	0.43	0.21	0.1
DDVP	0.15	0.15	0.01
DichhloroBiphnyl	0.18	0.18	0.2
Dichlorvos	0.18	0.12	0.01
g-chlordane	0.10	0.10	0.01
Glyphosphate	0.19	0.14	0.05
HCB	0.15	0.10	0.5
Isopropylamine	ND	0.01	0.1
Lindane	0.15	0.15	0.02
P'p'-DDD	0.11	0.07	0.01
Profenofos	0.34	0.34	0.05

4.1.7: Estimation of Health Risk of Pesticides

Table 4.14: Acceptable Daily Intake (ADIs), Estimated Daily Intake (EDIs) and Health Risk Index (HRIs) of the various pesticides in Iron Beans

Component	**EDI of Raw Iron Beans (mg/kg)**	**EDI of Parboiled Iron Beans (mg/kg)**	**ADI (mg/kg)**	**HRI of Raw Iron Beans**	**HRI of Parboiled Iron Beans**
2_4_dichloro	0.0001	0.0001	0.002	0.05	0.05
Aldrin	0.0004	ND	0.0001	4	ND
Carbofuran	0.0007	0.0007	0.001	0.7	0.7
DDVP	0.0002	ND	0.003	0.07	ND
Dichlorovos	0.0004	0.0004	0.004	0.1	0.1
Endosulfan	0.002	0.0005	0.006	0.3	0.08
Glyphosphate	0.0002	0.0002	1	0.0002	0.0002
HCB	0.002	0.0009	0.003	0.7	0.3
Heptaclor	0.0007	ND	0.0001	7	ND
Pp-DDD	0.0002	0.0002	0.01	0.02	0.02
Profenofos	0.002	0.001	0.03	0.07	0.03
t-nonachlor	0.0009	0.0008	0.05	0.02	0.02

Table 4.15: Acceptable Daily Intake (ADIs), Estimated Daily Intake (EDIs) and Health Risk Index (HRIs) of the various pesticides in Patisco Beans

Component	**EDI of Raw Patisco (mg/kg)**	**EDI of Parboiled Patisco (mg/kg)**	**ADI (mg/kg)**	**HRI of Raw Patisco Beans**	**HRI of Parboiled Patisco Beans**
2_4_dichloro	0.001	0.0005	0.002	0.5	0.25
Aldrin	0	0.0009	0.001	0	0.9
Carbofuran	0.001	0.0007	0.001	1	0.7
DDVP	0.0007	0.0002	0.003	0.23	0.07
DichloroBiphnyl	0.0007	0.0006	0.0001	7	6
Dichlorvos	0.0004	0.0003	0.004	0.1	0.075
Endosulfan	0.0003	ND	0.006	0.05	ND
g-chlordane	0.0004	0.0003	0.0005	0.8	0.6
Glyphosphate	0.00009	ND	1	0.0009	ND
HCB	0.0002	ND	0.003	0.07	ND
Heptachlor	0.003	0.002	0.0001	30	20
Lindane	0.0007	0.0004	0.005	0.14	0.08
P'p'-DDD	0.0009	0.0005	0.01	0.09	0.05
t-nonachlor	0.00002	ND	0.05	0.004	ND

Table 4.16: Acceptable Daily Intake (ADIs), Estimated Daily Intake (EDIs) and Health Risk Index (HRIs) of the various pesticides in Brown Beans

Component	**EDI of Raw Brown Beans (mg/kg)**	**EDI of Parboiled Brown Beans (mg/kg)**	**ADI (mg/kg)**	**HRI of Raw Brown Beans**	**HRI of Parboiled Brown Beans**
2_4-dichloro	0.0006	0.0003	0.002	0.3	0.15
Biphenyl	0.0004	0	0.4	0.01	0
Carbofuran	0.0004	0.0008	0.001	0.4	0.8
DDVP	0.0006	0.0006	0.003	0.2	0.2
DichhloroBiphnyl	0.001	0.001	0.0001	10	10
Dichlorvos	0.0004	0.0003	0.004	0.1	0.075
Endosulfan	ND	0.0003	0.006	ND	0.05
g-chlordane	0.0007	0.0003	0.0005	1.4	0.6
Glyphosphate	0.0004	0.0003	1	0.0004	0.0003
Heptachlor	0.0007	0.0007	0.0001	1	1
Profenos	0.0006	0.0003	0.03	0.02	0.01
t-nonachlor	0.004	0.004	0.05	0.08	0.08

Table 4.17: Acceptable Daily Intake (ADIs), Estimated Daily Intake (EDIs) and Health Risk Index (HRIs) of the various pesticides in Local Beans

Component	**EDI of Raw Local Beans (mg/kg)**	**EDI of Parboiled Local Beans (mg/kg)**	**ADI (mg/kg)**	**HRI of Raw Local Beans**	**HRI of Parboiled Local Beans**
2_4-dichloro	0.0005	0.0003	0.002	0.25	0.15
Carbofuran	0.001	0.0006	0.001	1	0.6
DDVP	0.0004	0.0004	0.003	0.13	0.13
DichhloroBiphnyl	0.0005	0.0005	0.0001	0.5	0.5
Dichlorvos	0.0005	0.0003	0.004	0.125	0.075
g-chlordane	0.0003	0.0003	0.0005	0.6	0.6
Glyphosphate	0.005	0.004	1	0.005	0.004
HCB	0.0004	0.0003	0.003	0.13	0.13
Isopropylamine	ND	0.00005	0.5	ND	0.0001
Lindane	0.0004	0.0004	0.005	0.08	0.08
P'p'-DDD	0.0003	0.0002	0.01	0.03	0.02
Profenofos	0.001	0.001	0.03	0.03	0.03

4.1.8: Summary of Pesticide residues result

Table 4.18: Comparison of the HRI of the various samples

Component	**HRI of Raw Iron Beans**	**HRI of Parboiled Iron Beans**	**HRI of Raw Patisco Beans**	**HRI of Parboiled Patisco Beans**	**HRI of Raw Brown Beans**	**HRI of Parboiled Brown Beans**	**HRI of Raw Local Beans**	**HRI of Parboiled Local Beans**
2_4-dichloro	0.05	0.05	0.5	0.25	0.3	0.15	0.25	0.15
Carbofuran	0.7	0.7	1	0.7	0.4	0.8	1	0.6
DDVP	0.07	ND	0.23	0.07	0.2	0.2	0.13	0.13
DichhloroBiphnyl	ND	ND	7*****	6*****	10*****	10*****	0.5	0.5
Dichlorvos	0.1	0.1	0.1	0.08	0.1	0.08	0.13	0.075
g-chlordane	ND	ND	0.8	0.6	1.4*****	0.6	0.6	0.6
Glyphosphate	0.0002	0.0002	0.0009	ND	0.0004	0.0003	0.005	0.004
HCB	0.7	0.3	0.07	ND	ND	ND	0.13	0.13
Isopropylamine	ND	ND	ND	ND	ND	ND	0.0001	ND
Lindane	ND	ND	0.14	0.08	ND	ND	0.08	0.08
P'p'-DDD	0.02	0.02	0.09	0.05	ND	ND	0.03	0.02
Profenofos	0.07	0.03	ND	ND	0.02	0.01	0.03	0.03
Aldrin	4*****	ND	0	0.9	ND	ND	ND	ND
t-nonachlor	0.02	0.02	0.004	ND	0.08	0.08	ND	ND
Heptaclor	7*****	ND	30*****	20*****	1	1	ND	ND
Endosulfan	0.3	0.08	0.05	ND	0.05	ND	ND	ND

Values with asterisk (*****) have HRI greater than 1 and pose health risk

4.2 Discussion

4.2.1 Proximate Composition of Beans Samples

The results of the proximate analysis of the samples are presented in Table 4.1.

The crude protein content of the samples ranged from 23.49±2.17 to 34.70±2.07. Raw iron beans had the highest concentration which could be attributed to the amino acid composition of the sample. The result in this work agrees with the protein content Nwadike *et al.,*(2018) observed which ranged from 21.23 to 23.83. It is also in slight agreement with the work of Aletan *et al.,*(2018) that observed crude protein in three beans varieties to range from 19.63 to 23.48. This is an indication that consumption of iron beans will help to supply adequate amount of amino acids to the body, which in turn will help in replenishing loss body tissues.

The Fat content of the samples ranged from 1.76±0.01 to 2.10±0.20. Raw Local Beans had the highest fat content which implies it is a good source of energy and aids in transport of fat soluble vitamins. The result in this work agrees with the fat content Nwadike *et al.,*(2018) observed which ranged from 1.63 to 2.03. This result does not agree with the work done by Aletan *et al.,* (2018), which observed fat in white beans to be 4.75±0.15 and slightly agreed with Olopade *et al.,* (2017) that observed crude fat in three beans varieties to range from1.86±2.97.

Fibre ranged from 8.45 ± 0.18 to 8.95 ± 0.04. Fibre helps in the removal of waste products from the body, thereby preventing constipation and many health disorders. It will also help to prevent diseases such as diabetes mellitus, cardiovascular disease, obesity and colon cancer (Ezike *et al.,* 2020). The result in this work does not agree with the work of Olopade *et al.,* (2017) that observed fibre in three beans varieties ranged from 2.22 to 3.20.

Ash content ranged from 2.49±0.04 to 3.90±0.17. Ash content is an indication of the level of minerals present in a particular food sample (Ezike *et al.,* 2020). The result from this work is in slight agreement with the work of Olopade *et al.,* (2017) which observed the ash content of three beans varieties to be 3.94 and 3.96. It was observed that parboiling reduced the ash content of the samples.

Carbohydrate content ranged from 42.19±1.97 to 56.86±1.62. Carbohydrates are major components of dry beans (Jepleting *et al.,* 2022). The carbohydrate content observed was not within the range of 50-60% reported by Los *et al.,* (2018) for various bean varieties. The carbohydrate content of beans has a low glycemic index which is considered a therapeutic diet for diabetes patients (Hayat et al., 2014).

Moisture content ranged from 5.42±0.30 to 10.35±0.15. Variations in moisture content can be attributed to the difference in the bean variety and location (Jepleting *et al.,* 2022).The low moisture content of dried beans facilitates their transportation and storability as well as prolongs their shelf life (Kyomugasho *et al.,* 2021). The result agrees with the work of Olopade *et al.,* (2017) that observed moisture content to range from 5.99 to 6.35. As reported by (Nagrale *et al.,* 2018) the sixteen mung beans varieties moisture also varied from 5.26 to 10.90 which is in accordance with the present findings. It was observed that parboiling increased the moisture content of the samples.

4.2.2 Toxic Metals Concentration of Beans Samples

Heavy metals, when present even in very low concentrations in foods, have the ability to cause human health complications and as such information about the dietary intake of these metals is important to evaluate dangers to consumers.

Toxic metals such as As, Ag, Hg, Cd and Pb are of no biological relevance to plants and animals; rather they are harmful (Okereafor *et al.,* 2020).

Lead is a far-reaching and non-biodegradable poison that is critically dangerous to human beings, with several sources for exposure (Kutry *et al.,* 2020). The result of this study showed no significant difference between in Pb concentration of raw and parboiled beans samples. This means that parboiling had no significant effect on the Pb concentration of the samples. The concentrations of Pb in the raw and parboiled beans samples were below WHO established permissible limit of 0.5 mg/kg (WHO, 2001). This implies that consuming this beans samples does not pose the threat of lead toxicity. The work report by Gargouri *et al.,* (2018) showed that rats treated with lead at doses of 0.344 g/kg BW for 30 days had renal damage with significant increases in hematological parameters, oxidative stress-related parameters, creatinine, urea levels in plasma, and uric acid level in urine and this could happen in human.

The concentration of mercury exceeded WHO established permissible limit of 0.0005 mg/kg (WHO, 2001). The result from this section shows there was no statistical difference in the mercury content of these samples. This implies that consuming these samples puts the individual at the risk of mercury toxicity. High Hg concentration affects the function of gonads, where female gametes (oocytes), male gametes (spermatozoa), and sex hormones are formed (Massányi *et al.,* 2020). Lee et al.,(2020) also observed that high blood mercury was associated, respectively, with 10.5% and 34.5% increases in

risk of hyperlipidemia and liver injury (elevated plasma levels of liver enzymes). Mercury can be converted to methylmercury (MeHg), (Sun *et al.,* 2020) and in vitro and in vivo experimental data strongly attest to thenegative effect methylmercury has on the development of the brain(Nogara *et al.,* 2021, Rai *et al.,* 2022, Skalny *et al.,* 2022, Wildner *et al.,* 2022).

Cadmium enters the body through inhalation or ingestion and causes acute and chronic effects(Okereafor *et al.,* 2020).In this study, cadmium was detected only in raw patisco and raw local beans with concentration above WHO permissible limit. However, Cd was not detected in any of the parboiled samples and the concentration of the samples were below the permissible limit of 0.02 mg/kg as reported by WHO (2001). This implies that parboiling of food reduces cadmium toxicity.Cadmium at a toxic level can bind to metallothionein (MT) and other metal transporters in the small intestine and accumulates in the GI tract (Ohta *et al.,* 2020).

Arsenic is one of the most toxic heavy metals in the world (Samira *et al.,*2021). The As concentration in the samples were below the WHO established maximum allowable daily level of arsenic in foodstuff 0.22 mg (WHO, 2009). This implies that consuming these beans samples will not lead to arsenic toxicity. When the concentration of arsenic is above the permissible limit in the body, can inhibit sulfhydryl group containing enzymes which leads to their dysfunction (Balali-Mood *et al.,* 2021).The study also shows parboiling decreased the arsenic concentration of all the samples.

Chromium can enter the human body via breathing, drinking, skin contact or eating food containing chromium (Bahiru *et al.,* 2019). Chromium plays a significant function in the body system in little amount but becomes toxic when it exceeds the acceptance limit. Cr (VI) is related to a series of diseases and pathologies while Cr (III) is required in trace amounts for natural lipid and protein metabolism and also as a cofactor for insulin action (Achmad *et al.,* 2017; Vincent, 2017; Vincent, 2019).The value obtained in the analysis was however lower than permissibility level set by WHO (1999) which is 2.3mg/kg (Bahiru*et al.,* 2019). This implies that consuming these samples will not lead to chromium toxicity.

4.2.3 Macro- and Micro- Element concentrations of Beans Samples

Zinc is required for the catalytic activity of hundreds of enzymes, and it plays a role in enhancing immune function, protein and DNA synthesis, wound healing, and cell signaling and division (Ryu *et al.,* 2020; King *et al.,* 2014). Zinc also supports healthy growth and development during pregnancy, infancy, childhood, and adolescence and is involved in the sense of taste (Nagraj *et al.,* 2017; Ryu *et*

al., 2020; King *et al.,* 2014). The result of this study shows that the concentration of zinc in the samples were below the WHO recommended value (WHO 2001). This means that consuming these samples alone will not be sufficient to supply the zinc requirement of the body. It was also observed that parboiling decreased the zinc content of all the samples.

Manganese is a trace mineral. It is vital for the human body, but people only need it in small amounts. Manganese is a cofactor for many enzymes, including manganese superoxide dismutase, arginase, and pyruvate carboxylase (Buchman *et al.,* 2014). Manganese is involved in amino acid, cholesterol, glucose, and carbohydrate metabolism; reactive oxygen species scavenging; bone formation; reproduction; and immune response (Li l*et al.,* 2018; Chen *et al.,* 2018). The manganese concentration in the samples were below the WHO recommended value. This means that consuming these samples alone will not be sufficient to supply the manganese requirement of the body. It was also observed that parboiling decreased the manganese concentration in all the samples.

Copper is required in trace amounts, sufficient quantities of this metal are needed to sustain growth and development in humans and other mammals (Shanbhag *et al.,* 2020). Copper (Cu) is an essential micronutrient required for the activity of redox-active enzymes involved in critical metabolic reactions, signaling pathways, and biological functions (Maung *et al.,* 2021). Copper deficiency is uncommon in humans (Prohaska 2012). The concentration of copper in the samples were below the WHO recommended value. This means that these samples are poor supplier of copper to the body.

Selenium is an essential trace element for mammalian redox biology (Guillin *et al.,* 2019). Studies have revealed an association between selenium deficiencies and the increased risks of developing several pathologies, including cancers, neurogenerative diseases, cardiovascular disorders (Vindry *et al.,* 2018; Avery *et al.,* 2018). Selenium is an essential micronutrient that plays a crucial role in development and a wide variety of physiological processes including effect immune responses (Avery *et al.,* 2018). Selenium concentration of the samples were below the WHO recommended value. This implies that consuming these samples alone, will not supply the required selenium the body need.

Iron is biologically essential, but also potentially toxic; as such it is tightly controlled at cell and systemic levels to prevent both deficiency and overload (Camaschella *et al.,* 2019; Yook *et al.,* 2021). The result of this study shows that the iron concentration of the samples are above 0.80mg/kg which is the WHO recommended iron concentration values in food (WHO 2010). This implies that consuming the beans samples can supply the iron requirement of the body. From the result, it is also

observed that parboiling of the raw beans samples, had little effect on the iron concentration of the samples.

Magnesium plays an important role in many physiological functions. Habitually low intakes of magnesium and in general the deficiency of this micronutrient induce changes in biochemical pathways that can increase the risk of illness and, in particular, chronic degenerative diseases (Fiorentini *et al.,* 2021). Reduced intracellular magnesium level can lead to increased calcium entry into adipocytes followed by increase oxidative stress, inflammation, and increase insulin resistance (Nielsen 2018). Hypermagnesemia is usually iatrogenic and is reported along with impaired kidney function, bowel disorders, and old age (Al Alawi *et al.,* 2018). From this study, significant difference was observed in magnesium after parboiling. This indicates that parboiling affected the magnesium concentration of the samples.

Potassium is present in all body tissues and is required for normal cell function because of its role in maintaining intracellular fluid volume and trans membrane electrochemical gradients (Stone *et al.,* 2016; Hinderling 2016). From this study, significant difference was observed in potassium after parboiling. This indicates that parboiling affected the potassium concentration of the samples.

Calcium is an essential mineral with critical functions in the skeletal, cardiovascular, endocrine, and neurological systems (Gomes *et al.,* 2022). Studies show long-term consequences of inadequate calcium intakes are related to bone health, especially rickets in children and fractures, osteopenia, and osteoporosis in older adults (Cormick *et al.,* 2019; Office of Dietary Supplements). From this study, significant difference was observed in calcium after parboiling. This indicates that parboiling affected the calcium concentration of the samples.

High sodium consumption can raise blood pressure, and high blood pressure is a major risk factor for heart disease and stroke (National Academies of Sciences, Engineering, and Medicine 2019).
Therefore, ,a reduction in dietary sodium not only decreases the blood pressure and the incidence of hypertension, but is also associated with a reduction in morbidity and mortality from cardiovascular diseases (Grillo *et al.,* 2019). From this study, significant difference was observed in sodium after parboiling. This indicates that parboiling affected the sodium concentration of the samples.

4.2.4: Pesticides Concentration of Beans Samples

The use of pesticides for the preservation of agricultural food crops is a common practice in Nigeria. Therefore studies on the concentration of pesticide residues absorbed by food crop are necessary as it provides an insight on the safety of the population consuming such food crop.

In this study, 17 pesticide residues were detected in the samples of *Phaseolus vulgaris L* (Table 4.6-Table 4.9). The pesticide residues belong to four major classes, which are Organochlorines, Organophosphates, Carbamates and Polychlorinated biphenyls.

The organochlorine pesticides detected include Aldrin, 2-4-dichloro, DDVP, Lindane, Endosulfan, p,p DDT, t-nonachlor, g-chlordane and Heptachlor.

2-4-dichloro and DDVP were detected in all samples of beans with concentrations ranging from 0.422± 0.009 mg/kg to 0.048 ± 0.002 mg/kg and 0.236 ± 0.019 mg/kg to 0.0002 ± 0.00 mg/kg respectively. The value of 2,4-dichloro and DDVP were below the EUs MRL in only Iron beans samples. This implies that the pesticides 2,4-dichloro and DDVP were still used in preserving the samples. Studies indicate that consumption foodstuffs contaminated with 2,4-dichloro and DDVP pesticides could have health effects such as neurological damage, hypertension, cardiovascular diseases and skin disorders and cancer in human (Ujowundu *et al.,* 2017; Okoroiwu *et al.,* 2018).

Endosulfan and t-nonachlor were detected in all the samples except the local beans samples. The residues of this pesticides occurred at concentrations ranging from 0.11 ± 0.016 mg/kg to 0.18 ± 0.009 mg/kg and 0.006 ± 0.00 mg/kg to 0.315 ± 0.0009 mg/kg respectively in the raw samples and 0 to 0.085 ± 0.01 and 0 to 0.22 ± 0.02 in the parboiled samples. These values were above the EUs MRL. The presence of endosulfan and t-nonachlor in these beans samples, which are banned organochlorine pesticides indicate their continued use in preserving foodstuff in Nigeria. Exposure to endosulfan and t-nonachlor has been linked to severe health conditions like liver lesions and reproductive disruptions, as well as presenting potential carcinogenic risks (Okoroiwu *et al.,* 2018; Sosan *et al.,* 2017).

A study by Sosan *et al.,* (2017) identified similar organochlorine pesticides in beans from markets in Ile-ife, Nigeria. Other research by Obida *et al.,* (2012) and Ogah *et al.,* (2012) detected organochlorine pesticides like Aldrin, dieldrin, and DDT at concentration exceeding the European Union's maximum residue level (MRL) in bean samples from Maiduguri and Lagos respectively. Furthermore, Iliya *et al.,* (2012) reported high concentrations of endosulfan in samples of beans in Jos, Nigeria. Consumption of beans contaminated with DDT and other organochlorine pesticides could cause liver lesions and may also disrupt reproductive functions as well as carcinogenic risks (Adeoluwa *et al.,* 2019; Integrated Risk Information System, 2019).

Organophosphates detected in this study include Dichlorovos and glyphosate. They were detected in all the samples and occurred at concentrations ranging from 0.15 ± 0.002 mg/kg to 0.177 ± 0.046 mg/kg and 0.0003 ± 0.00 mg/kg to 0.076 ± 0.01 mg/kg in the raw samples respectively and 0.10 ± 0.002 mg/kg to 0.13 ± 0.01 mg/kg and 0 to 0.075 ± 0.01 mg/kg in parboiled samples respectively. Iliya *et al.*,(2012) have previously detected organophosphates in samples of beans sold in Jos, Nigeria. The results of their work, showed the residues to be below their respective EU's MRL, which does not agree with the results of this study.

Carbamates detected in this study, include Carbofuran. Carbofuran was detected in all samples of beans ranging from 0.21 ± 0.06 mg/kg to 0.50 ± 0.02 mg/kg in the raw samples and 0.21 ± 0.01 mg/kg to 0.25 ± 0.01 mg/kg in the parboiled samples. The residues were above EU's MRL value of 0.1 mg/kg. This result agrees with the work of Oshatunberu *et al.,* (2023) that found carbofuran as the only carbamates in some selected beans samples and carbofuran concentration in the beans samples of this study was also above the EU's MRL value. The low concentration of organophosphate and carbamate residues in this study suggests good storage practices and safe pesticide application was observed to prevent insect infestation.

The polychlorinated biphenyls pesticides detected in this study include dichloroBiphnyl and biphenyl. DichloroBiphnyl was detected in all samples of beans except in Iron beans with concentrations ranging from 0.18 ± 0.01 mg/kg to 0.39 ± 0.01 mg/kg in the raw samples and 0.18 ± 0.00 mg/kg to 0.36 ± 0.00 mg/kg in the parboiled samples. These residues were above the EUs MRL in brown and patasco beans samples. The result of this study is in tandem with the work of Usman *et al.,*(2021) that reported polychlorinated biphenyls to be above the EUs MRL in some beans samples in Gombe State, Nigeria. Previous studies linked PCB species to endocrine disruption and toxic health effects, cancerous and noncancerous in animals (Arshad *et al.,* 2022).

The MRL is not expected to be exceeded in any foodstuff if the pesticide was applied in accordance with directions for its safe use (Ogar *et al.,* 2012). If, however, a residue in a food sample exceeds the MRL, the food commodity is unsafe for consumption because it contains an unsafe or illegal amount of the residue (Otitoju *et al.,* 2021). Also the detection of organochlorines in beans suggests that these persistent pesticides may still be in use even though they have been banned in Nigeria and most countries of the world. The goal of monitoring of pesticide use in agriculture should therefore be directed at ensuring appropriate use of pesticide products and banned pesticides are no longer used.

The residues investigated in the study could pose potential risk to liver and kidney toxicity for adult consumers of beans (Integrated Risk information System, 2019). Consumption of beans contaminated with DDT and other organochlorine pesticides could cause liver lesions and may also disrupt reproductive functions as well as carcinogenic risks (Adeoluwa *et al.,* 2019; Integrated Risk information System, 2019).

4.2.5: Estimation of Health Risk of Pesticides

The estimated health risk of a sample is the risk associated with consuming the sample. According to Akoto *et al.,* (2015), when HRI is greater than one, lifetime consumption of the food sample could pose significant health risks.

Aldrin and Heptaclor in raw iron beans have a health risk index (HRI) of 4 and 7 respectively, but were not detected in the parboiled iron beans sample. This implies that parboiling and decanting the water, removed aldrin from the sample. Other pesticide residues had HRI below 1.

In raw Patisco beans, DichloroBiphnyl and Heptaclor have HRI of 7 and 30 respectively, but after parboiling the HRI reduced to 6 and 20 respectively. This means that parboiling and decanting the water, reduced these pesticides residue but not to a level less than 1. The residues of dichloroBiphnyl and heptaclor investigated in this study could pose potential toxicity risk to liver and kidney for adult consumers of beans. This is in line with the study of IntegratedRiskInformationSystem (2019).

In raw Brown beans, DichloroBiphnyl and Heptaclor have a HRI of 10 and 1 respectively, but after parboiling, the HRI reduced to 8 and 0.8 respectively. This implies that parboiling and decanting the water, reduced these pesticide residues although the HRI value of DichloroBiphnyl was still greater than 1. Consuming this sample could pose significant health risk on the consumers which include liver lesions IntegratedRiskInformationSystem (2019).

In raw Local beans, Carbofuran has a HRI value of 1, but after parboiling it reduced while other residues have HRI below 1. This means that parboiling and decanting the water, affected the carbofuran concentration. It is observed that the HRI value of the pesticide residues in this sample after parboiling was below 1. Therefore consuming this sample poses no significant health risk.

4.2.6: Effect of Heat

Raw agricultural commodities are often processed in one way or the other before consumption and this affect the pesticide residue content. Processing may involve peeling, washing, soaking, boiling etc. Washing and soaking may have been found to reduce pesticide residue content of fruits by 18% and 85% respectively (Fytianos *et al.*, 2006). In this study, the effect of parboiling on the pesticide residue of the beans samples was observed.

It was found in most of the samples, pesticide residue content were decreased by boiling. This agrees with the results of similar studies (Soliman 2001; Rasmussen *et al.,* 2003). The extent of reduction is higher in organophosphate and carbamates than in organochlorines. This may be due to the higher stability of organochlorine compounds to heat treatment. Only carbofuran disappeared completely after boiling, that is 100% reduction.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The proximate analysis (moisture content, ash content, protein content, fat content, fiber content, carbohydrate content) of the various beans samples that were analysed showed that all of the contents of the beans samples except moisture, decreased when parboiled.

The levels of selected metals in the beans samples analysed showed that the level of lead across the samples were below WHO maximum permissible limit of 0.5mg/kg and further parboiling of the samples decreased its level.

Cadmium levels were detected in only Raw Patisco beans and Raw Local beans samples, and at a level slightly above the WHO maximum permissible limit of 0.02mg/kg. Parboiling of the samples reduced the cadmium levels and they were not detected.

Mercury levels were detected across the samples and at levels above the WHO maximum permissible limit of 0.0005mg/kg. It was observed that parboiling did little in reducing the mercury contents.

Arsenic levels were detected in all the beans samples but at levels below WHO maximum permissible limit of 0.22mg/kg. Parboiling of the samples also reduced the arsenic levels.

Iron levels were detected in all the beans samples and at levels above the WHO maximum permissible limit of 0.80mg/kg. Parboiling of the samples increased the iron level in Iron beans, Brown beans and reduced the iron content of Patisco beans and Local beans.

Zinc levels were detected in all the beans samples and at levels below the WHO maximum permissible limit of 1.50mg/kg. Parboiling reduced the of zinc levels in all the samples.

Manganese levels were detected in all the beans samples but at levels below the WHO maximum permissible limit of 5.0mg/kg. Parboiling reduced the manganese levels in all the samples.

Copper levels were detected across the samples and at levels below the WHO maximum permissible limit of 0.50mg/kg. Parboiling reduced the copper levels of the samples

Selenium levels were detected in all the beans samples and at levels below the permissible limit of 2mg/kg. Parboiling reduced the selenium levels of the samples, only in Local beans was it increased.

Chromium levels were detected in all the beans samples except Raw Local beans sample. Parboiling of the samples reduced chromium levels in all the samples.

Sodium was detected across all the beans samples. Parboiling increased the sodium level in all the samples except Local beans.

Potassium was also detected in all the beans samples. Parboiling increased the potassium levels across the samples except in Patisco beans.

Phosphorus levels were detected in all the beans samples. Parboiling of the samples increased the phosphorus content in the samples.

Other metals like magnesium, and calcium were detected in all the beans samples and their levels decreased when the samples were parboiled.

The result of this study also showed that there is high incidence of pesticide residues in beans sold in Mile 3 market, Port Harcourt. Pesticide residue occurred more often in Iron beans, Patisco beans, Brown beans than in Local beans.

This study also reveals that the concentration of pesticide residues is decreased by boiling. Effect of heating was more pronounced on carbamate pesticides than on organochlorine compounds. This means that organochlorine are more likely to accumulate in the human body and cause chronic poisoning than organophosphates and carbamate. This implies that beans should be parboiled and the water discarded prior to subsequent cooking as this could help reduce pesticide and metal toxicity.

From the results of this study, it is observed that in Local beans, none of the pesticide residue was above HRI 1 and only one pesticide residue had an EDI greater than the ADI. Therefore one can say that Local beans had the least pesticide toxicity. While Patisco beans that had two pesticide residues having EDI above ADI and two pesticide residues having HRI greater than 1, contained the most toxic.

The results in this study also showed that organochlorine pesticides were prevalent across all samples. There is therefore need for more stringent monitoring of the use of pesticides in agriculture and food storage in Nigeria. The residue concentration found for three of the pesticides (aldrin, dieldrin and dichlorovo) were above safety levels. They may pose serious threat to human health due to chronic toxicity.

5.2 Recommendations

Fertilizers and pesticides are useful in food production and eradication of pests respectively but they

are poisonous and can contaminate the environment. There use in agriculture should be highly monitored and regulated by the government of Nigeria. Pesticide monitoring bodies with programs should be established to carry out the following:

a. The sources of highly toxic metals like, Pb, Hg, Cd, and Cr in beans be further investigated.
b. All beans should be well parboiled for few minutes andwater discarded before cooking. If possible it should be further rinsed after parboiling with warm water before cooking.

5.3 Contribution to Knowledge

This study has made the following contribution to knowledge:

a. The three main classes of pesticides in the four commonest beans variety consumed by Port Harcourt residents have been analyzed simultaneously.

b. The beans variety (among the four most consumed beans samples) that showed the least susceptibility to heavy metals and pesticides has been determined.

c. Attention should be drawn to the government of the possibility of continuous use of banned pesticides by farmers and the need to upgrade measures and means of apprehending the culprits and curbing further risk to life.

References

Achmad, R. T., Budiawan, B., and Ibrahim Auerkari, E. (2017). Effects of chromium on human body. *Annu. Res. Rev. 13*, 1–8. doi:10.9734/arrb/2017/33462

Adeoluwa OA, Mosudi BS, John A, Oyedele O (2019). Occurrence and Human Health Risk of Dichlorodiphenyltrichloroethane (DDT) and Hexachlorocyclohexane (HCH) Pesticide Residues in Commonly Consumed Vegetables in Southwestern Nigeria.*Journal of Health and Pollution 9*(23):1-13

Aktar MW, Sengupta D, Chowdhury A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology 2*(1): 112.

Al Alawi M.A , Majoni W.S, and Falhammar H. (2018). Magnesium and Human Health: Perspectives and Research Directions. *International Journal of Endocrinology.*

Aletan, U.I. (2018) Comparison of the proximate and mineral composition of two cowpea varieties obtained from Mile 12 market, Lagos, *Communication in Physical Sciences 3*(1): 43-48.

Alewu B, Nosiri C (2011) Pesticides and human health. In: Stoytcheva M (ed) Pesticides in the modern world—effects of pesticides exposure. In Tech, Rijeka, pp 231–250

Ali, Mir & Hossain, Delower & Al-Imran, & Khan, Md & Begum, Maksuda & Osman, Mahadi. (2021). Environmental Pollution with Heavy Metals: A Public Health Concern. 10.5772/intechopen.96805.

Alina M, Azrina A, Mohd Yunus AS, Mohd Zakiuddin S, Mohd Izuan Eff endi H, Muhammad Rizal R. (2012). Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. *Int Food Res J 19*(1): 135140.

Aggett S, Dunn A, Vincent R. (2012). Engaging with impact: how do we know if we have made a difference?London: Wellcome Trust.

American Public Health Association (1998) 3111B, *Direct Air- Acetylene Flame Method, Standard Methods for the examination of metals in blood 20th Edition,* APHA, AWWA, WEF.

Amir, Y., Haenni, A. L. and Youyou, A. (2006).Differences in the biochemical composition of dry legumes cultivated in north Algeria.Electron.*J. Environ. Agric. Food Chem. 5*(3):14111418.

AOAC(1990)Association of Analytical Chemistry. Methods for chemical Analysis.2217 2280.

Aparicio-Fernandez, X., Yousef, G. G., Loarca-Pina, G., Gonzalez de Mejia, E. and Lila, M. A. (2005). Characterization of polyphenolics in the seed coat of black jamapa bean (Phaseolus vulgaris L.). *J. Agric. Food Chem. 53*:46154622.

Aparicio-Fernandez, X., Garica-Gasca, T., Yousef, G. G., Lila, M. A., Gonzales de Mejia, E. and Loarca-Pina, G. (2006). Chemopreventive activity of polyphenolics from black jamapa bean (Phaseolus vulgaris L.) on HeLa and HaCaT cells. *J. Agric. Food Chem. 54*:21162122.

Aschner JL, Aschner M, 2005. Nutritional aspects of manganese homeostasis. *Molecular Aspects of Medicine 26*, 353–362.

Ashraf, U.; Kanu, A.S.; Mo, Z.; Hussain, S.; Anjum, S.A.; Khan, I.; Abbas, R.N.; Tang, X. (2015). Lead toxicity in rice: Effects, mechanisms, and mitigation strategies—a mini review. *Environ. Sci. Pollut. Res.* 22, 18318–18332.

Ashraf, U.; Kanu, A.S.; Deng, Q.; Mo, Z.; Pan, S.; Tian, H.; Tang, X.(2017). Lead (Pb) toxicity; physio-biochemical mechanisms, grain yield, quality, and Pb distribution proportions in scented rice.*Front. Plant Sci.* 8, 259.

ATSDR.CERCLA. (1999). List of priority hazardous substances. Atlanta, GA: Agency for Toxic Substances and Disease Registry.

Augustin J, Beck C. B., Kalbfleish, G., Kagel, L. C. and Matthews, R. H. (2000).Variation in the vitamin and mineral content of raw and cooked commercial Phaseolus vulgaris classes.*J. Food Sci. 46*:1701–1706.

Avery JC, Hoffmann PR. (2018). Selenium, Selenoproteins, and Immunity. *Nutrients 10*(9):1203. https://doi.org/10.3390/nu10091203

Babula P, Vojtech A, Opatrilova R, Zehnalek J, Havel L, Kizek R. (2008). Uncommon heavy metals, metalloids and their plant toxicity: a review. *Environ Chem Lett;6*: 189213.

Bahiru DB, Teju E, Kebede T, Demissie N. (2019). Levels of some toxic heavy metals (Cr, Cd and Pb) in selected vegetables and soil around eastern industry zone, central Ethiopia. *African Journal of Agricultural Research. 14*(2):92-101.

Balali-Mood Mahdi, Naseri Kobra, Tahergorabi Zoya, Khazdair Mohammad Reza, Sadeghi Mahmood. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Frontiers in Pharmacology; 12*: 1663-9812 DOI=10.3389/fphar.2021.643972

Baldwin BC, Bray MF, Geoghegan MJ.(1966). the microbial decomposition of paraquat.*Biochemical Journal 101*: 15

Banjo, A. D., Aina, S. A., & Rije, O. I. (2010). Farmers' knowledge and perception towards herbicides and pesticides usage in Fadama area of Okun-Owa, Ogun state of Nigeria. *African Journal of Basic and Applied Science.2*(56), 188194

Batista K. A, Prudencio, S. H, Fernandes K. F. (2011). Wheat bread enrichment with hard-to-cook bean extruded flours: nutritional and acceptance evaluation. *Journal of Food Science; 76(*1):108-S113 http://dx.doi.org/10.1111/j.1750-3841.2010.01969.x

Beninger, C. W. and Hosfield, G. L. (2003).Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from Phaseolus vulgaris L. seed coat color genotypes.*J. Agric. Food Chem. 51*:78797883.

Bertinato J. , Wu Xiao C, Ratnayake W.M. (2015). “Lower serum magnesium concentration is associated with diabetes, insulin resistance, and obesity in South Asian and white Canadian women but not men,” *Food & Nutrition Research*, vol. 59, no. 1, article 25974

Berrios J. J, Swanson B. J and Chaong W. A. (1999). Physico-chemicalcharacterization of stored black beans (Phaseolus vulgaris L.). *Food Res. Int*.669–676.

Bliss FA, Brown JWS (1983) Breeding common bean for improved quantity and quality of seed proteins. In: Janick J (ed) Plant Breeding reviews, vol 1. AVI, Westport Conn, USA, pp 59–102

Bini C.W, Dr. Mohammad F, Silvia M, Laura. (2012). Effects of heavy metals on morphological characteristics of Taraxacum officinale Web growing on mine soils in NE Italy. *Journal of Geochemical Exploration. 10*.1016/j.gexplo.2012.07.009.

Brama M, Politi L, Santini P, Migliaccio S, Scandurra R. (2012). Cadmium-induced apoptosis and necrosis in human osteoblasts: role of caspases and mitogen-activated protein kinases pathways. *J Endocrinol Invest*; 35:198–208.

Buchman AR. Manganese. In: A. Catharine Ross BC, Robert J. Cousins, Katherine L. Tucker, Thomas R. Ziegler. (2014). Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 238-44.

Biester H, Müller G, Schöler HF. (2002). Binding and mobility of mercury in soils contaminated by emissions from chlor-alkali plants. *Sci Total Environ;284*:191–3.

Bitocchi E, Rau D, Rodriguez M, and Murgia M. L. (2016). Crop improvement of Phaseolus spp. through interspecific and intraspecific hybridization, in Polyploidy and Hybridization for Crop Improvement, ed. A. Mason (Boca Raton, FL:CRC Press), 218280. doi: 10.1201/9781315369259-11.

Bluskov, S., Arocena J.M., Omotoso O.O., Young, J.P. (2005).Uptake, distribution, and speciation of chromium in Brassica Juncea.*Int J Phytorem;7*:1535.

Bodnar, M., Konieczka, P., and Namiesnik, J. (2012).The properties, functions, and use of selenium compounds in living organisms.*J. Environ. Sci. Health Part C 30,* 225–252. doi: 10.1080/10590501.2012.705164

Bojinova P, Georgiev B, Krasteva V, Chuldjian H, and Stainislavova L. (1994). Investigation about the heavy metal pollution in soils and agricultural crops in region of nonferrous metal works, D. Bagoev, Soil Science, Agrochemistry and Ecology, 4-6, p 32.

Booth N.H, McDowell J.R. (1975). Toxicity of hexachlorobenzene and associated residues in edible animal tissues. *J. Am. Vet. Med. Assoc; 166*, 591595.

Bravo, L., Siddhurahu, P. and Saura-Calixto, F. (1998). Effect of various processing methods on the in vitro starch digestibility and resistant starch content of Indian pulses.*J. Agric. Food Chem. 46*:4667–4674.

Bressani, R. (1993). Grain quality of common beans. *Food Rev. Int. 9*(2):237297.

Broughton, W. J., Hernández, G., Blair, M., Beebe, S., Gepts, P, and Vanderleyden, J. (2003).Beans (Phaseolus spp.): model food legumes.*Plant Soil 252*, 55128.doi: 10.1023/A:1024146710611

Butt, S.M. and Batool, R. (2010) Nutritional and Functional Properties of Some Promising Legumes Protein Isolates. *Pakistan Journal of Nutrition, 9,* 373-379. http://dx.doi.org/10.3923/pjn.2010.373.379

Camaschella, C., Nai, A., & Silvestri, L. (2020). Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica, 105(2), 260–272. https://doi.org/10.3324/haematol.2019.232124

Campos-Vega, R., Loarca-Pina, G. and Oomah, B. D. (2010).Minor components of pulses and their potential impact on human health.*Food Res. Int. 43*:461–582.

Castro E, Manas P, Heras JDL (2009). A comparison of the application of different waste products to a lettuce crop effects on plant and soil properties. *Sci Hortic;123*:148-155

Caussy,D., Gochfeld, M., Gurzau, E., Neagu, C., Ruedel, H. (2003). Lessons from case studies of metals: investigation exposure, bioavailability, and risk. *Ecotoxicol Environ Safe;56*:45–51.

Chao Y, Hamel C, Vujanovic V, Gan Y. (2011). Fungicide: Mode of action and possible impact on non-target microorganisms. IRSN Ecology.Article ID 130239. http: //dx.doi.org/10.5402/2011/ 130289.

Chaves LHG, Estrela MA, Sena de Souza R (2011). Effect on plant growth and heavy metal accumulation by sunflower. *J Phytol; 3*(12):04-09

Chen P, Bornhorst J, Aschner M. Manganese metabolism in humans. Front Biosci (Landmark Ed) 2018;23:1655-79.

Chen CW, Chen CF, Dong CD. (2012). Distribution and Accumulation of Mercury in Sediments of Kaohsiung River Mouth, Taiwan. *APCBEE Procedia 1*:153–158.

Chen W, Chang AC, Wu, L. (2007). Assessing long term environmental risks of trace elements in phosphate fertilizers.*Ecotoxicol Environ Safe;67*:48–58.

Choung, M. G., Choi, B. R., An, Y. N., Chu, Y. H. and Cho, Y. S. (2003). Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris L.*). *J. Agric. Food Chem. 51*:70407043.

Chung, H. J., Liu, Q., Pauls, K. P., Fan, M. Z. and Yada, R. (2008).In vitro starch digestibility, expected glycemic index and some physicochemical properties of starch and flour from common bean (*Phaseolus vulgaris L.*) varieties grown in Canada.*Food Res. Int. 41*:869875.

Codex Alimentarius Commission (CAC). Joint FAO/WHO Food Standards Program. 1993;391.

Collins JF. Copper. In: Ross AC, Caballero, B., Cousins, R.J., Tucker ,K.L., Ziegler,T.R. (Eds).(2014).*Modern Nutrition in Health and Disease.* (11thed.). Baltimore, MD: Lippincott Williams & Wilkins; 206-16.

Commission Regulation (EC) No 1881/2006.*Setting Maximum Levels for Certain Contaminants in Foodstuffs; Official Journal of the European Union; European Union:* Brussels, Belgium, 2006. Retrieved from https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF (accessed on 19 November 2021).

Cormick G, and Belizán J.M. (2019). Calcium intake and health. Nutrients 11: E1606.
Office of Dietary Supplements - Calcium. Accessed July 7, 2021.

Costa G. E. A, Queiroz-Monici K. S, Reis S. M. P. M. and Oliveira A. C. (2006). Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. *Food Chem.94*:327330.

De Baaij J. H. F., Hoenderop J. G. J., Bindels R. J. M. (2015). Magnesium in man: implications for health and disease. *Physiological Reviews; 95*(1):1–46.

Deng Q, Liu J, and Li Q. Interaction of occupational manganese exposure and alcohol drinking aggravates the increase of liver enzyme concentrations from a cross-sectional study in China, Environmental Health, vol. 12, no. 1, p. 30, 2013.

Driscoll C., T., Yah, C., Schofield, C., L., Munson, R., Holsapple, J. (1994).The mercury cycle and fish in the Adirondack lakes.*Environ Sci Technol;28*:136A43A.

Duffus, J., H. (2002). Heavy metals a meaningless term?(IUPAC Technical Report). *Pure and applied chemistry.74*:793-807.

Duruibe, J., O., Ogwuegbu, M., O., C., Egwurugwu J., N. (2007).Heavy metal pollution and human biotoxic effects. *International Journal of physical sciences*. *2*:112-8.

El Kutry and El-Sahar Beni-Suef. (2020). University Journal of Basic and Applied Sciences; 9:18 https://doi.org/10.1186/s43088-020-00043-1

Egounlety M, and Aworh O.C. (2003).Effectofsoaking,dehulling,cookingandfermentationwithRhizopusoligosporusonthe oligosaccharides,trypsininhibitor,phyticacidandtanninsofsoybean(GlycinemaxMerr.),cow pea(VignaunguiculataL.Walp)andgroundbean(MacrotylomageocarpaHarms).*J. Food Eng; 56* (2-3):249-254

Evan H, Matt B, and Kate J. (2007). The Fate and Effect of Gyphosphate on Amphibians.Final Report ENAC 202.

European Union. Regulation Number 396/2005 of the European parliament and the Council on Maximum Residue Level of Pesticides in or on Food and Feed of Plant and Animals Origin and Amending council Directive 91/414 EEC. 2005.

Ezeji E.U, Ogbulie T.E, Onwurah I.N.E. and Ujowundu, C.O. (2014). Effects of Dichlorvos on Reduced Glutathione, Glutathione S-Transferase and Lipid Peroxidation of Egg Laying Hens.*International Journal of Chemical and Biological Sciences. 1* (1) 46

Ezike N.O, Munonye J and Esiegwu A. (2020). Comparative Evaluation of the Proximate Composition, Anti-Nutrients and Functional Properties of Some Underutilized Pulses. *Food Science and Quality Management.* 2225-0557.

FAO.(2005). Proceedings of the Asia Regional Workshop, Regional Office for Asia and the Pacific, Bangkok.

FAO/WHO. Joint meeting on Pesticide. Residues. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/39358/9241543434-eng.pdf?sequence=1&isAllowed=y (accessed on 19 November 2021).

FAO/WHO. Pesticide residues in food and feed: maximum residual limits;2013. Codex alimentarius.net/pesters/data/pesticides/details.

FAO. Regulation (EC) No. 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Rome, Italy: Food and Agriculture Organization of the United Nations; 2005. [Google Scholar]

FAOSTAT (2017) Food and Agriculture Organization of the United Nations. In: FAOSTAT Database Gatew

Fergusson JE, editor. The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Oxford: Pergamon Press; 1990.

Fiorentini D, Cappadone C, Farruggia G, and Prata C. (2021). Magnesium: Biochemistry, Nutrition, Detection, and Social Impact of Diseases Linked to Its Deficiency. *Nutrients; 13*(4), 1136. https://doi.org/10.3390/nu13041136

Food and Agriculture Organization (FAO).Working Party of Experts and the WHO ExpertCommittee on Pesticide Residues. Retrieved from https://inchem.org/documents/jmpr/jmpmono/v067pr14.htm (accessed on 19 November 2021).

Funderburk HH. (1969). Diquit and paraquat.In PC Kearney and DD Kaufman (Ed.) *Degradation of herbicides*. Marcel Dekker, New York. 283298.

Fytianos K, Raikos N, Theodoridis G, Velinova Z, and Tsoukali H. (2006). Solid phase microextraction applied to the analysis of organophosphorous insecticides in fruits. *Chemosphere; 65* (11): 2090-2095.

Gargouri M, Soussi A, Akrouti A, Magné C, El Feki A (2018) Ameliorative effects of spirulina platensis against lead-induced nephrotoxicity in newborn rats: modulation of oxidative stress and histopathological changes. *EXCLI J 17*:215–232.

Gepts P, and Bliss F A. (1984). Enhanced available methionine concentration associated with higher phaseolin levels in common bean seeds. *Theor. Appl. Genet. 69*, 47–53.

Gepts, P. and Dpbouk, D. (1991). Origin, domestication, and evolution of the common bean (Phaseolus vulgaris L.). In: Common Beans: Research for Crop Improvement, pp. 753. Van Schoonhoven, A. and Voyset, O., Eds., CAB International, Wallingford, UK.

Goel A, Aggarwal P. (2007). Pesticide Poisoning. *The National Medical Journal of India; 20*:182–191.

Gomes F, Khadilkar A, Owino V, Pettifor J. M, Rana Z. H, Roth D. E, and Weaver C. (2022). Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes. *Annals of the New York Academy of Sciences; 1512*(1), 10–28. https://doi.org/10.1111/nyas.14758

Gonçalves A., Goufo P., Barros A., Domínguez-Perles R., Trindade H., Rosa E. A., et al.. (2016). Cowpea (Vigna unguiculata L. Walp), a renewed multipurpose crop for a more sustainable Agri-food system: nutritional advantages and constraints. *J. Sci. Food Agric; 96*, 2941–2951.
doi: 10.1002/jsfa.7644,

Goyer RA. (2001). Toxic effects of metals. In: Klaassen CD, editor. Cassarett and Doull's Toxicology: The Basic Science of Poisons. New York: McGraw-Hill Publisher; pp. 811–867.

Granito M, Palolini M, and Perez S. (2008). Polyphenols and antioxidant activity of Phaseolus vulgarisstored under extreme conditions and processed. LWT.41:994999.

Grillo A, Salvi L, Coruzzi P, Salvi P, and Parati G. (2019). Sodium Intake and Hypertension. *Nutrients.11*(9):1970.
https://doi.org/10.3390/nu1109197

Grober U, Schmidt J, and Kisters K. (2015). Magnesium in prevention and therapy," Nutrients, vol. 7, no. 9, pp. 8199–8226.

Guillin O. M, Caroline V, Théophile O, and Laurent C. (2019). "Selenium, Selenoproteins and Viral Infection" Nutrients 11, no. 9: 2101. https://doi.org/10.3390/nu11092101

Gupta PK. (2004). Pesticide exposureIndian scene.*Toxicology* 198: 83 90.

Hambidge K.M. (1987). Zinc. In: Trace elements in human and animal nutrition. Mertz, W., ed. 5th, Vol. 1., p.1-137. Orlando, Florida, Academic Press, Inc.

Handa S., Agnihotri N. and Kulshrestha G. (1999). Pesticide Residues: Significance, Managemen and Analysis. Research Periodical and Books Publishing House, USA.p1-31.

Hanisch C. (1998). Where is mercury deposition coming from? Uncertainties about the roles of different natural and synthetic sources are fueling the debate on how to regulate emissions. *Environ Sci Technol; 32*: 176A–9A.

Hartikainen H. (2005). Biogeochemistry of selenium and its impact on food chain quality and human health. *J. Trace Elem. Med. Biol. 18*, 309–318. doi: 10.1016/j.jtemb.2005.02.009

Hatfield D.L, Tsuji P.A, Carlson B.A, and Gladyshev V.N. (2014). Selenium and selenocysteine: roles in cancer, health, and development. *Trends.Biochem. Sci; 39*, 112–120. doi: 10.1016/j.tibs.2013.12.007

Henke K.R, (2009). Arsenic in natural environments. In: Henke, K. (Ed.), Arsenic: Environmental Chemistry, Health Threats and Waste Treatment. John Wiley & Sons Ltd., Chichester, UK, pp. 69–235.

Henson MC, Chedrese PJ. (2004). Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. Exp Biol Med (Maywood);229(5):383–392.

Herawati N, Suzuki S, Hayashi K, Rivai IF, Koyoma H. (2000). Cadmium, copper and zinc levels in rice and soil of Japan, Indonesia and China by soil type. *Bull Env Contam Toxicol;64:*33–39.

He ZL, Yang XE, Stoffella PJ. (2005). Trace elements in agroecosystems and impacts on the environment. *J Trace Elem Med Biol;19*(2–3):125–140.

Hinderling PH. (2016). The pharmacokinetics of potassium in humans is unusual. *J Clin Pharmacol;56*:1212-20.

Hosfield, G. L., Varner, G. V., Uebersax, M. A. and Kelly, J. D. (2004).Registration of Merlot small red bean. *Crop Sci. 44*:351352.

Iliya HA, Wannang NN, Falang KD, Nehemiah JA (2012).Evaluation of some pesticide residues in stored beans in Jos, Nigeria. *West African Journal of Pharmacology and Drug Research 28*:1-5

Imran H, Asif A , Tariq M , Anwaar A and Shaukat B. (2014) Nutritional and Health Perspectives of Beans (Phaseolus vulgaris L.): An Overview, Critical Reviews in Food Science and Nutrition, 54:5, 580-592, DOI:10.1080/10408398.2011.596639

Integrated risk information system (IRIS) (2019). Assessments: list A to Z chemical assessments [Internet]. Washington D.C.: Environmental Protection Agency; [updated 2019 Apr 17; cited 2019 Jun 11].

Institute of Medicine.Food and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press; 1998.

Institute of Medicine.Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc : a Report of the Panel on Micronutrients. Washington, DC: National Academy Press; 2001.

2015–2020 Dietary Guidelines for Americans.8th Edition. December 2015

Integrated risk information system (IRIS) (2019). Assessments: list A to Z chemical assessments [Internet]. Washington D.C.: Environmental Protection Agency; [updated 2019 Apr 17; cited 2019 Jun 11].

Available from: https://cfpub.epa.gov/ncea/iris_drafts/atoz.cfm?list_type=alpha

International Food Standards/Codex Alimentarius FAO/WHO. Available online: https://en.wikipedia.org/wiki/Pesticide_residue (accessed on 19 November 2021).

International Food Standards/Codex Alimentarius FAO/WHO. Available online: https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/en/?p_id=171 (accessed on 19 November 2021).

International Food Standards/Codex Alimentarius FAO/WHO. Available online: https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/en/?p_id=43 (accessed on 19 November 2021).

Jarup, L. (2003). Hazards of heavy metal contamination.*Br Med Bull;68*:167–82.

Jenkins A. L. (2007). The glycemic index: Looking back 25 years. *Cereal Foods World; 52*:5053.

Jepleting N, Sila D. N, Orina I. N. (2022). Nutritional Composition and Antinutrient to Mineral Molar Ratios of Selected Improved Common Beans Grown in Kenya. *Curr Res Nutr Food Sci; 10*(3).

doi : http://dx.doi.org/10.12944/CRNFSJ.10.3.35

Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Available online: https://nj.gov/health/eoh/rtkweb/documents/fs/0596.pdf (accessed on 19 November 2021).

Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Available online: https://ec.europa.eu/food/system/files/202012/scicom_scf_out03_en.pdf (accessed on 19 November 2021).

Jones G. (2008).Pharmacokinetics of vitamin D toxicity.*American Journal of Clinical Nutrition.88*(2):582S6S.

Jone K.C. and de Voogt P. (1999) Persistent Organic Pollutants (POPs): State of the Science. *Environmental Pollution; 100*, 209-221.

José F, Cruz R, Júnior De Almeida H, Maria D and Dos SantosM, Growth, nutritional status and nitrogen metabolism in Vignaunguiculata (L.) Walp is affected by aluminum. Aust J Crop Sci8:1132– 1139 (2014)

Kabata-Pendias A. (2004). Soil–plant transfer of trace elements—an environmental issue.Geoderma.122:143–9.

Kamrin, M.A. (1997). Pesticide Profiles: Toxicity, Environmental Impact, and Fate (1st ed.). CRC Press.
https://doi.org/10.1201/9780367802172

Karikari S.K. and Molatakgosi, G. (1999) Response of Cowpea (Vigna unguiculata (L) Walp) Varieties to Leaf Harvesting on Botswana. UNISWA Journal of Agriculture, 8, 5-11. https://doi.org/10.4314/uniswa.v8i1.4611

Kaur N, Sharma S, and Kaur S. (2014). Selenium in agriculture: a nutrient or contaminant for crops? *Arch. Agron. Soil Sci. 60*, 1593–1624. doi: 10.1080/03650340.2014.918258

Kelly J.D, Hosfield G.L, Varner G.V, Uebersax M.A, Ender M, and Taylor J. (2003). Registration of Seahawk navy bean. *Crop Sci; 43*: 23072308.

Khan M, Muhammad S, Malik R.K, Sabaz A, and Tariq M. (2015). Heavy metals potential health risk assessment through consumption of wastewater irrigated wild plants: A case study. *Human and Ecological Risk Assessment.* 22(1)
DOI 10.1080/10807039.2015.1056292.

Khalid II and Elharadallou SB. (2013). Functional properties of cowpea (Vignaungiculata L. Walp), and Lupin (Lupinus termis) flour and proteinisolates. *J Nutr Food Sci* 3:1–6.

King JC, Cousins RJ. (2014). Zinc. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, eds. Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins;189-205.

Kirse A and Karklina D. (2015). Integrated evaluation of cowpea (Vignaunguiculata (L.) Walp.) and maple pea (Pisum sativum var. arvenseL.) spreads. *Agron Res;13*:956–968

Kutos T, Golob T, Kac M, and Plestenjak A. (2003). Dietary fibre content of dry and processed beans. *Food Chem. 80*:231235.

Kyomugasho C, Kamau P, Aravindakshan. (2021). Evaluation of Storage Stability of Low Moisture Whole Common Beans and Their Fractions Through the Use of State Diagrams. *Food Res Int;140*:109794.

Lallas P. (2001). Reproductive Eff ects in Birds Exposed to Pesticides and Industrial Chemicals. The Stockholm Convention on persistent organic pollutants. *American Journal of International Law 95*: 692708.

Lee P-K, Kang M-J, Yu S, Ko K-S, Ha K, Shin S. (2017).Enrichment and geochemical mobility of heavy metals in bottom sediment of the Hoedong reservoir, Korea and their source apportionment. *Chemosphere;184*:74-85.

Lee S, Cho S.R, Jeong l, Park J.B, Shin M.Y, Kim S, Kim J.H. (2020). Mercury Exposure and Associations with Hyperlipidemia and Elevated Liver Enzymes: A Nationwide Cross-Sectional Survey. *Toxics* ; 8, 47

Li L, Yang X. The Essential Element Manganese, Oxidative Stress, and Metabolic Diseases: Links and Interactions. Oxid Med Cell Longev 2018: 7580707.

Lin L.Z, Harnly J.M, Pastor-Corrales M.S, and Luthria D.L. (2008). The polyphenolic profiles of common beans (*Phaseolus vulgaris L.*). *Food Chem;107*: 399410.

Liu R, Bao K, Yao S, Yang F, Wang X. (2018). Ecological risk assessment and distribution of potentially harmful trace elements in lake sediments of Songnen Plain, NE China. *Ecotoxicology and environmental safety; 163*:117-24.

Los F.G.B, Zielinski A.A.F, Wojeicchowski J.P, Nogueira A, Demiate I.M. Beans (Phaseolus vulgaris L.): Whole Seeds with Complex Chemical Composition. Curr Opin Food Sci. 2018;19:63-71. doi:10.1016/J.COFS.2018.01.010

Luthria D.L, Pastor-Corrales M.A. (2006). Phenolic acids content of fifteen dry edible bean (Phaseolus vulgaris L.) varieties. *J. Food Compos. Anal; 19*, 205–211.

Mabaleha M.B, and Yeboah S.O. (2004). Characterization and compositional studies of the oils from some legume cultivars, Phaseolus vulgaris, grown in Southern Africa. *J. Am. Oil Chem. Soc; 81*, 361-364.

Mahaffey KR. (1990). Environmental lead toxicity: nutrition as a component of intervention. *Environ Health Perspect; 89*:75–78.

Malagoli M, Schiavon M, dall'Acqua S, and Pilon-Smits E.A. (2015). Effects of selenium biofortification on crop nutritional quality. *Front Plant Sci.* 6:280.
doi: 10.3389/fpls.2015.00280

Mansour SA (2004) Pesticide exposure Egyptian scene. *Toxicology 198*: 91-115.
Link: https://tinyurl.com/y7v4as6t

Martin S, Griswold W. (2009). Human health effects of heavy metals. Environmental Science and Technology Briefs for Citizens;(15):1–6.

Marchiol L, Assolari S, Sacco P, Zerbi G. (2004). Phytoextraction of heavy metals by canola (Brassica napus) and radish (Raphanus sativus) grown on multicontaminated soil. *Environ Pollut. 132*:21–7

Massányi P, Massányi M, Madeddu R, Stawarz R, Lukáč N. (2020). Effects of Cadmium, Lead, and Mercury on the Structure and Function of Reproductive Organs. Toxics, 8, 94.

Massanyi P, Lukac N, Uhrin V, Toman R, Pivko J, Rafay J, Forgács Z, Somosy Z. 2007). Female reproductive toxicology of cadmium. *Acta Biol. Hung;58*:287–299.
doi: 10.1556/ABiol.58.2007.3.5.

Maung M.T, Carlson A, Olea-Flores M, Elkhadragy L, Schachtschneider K.M, Navarro-Tito N, Padilla-Benavides T. (2021). The molecular and cellular basis of copper dysregulation and its relationship with human pathologies. *FASEB J; 35*, 1–50.

McCarty K.M, Hanh H.T, Kim K.W. (2011). Arsenic geochemistry and human health in South East Asia. *Rev. Environ. Health 26*, 71–78.

Méplan C. (2011). Trace elements and ageing, a genomic perspective using selenium as an example. *J. Trace Elem. Med. Biol. 25*, S11–S16.
doi: 10.1016/j.jtemb.2010.10.002

Messina V. (2014). Nutritional and health benefits of dried beans. Am. *J. Clin. Nutr.100*, 437S442S.
doi: 10.3945/ajcn.113.071472.

Monkiedje A, and Spiteller M. (2002). Sorptive behavior of the phenylamide fungicides, mefenoxam and metalaxyl, and their acid metabolite in typical Cameroonian and German soils. *Chemosphere; 49*(6), 659–668.
https://doi.org/10.1016/s0045-6535(02)00325-9

Morais S, Costa FG, Pereira ML. (2012). Heavy metals and human health, in Environmental health – emerging issues and practice (Oosthuizen J ed), pp. 227–246, InTech.

Murray-Kolbe LE, Beard J. Iron. In: Coates PM, Betz JM, Blackman MR, et al., eds. Encyclopedia of Dietary Supplements. 2nd ed. London and New York: Informa Healthcare; 2010:432-8.

NAFDAC (2003). National Agency for Food and Drug Administration and Control. Consumer Safety Bulletin; 2 (3): 6-17.

Nagraj SK, Naresh S, Srinivas K, George RP, Shetty N, Levenson D. (2017). Interventions for the managing taste disturbances. Cochrane Database Syst Rev:CD010470.

National Academies of Sciences, Engineering, and Medicine.(2019). Dietary Reference Intakes for Sodium and Potassium. Washington, DC: The National Academies Press.

National Research Council Committee on Copper in Drinking Water. Copper in Drinking Water. Washington, DC: National Academies Press; 2000.

Nielsen F.H. (2018). "Magnesium deficiency and increased inflammation: current perspectives," Journal of Inflammation Research, vol. 11, pp. 25–34.

Nogara P.A, Oliveira C.S, Schmitz G.L, Piquini P.C, Farina M, Aschner M. (2019). Methylmercury's chemistry: From the environment to the mammalian brain. Biochim. Biophys. Acta. Gen. Subj. 1863, 129284. 10.1016/j.bbagen.2019.01.006

Novoa-Munoz JC, Pontevedra-Pombal X, Martinez-Cortizas A, Garcia-Rodeja Gayoso E. (2008). Mercury accumulation in upland acid forest ecosystems nearby a coal-fired power-plant in Southwest Europe (Galicia, NW Spain). *Sci Total Environ; 394*:303–12.

Nriagu JO. A global assessment of natural sources of atmospheric trace metals. Nature. 1989;338:47–49.

Nwadike C, Okere A, Nwosu D, Okoye C, Vange T, Apuyor B. (2018). Proximate and Nutrient Composition of Some Common Bean (Phaseolus vulgaris L.) and Cowpea (Vigna unguiculata L. Walp.) Accessions of Jos- Plateau, Nigeria. *Journal of Agriculture and Ecology Research International. 15.* 1-9. 10.9734/JAERI/2018/42138.

Nwankwoala H.O. and T Warmate T. (2014). Subsurface Soil Characterization of a Site for Infrastructural Development Purposes in D/Line, Port Harcourt, Nigeria. American *International Journal of Contemporary Research, 4* (6):139-148.

Obida MG, Stephen SH, Goni AD, Victor OO (2012). Pesticide Residues in Bean Samples from Northeastern Nigeria. *ARPN Journal of Science and Technology; 2*(2):34-37.

Okoroiwu H.U, and Iwara I.A. (2018). Dichlorvos toxicity: A public health perspective. *Interdisciplinary toxicology, 11*(2),129137. https://doi.org/10.2478/intox-2018-0009

Ogah CO, Coker HB (2012). Quantification of Organophosphate and Carbamate Pesticide Residues in Maize. *Journal of Applied Pharmaceutical Science 2*: 093-097.

Odewale G.O, Sosan M.B, Oyekunle J.A.O, Adeleye A.O. (2021). Human health risk assessment of dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) pesticide residues in fruits and vegetables in Nigeria. *Environ. Sci. Pollut. Res. 28,* 3313333145.

Ogah CO, Tettey J, Coker HB and Adepoju-Bello AA. (2012). Analysis of Organochlorine pesticide residues in beans from markets in Lagos State, Nigeria. *West African Journal of Pharmacy.*23(1): 60 68.

Ohta, H., and Ohba, K. (2020). Involvement of metal transporters in the intestinal uptake of cadmium. *J. Toxicol. Sci; 45* (9), 539–548. doi:10.2131/jts.45.539

Okereafor U, Makhatha M, Mekuto L, Uche-Okereafor N, Sebola T, Mavumengwana V. (2020). Toxic Metal Implications on Agricultural Soils, Plants, Animals, Aquatic life and Human Health. *International Journal of Environmental Research and Public Health; 17*(7):2204. https://doi.org/10.3390/ijerph17072204

Olopade O, Odeniyi I, Iwuala S.O, Kayode O.O, Fasanmade O, Ajala M, Po C, and Ohwovoriole A. (2017). Comparison of glycaemic indices of some local beans (Vigna unguiculata [Linn] Walp varieties) in Nigerians. *Journal of Endocrinology, Metabolism and Diabetes of South Africa. 22*. 1-5. 10.1080/16089677.2017.1378461.

Oshatunberu M, Oladimeji A, Sawyerr HO, Raimi MO (2023). Searching for What You Can't See - Evaluation of Pesticide Residues in Grain Sold at Selected Markets of Southwest Nigeria. *Current Research in Public Health. 3*. 10-36. 10.31586/crph.2023.566

Otitoju O, and Lewis C.C. (2021). Health risk assessment of pesticide residues in bean samples from Wukari, Taraba State, Nigeria. *Journal of Environmental Chemistry and Ecotoxicology, 13*(1), 1-13.

Ovando-Martinez M, Luis A, Bello-Pereza L.A, Whitney K, Osorio-Diaz P, Senay S. (2011). Starch characteristics of bean (*Phaseolus vulgaris L*.) grown in different localities. *Carboh.Polym. 85*:54–64.

Parbhu B, Rodgers G, Sullivan J. E. (2009). Death in a toddler following endosulfan ingestion. *Clin Toxicol (Phila). 47*(9):899-901

Papa R, Acosta J, Delgado-Salinas A, and Gepts P. (2005). A genome-wide analysis of differentiation between wild and domesticated *Phaseolus vulgaris* from Mesoamerica. *Theor. Appl. Genet.111*:11471158.

Papa R, and Gepts P. (2003). Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris L.*) from Mesoamerica. *Theor. Appl. Genet;106*:239250.

Preuss HG, Clouatre DL. (2012). Sodium, chloride, and potassium. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell; 475-92.

Prohaska JR. (2012). Copper. In: Erdman JW, Macdonald IA, Zeisel SH, eds. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell; 540-53.

Rada RG, Wiener JG, Bailey PA, Powell DE. (1990). Recent influxes of metals into Lake Pepin, a natural lake on the Upper Mississippi River. *Arch Environ Contam Toxicol. 19*:712–6.

Rai Y, Pathak R, Kumari N, Sah DK, Pandey S, Kalra N, Soni R, Dwarakanath BS, Bhatt AN. (2018). Mitochondrial biogenesis and metabolic hyperactivation limits the application of MTT assay in the estimation of radiation induced growth inhibition. *Scientific Reports 8*(1):1531

Rasmussen R.R, Poulsen M.E, and Hansen H.C. (2003). Distribution of multiple pesticide residues in apple segments after home processing. *Food addictives and Contaminates; 20*(11): 1044-63.

Reid M.E, Duffield-Lillico A.J, Slate E, Natarajan N, Turnbull B, Jacobs E. (2008). The nutritional prevention of cancer: 400 mcg per day Se treatment. *Nutr.Cancer.60*, 155–163. doi: 10.1080/01635580701684856

Reilly C. (2007). Pollutants in Food – Metals and Metalloids, in Mineral Components in Foods, (Szefer P, Nriagu JO eds), pp. 363–388, Taylor & Francis Group, Boca Raton, FL.

Reynoso-Camacho R, Ramos-Gomez M, and Loarca-Pina G. (2006). Bioactive components in common beans (*Phaseolus vulgaris L.*). *Advances Agric. Food Biotechnol., 217*–236.

Riaz U, Sajid I, Parwai, AB, Abbas B and Akhlaq A. (2015). Gas Chromatographic Multi-Residue Pesticide Determination Method for Cereal Grains. *American-Eurasian Journal of Agricultural and Environmental Science. 15*(8): 1617-1624.

Ricci AJ, Wu YC, Fettiplace R. (1998). The endogenous calcium buffer and the time course of transducer adaptation in auditory hair cells. J. Neurosci 18, 8261–77.

Riedt CS, Cifuentes M, Stahl T, Chowdhury HA, Schlussel Y, Shapses SA. Overweight postmenopausal women lose bone with moderate weight reduction and 1 g/day calcium intake. Journal of Bone and Mineral Research. 2005;20(3):455–63.

Rubin R, Strayer DS. Rubins pathology; Clinicopathologic Foundations of Medicine. 5th ed. Lippincot Williams & Wilkins; 2008. Environmental and Nutritional pathology.

Ryu M-S, Aydemir TB. Zinc. In: Marriott BP, Birt DF, Stallings VA, Yates AA, eds. Present Knowledge in Nutrition. 11th ed. Cambridge, Massachusetts: Wiley-Blackwell; 2020:393-408.

Saifullah M, andRabbani M.G.(2009)EvaluationandCharacterizationofOkra(AbelmoschusesculentusL.Moench.)Genotypes.SAARCJournalofAgriculture,7,92-99.

Sai-Ut S, Ketnawa S, Chaiwut P, and Rawdkuen S. (2009). Biochemical and functional properties of protein from red kidney, navy and adzuki beans. As. *J. Food Agric. Ind. 2*(04):493-504.

Samira N, Mohammad S, Mohadeseh P, Mahsa A, Parisa S, Mohammad D, Mohammadreza N. (2021). Potential Harmful Effects of Heavy Metals as a Toxic and Carcinogenic Agent in Marine Food-An Overview. Egyptian Journal of Veterinary Sciences. Article 13, Volume 52, Issue 3.

DOI: 10.21608/ejvs.2021.83716.1245

Sataka MY, Mido MS, Sethi SA, Iqbal HY and Taguchi S (1997) Environmental toxicology. Discovery Publishing House, New Delhi, India. Pp. 185-197.

SebethaET,ModiATandOwoeyeLG,Cowpeacrudeproteinasaffectedbycroppingsystem,siteandnitrogenfertilization.JAgricSci7:224–234(2014)

Sehirali S. (1998). Leguminosae Book. Ankara University, Faculty of Agriculture, 1089. Lesson Book: 314, Ankara, pp: 435.

Shanbhag V.C , Gudekar N., Jasmer K., Papageorgiou C., Singh K., Petris J.M., (2020). Copper metabolism as a unique vulnerability in cancer.

https://doi.org/10.1016/j.bbamcr.2020.118893

ShankarAH,PrasadAS. (1998). Znandimmunefunction:thebiologicalbasisofalteredresistancetoinfection.*Am J Clin Nutr;68*:447S–463S.

Shimelis E. A. and Rakshit, S. K. (2005).Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris L.)* varieties grown in Ethiopia. *LWT.38*:331338.

Siddiq M, Ravi R, and Dolan K.D. (2010). Physical and functional characteristics of selected dry bean (Phaseolus vulgaris L.) flour. LWT-Food Sci Technol. 43:232237.

Singh K. (2018). Human Health Risks of Persistent Organic Pollutant Exposures in the Canadian Arctic. Ph.D. Thesis, Department of Biology, University of Ottawa, Ottawa, ON, Canada; p. 236.

Sokol RZ, Berman N. (1991). The effect of age of exposure on lead-induced testicular toxicity. *Toxicology;69:*269–78.

Stolarz-Skrzypek K, Kuznetsova T, Thijs L, Tikhonoff V, Seidlerová J, Richart T. (2011). European Project on Genesin Hypertension (EPOGH) Investigators. Fatal and nonfatal outcomes,incidence of hypertension, and blood pressure changes in relation tourinary sodium excretion. *JAMA;305:*1777-85

Soliman K.M. (2001) Changes in concentration of pesticide residues in potatoes during washing and home preparation.*Food and Toxicology; 39*: 887-891.

Sors T.G, Ellis D.R, Na G.N, Lahner B, Lee S, Leustek T. (2005). Analysis of sulfur and selenium assimilation in Astragalus plants with varying capacities to accumulate selenium. *Plant J. 42*,785–797. doi: 10.1111/j.1365-313X.2005.02413.

Sosan MB, Oyekunle JAO (2017). Organochlorine pesticide residue levels and potential human risks in kolanut from selected markets in Osun State, South Western Nigeria.*Asian Journal of Chemical Science 2*(4):1-11

Stone MS, Martyn L, Weaver CM. (2016). Potassium intake, bioavailability, hypertension, and glucose control. *Nutrients* ;8

Sun T, Wu H, Wang X, longJi C.H, Shan X. (2020). Evaluation on the biomagnification or biodilution of trace metals in global marine food webs by meta-analysis. Envir. Pollution, 264, 113856, p:1-11.
DOI: 10.1016/j.envpol.2019.113856

Tangahu B. V, Sheikh Abdullah S.R, Basri H, Idris M, Anuar N, Mukhlisin M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int. J. Chem. Eng*; 1–31.
doi: 10.1155/2011/939161

Trasande L, Landrigan PJ, Schechter C. (2005). Public health and economic consequences of methyl mercury toxicity to the developing brain. *Environ Health Perspect 113*(5): 590–596.

Trehan I, Benzoni N.S, Wang A.Z, Bollinger L.B, Ngoma T.N, Chimimba U.K. (2015). Common beans and cowpeas as complementary foods toreduce environmental enteric dysfunction and stunting in Malaw-ian children: study protocol for two randomized controlled trials.*Trials 16*:520.

Uduak .I. Aletan. (2018). Comparison of the Proximate and Mineral Composition of two Cowpea Varieties obtained from Mile 12 Market, Lagos. Communication in physical science 3(1):44-48.

Ugbeye G. (2004). Regulatory control and monitoring of pesticides and pesticide residue. Seminar Paper presented at NAFDAC Central Laboratory, Oshodi, Lagos, Nigeria.

Ujowundu C.O, Koloseke D.M, Uba N.O, Achilike J. J, Ogbuagu H.D, and Ubuoh E.A. (2017). Ecotoxicological Impact of Sub-lethal Concentrations of Glyphosatebased Herbicide on Juvenile Clarias gariepinus.*Journal of Advances in Medicine and Medical Research; 22*(11): 1-14, 2017; ISSN: 2231-0614, DOI: https://doi.org/10.9734/JAMMR/2017/34374

Usman Y.M, Danbature W.L, and Zaliha, A.Y. (2021). Evaluation of Polychlorinated Biphenyl (PCB) Components of beans consumed in Gombe Metropolis Nigeria. *Journal of Chemical Society of Nigeria; 46*(5). https://doi.org/10.46602/jcsn.v46i5.661

US Department of Agriculture. What we eat in America. NHANES 2011–2012, individuals 2 years and over (excluding breast-fed children), day 1. Available at http://www.ars.usda.gov/SP2UserFiles/Place/80400530/pdf/1112/Table_1_NIN_GEN_11.pdf.

US Department of Health and Human Services. Healthy People 2020. Nutrition and weight status. Available at http://www.healthypeople.gov/2020/topics-objectives/topic/nutrition-and-weight-status/objectives.

U.S. Environmental Protection Agency (EPA). Available online: https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/103601/103601-247.pdf

Vadivel V, and Janardhanan K. (2000). Preliminary agrobotanical traits and chemical evaluation of Mucuna pruriens (itching beans): A less-known food and medicinal legume. *J. Medic. Aromatic. Plant Sci. 22*: 191 – 199.

Verma S, Dubey RS (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plant. *Plant Sci 164:*645-655

Vahter M. (2008). Health effects of early life exposure to arsenic. *Basic Clin.Pharmacol.Toxicol. 102*, 204–211.

Vargas-Torres A, Osorio-Dıaz P, Islas-Hern!andez J.J, Tovar J, Paredes- Lopez O, and Bello-Perez L.A. (2004). Starch digestibility of five cooked black bean (*Phaseolus vulgaris L.)* varieties. *J. Food Comp. Anal. 17*:605–612.

Vaz Patto M.C, Amarowicz R, Aryee A.N, Boye J.I, Chung H.J, Martín- Cabrejas M.A. (2015). Achievements and challenges in improving the nutritional quality of food legumes. *Crit. Rev. Plant Sci. 34*, 105143.
Doi: 10.1080/07352689.2014.897907.

Viltos A.J. (1952). Biological activation of sodium 2(2,4-dichlorophenoxy)ethylsulfate by Bacillus cereus var. mycoides, Contrb. *Boyce Thompson Inst. 16*: 435438.

Vincent J.B. (2017). New evidence against chromium as an essential trace element. *J. Nutr. 147* (12), 2212–2219. doi:10.3945/jn.117.255901

Vincent J.B. (2019). Effects of chromium supplementation on body composition, human and animal health, and insulin and glucose metabolism. Curr. Opin. Clin. Nutr. Metab. Care 22 (6), 483–489. doi:10.1097/mco.0000000000000604

Wallace K, Kelsey K.T, Schned A, Morris J.S, Andrew A.S, and Karagas M.R. (2009). Se and risk of bladder cancer: a population-based case-control study. Cancer Prev. Res. 2, 70–73. doi: 10.1158/1940-6207.CAPR-08-0046

Welch R.M, House W.A, Beebe S, and Cheng Z. (2000). Genetic selection for enhanced bioavailable levels of iron in bean (*Phaseolus vulgaris L.*) seeds. *J. Agric. Food Chem. 48*:3576–3580.

Wendie L.C, Jean-Francois S, Claude B, and Guy M. (2011). Exposure of several Belgian consumer groups to pesticides through fresh fruit and vegetable consumption.*Food Control., 22*, 508–516

Wessling-Resnick M. (2014). Iron. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler RG, eds. Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 176-88.

White P.J, Bowen H.C, Parmaguru P, Fritz M, Spracklen W.P, Spiby R.E. (2004).Interactions between selenium and sulphur nutrition in Arabidopsis thaliana. *J. Exp. Bot. 55*, 1927–1937. doi: 10.1093/jxb/erh192

WinkelL.H, Johnson C.A, Lenz M, GrundlT, Leupin O. X., Amini, M. (2012). Environmental selenium research: From microscopic processes to global understanding. *Environ. Sci. Technol. 46*, 571–579. doi: 10.1021/es203434d

World Health Organization (WHO). Permissible limits of heavy metals in soil and plants (Geneva: World Health Organization), Switzerland; 1999.

WHO (2009). Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks. Available online at: http://www.who.int/healthinfo/global_burden_disease/GlobalHealth2009:Risks_report_annex.pdf (Accessed March 5, 2014).

WHO (2004). Guidelines for drinking-water quality.Sixty-fi rst meeting, Rome, 10–19 June 2003.Joint FAO/WHO Expert Committee on Food Additives, Avaible from http://ftp.fao.org/es/esn/jecfa/jecfa61sc.pdf).

WHO 2010. The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2009 (Vol. NLM classification: WA 240). Geneva, Swizerland: World Health Organisation

WHO. Guidelines for drinking-water quality. Sixty-first meeting; 10–19 June 2003; Rome. 2004. Joint FAO/WHO Expert Committee on Food Additives, Avaible from http://ftp.fao.org/es/esn/jecfa/jecfa61sc.pdf)

WHO. Aluminium; Geneva: World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 194); 1997.

Wong S.C, Li Xiang-Dong, Zhang Gan, Qi Shu, Min Yunkyung. (2002). Heavy metals in agricultural soils of the Pearl River Delta, South China. *Environmental pollution 119 (1):* 33-44. DOI: 10.1016/S0269-7491(01)00325-6.

World Health Organization. Guideline: Sodium Intake for Adults and Children; World Health Organization: Geneva, Switzerland, 2012.

WHO. 2007. Preventing disease through healthy environments-exposure to mercury: a major public health concern. World Health Organization.

WHO/FAO (2001). Codex Alimentarius Commission. Food additives and contaminants. Joint FAO/WHO Food Standard Programme

Wu Z, Bañuelos G.S, Lin Z.Q, Liu Y, Yuan L, Yin X. (2015). Biofortification and phytoremediation of selenium in China. *Front. Plant Sci. 6*:136. doi: 10.3389/fpls.2015.00136

Yook J.S, You M, Kim J, Toney A.M, Fan R, Puniya B.L, Helikar T, Vaulont S, Deschemin J.C, Okla M, Xie L, Ghosh M.C, Rouault T.A, Lee J, and Chung S. (2021). Essential role of systemic iron mobilization and redistribution for adaptive thermogenesis through HIF2-α/hepcidin axis. Proceedings of the National Academy of Sciences of the United States of America, 118(40), e2109186118. https://doi.org/10.1073/pnas.2109186118

Yoshida H, Tomiyama Y, and Mizushina Y. (2005). Characterization in the fatty acid distributions of Triacylglycerols and phospholipids in kidney beans (*Phaseolus vulgaris L.*). *J. Food Lipids. 12*:169–180.

Zhang W, Pang F, Huang Y, Yan P, Lin W. (2008). Cadmium exerts toxic effects on ovarian steroid hormone release in rats. *Toxicol. Lett*;182:18–23.
doi: 10.1016/j.toxlet.2008.07.016.