IN-VITRO BIOACCESSIBILITY STUDIES AND HUMAN RISK ASSESSMENT OF POTENTIALLY TOXIC ELEMENTS IN CONTAMINATED SOILS AND VEGETABLES

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BY

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CERTIFICATION

This is to certify that the thesis

***IN-VITRO* BIOACCESSIBILITY STUDIES AND HUMAN RISK ASSESSMENT OF POTENTIALLY TOXIC ELEMENTS IN CONTAMINATED SOILS AND VEGETABLES**

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DEDICATION

This research work is dedicated to The ALMIGHTY GOD, The Most Beneficient, The Most Merciful and my dearest parents Alhaji L.O. Odujebe and Late Deaconness E.F. Odujebe.

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ABSTRACT

Potentially toxic elements (PTEs) contamination of urban soils is of increasing concern because of food safety issues, potential health risks, and detrimental effects on terrestrial ecosystems. The determination of the total concentration of elements alone is therefore not enough in assessing its potential health risk but the fraction absorbed into the body through oral ingestion. The present study was undertaken to assess the human health risk of potentially toxic elements (As, Cd, Cu, Cr, Mn, Ni, Pb, and Zn) through the oral ingestion of soil and locally grown edible vegetables viz: waterleaf (*Talinum triangulare*), spinach (*Basella alba),* pumpkin leaf (*Telfairia occidentalis),* okro leaf (*Abelmolschus esculentus)*, cockscomb (*Celosia argentea)* and green leaf (*Amaranthus viridis*) grown on some contaminated soil samples. The soil samples were obtained from seven locations in Lagos, Nigeria. The sites included MFM (a car park dump site), Orile (a dump site), Katangua (a dump site), Owode (a metal scrap market dump site), Ibafo (a road side dump site), FSS (a dump site) and Control (an uncontaminated soil). Soil and vegetable samples were acid digested using conventional hot plate (STUART C300) and microwave assisted digestion (MARS5, CEM) methods respectively. The quantification of PTE in the soils and vegetables were carried out with Perkin Elmer AAnalyst 200 Flame Atomic Absorption Spectrophotometer (FAAS) and Agilent 7700 Inductively coupled plasma mass spectrometer (ICP-MS) respectively. A range of PTE concentrations were observed from the results obtained for the soil samples used for planting the vegetables. The values ranged between < 0.002 – 20 $mgkg^{-1}$ for As, 0.01 – 20 $mgkg^{-1}$ for Cd, 0.1 – 2400 $mgkg^{-1}$ for Cr, 10 – 14900 $mgkg^{-1}$ for Cu, 42 – 3000 $mgkg^{-1}$ for Mn, 1.5 – 1050 $mgkg^{-1}$ for Ni, 2 – 6200 $mgkg^{-1}$ for Pb and 98 – 4800 $mgkg^{-1}$ for Zn, with most soil samples i.e. ORL, KATANG, OWD and FSS having values much higher than soil guideline values. The PTE concentrations in the edible part of the vegetables studied were observed to vary greatly between and within soil sites. Relatively high total concentrations of PTEs (As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn) were found with most of the values obtained higher than recommended tolerable safe limits established by FAO/ WHO for edible vegetables. The values obtained in the vegetable types for individual elements range from 0.0 – 2.2 mg/kg As, 0.0 – 12.5 mg/kg Cd, 0.2 – 12.4 mg/kg Cr, 10.4 – 277 mg/kg Cu, 38.6 – 1680 mg/kg Mn, 0.8 – 13.0 mg/kg Ni, 2.0 – 108 mg/kg Pb and 98.0 – 1040 mg/kg Zn respectively. The estimation of the daily intake rate and health index (HI) showed values obtained higher than recommended intake limits for most of the PTEs studied with values of health index greater than one in most of the vegetables. This suggests some potential risk associated with consumption of the vegetables. The major non-carcinogenic risk contributors were Cd, Cu and Pb while As, Cr, Ni, Zn did not pose any major potential risk through consumption of these vegetables. Bioaccessibility studies using the physiologically based extraction test (PBET) and simplified bioaccessibility extraction test (SBET) showed that PTE were easily solubilised only in the gastric phase while little or no bioaccessibility was observed in the intestinal phase values with concentrations obtained near or below detection limits of the instrument.Re-evaluation of risk using bioaccessibility studies revealed that though the PTEs were present in the gastric phase, the levels bioaccessible through the ingestion of these vegetables were unlikely to pose any major health risk to consumer. The values obtained for the PTE were within the tolerable safe limit for oral ingestion. The research has demonstrated the use of bioaccessibility studies as a more appropriate approach for evaluation of potential human health risks.

Keywords: Bioaccessibility, Daily intake, Potentially toxic elements, Risk assessment

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The World's urban communities continue to increase much faster than total global population as urbanisation progresses in less developed regions (UN-HABITAT, 2008). This urban expansion causes significant alterations in the physical environment and increases the accumulation of waste materials containing potentially toxic elements from human activities. Due to rapid industrialization in the last five decades, soil contamination with potentially toxic elements (PTEs)has become a serious environmental problem on a global scale (Chandra *et al.,* 2008). Potentially toxic elements (PTEs) are chemical elements in the environment posing a major health hazard due to their toxicity, persistence and bioaccumulation in human, animals and plants (Dong *et al.,* 2010). They are released into the ecosystem by both geogenic and anthropogenic activities but the presence of elevated levels in the environment originate mainly from human activities such as mining, smelting, lead-works, chemical production, foundries, incineration, transportation, illegal waste disposal practises amongst many others (Khan *et al.*, 2015).

The presence of elevated levels of PTEs in the environment raises a lot of health concern because these elements can be toxic, ubiquitous and cannot be degraded to non-toxic forms by any known method and as a result remain in the environment for decades. Water solubility of PTEs and lack of proper mechanism for their removal from the body make most of them extremely toxic even at low concentrations (Amin *et al.,* 2013). Thus, the effects to human health are as a result of the inability of the body to metabolise them leading to bioaccumulation and health hazards.

Due to their high degree of toxicity, PTEs such as arsenic, cadmium, lead and mercury rank among the priority PTEs of public health concern because they have no known biochemical function in the body even at very low concentrations (Gergen and Harmanescu, 2012). These elements are

considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as “human carcinogens” (known or probable) according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer (ATSDR, 2012).

Others PTEs such as Cu, Ni, Mn, Fe, Cr, V, Mo, Znare essential at low concentrations. They play an important role in different metabolic functions andhelp to ensure proper functioning of various enzyme systems in the human body but they are toxic at high concentrations in the body (Gergen and Harmanescu, 2012).

1.8 POTENTIALLY TOXIC ELEMENTS IN THE ENVIRONMENT

Potentially toxic elements in the environment are commonly referred to as heavy metals. Though, the term “heavy metal” isambiguous and imprecise, it is defined in different ways by different researchers (Duffus, 2002). The term “heavy metal” is widely used, but others such as “toxic metals”, “trace metals” or better still, “potentially toxic elements” are possible alternatives.According to Alloway (1995), heavy metals is a group name commonly adopted for metals and metalloids which are associated with pollution and toxicity, however, toxicity is usually a function of the concentration to which a human or organism is exposed to. The term “potentially toxic elements”(PTEs) has therefore become more appropriate, but it is still being used interchangeably with the term “heavy metals”.

The Dangerous Substances Directive of the European Union (76/464/EEC) therefore defines PTEs as those elements which are toxic, persistent and / or bioaccumulative, as they are elements that cannot be broken down and therefore persist in the environment (Albert*et al.,* 2010). Unlike organic pollutants, which eventually degrade to carbon dioxide and water, PTEsare not biodegradable and

hence accumulate in the environment and organisms as a result of direct uptake from the surroundings across the body wall, from respiration and from food.

Every element can be toxic to humans and organisms depending on the concentration to which they are exposed. While some areneeded in the body at very low concentrations but toxic at high concentration. For instance, chromium, copper, manganese, nickel and zinc are beneficial to organisms at low concentrations, others such as arsenic, cadmium and lead have no known health benefit at any concentration(Bosso and Enzweiler, 2008).

Humans are exposed to PTEs through various pathways, especially the food chain, and enter the human body through consumption of contaminated soil, vegetables and water; inhalation of dust; and – less commonly – direct dermal uptake (Monica *et al.*, 2011; El-Hamiani *et al.,* 2015).The presence of these PTEs in environmental matrices may have irreversible adverseeffects on humans particularly children due to their pica behaviour, physiology unique exposures and special vulnerabilities which put them at a higher risk because immature organs tend to be more susceptible to PTEs than other contaminants (Landrigan*et al.,* 2000). Bierkens*et al.* (2009) reported that the absorption rate of lead from environmental matrices by children is 40 % higher than adults. Children are exposed to PTEs in their homes, streets, roads, schools, recreation parks, and playground.

1.3 SOURCES OF PTEs IN THE ENVIRONMENT

Potentially toxic elementsenter the ecosystem through natural and anthropogenic progresses. They are mainly found in undisturbed environment (soil, air, water) in very low concentrations but when the environment is subjected to domestic, commercial and industrial activities,the resultis a release of higherlevels of PTEs whichconsequentlyresult in possible dangers to humans and other organisms in the ecosystem (Elom *et al*., 2014).

Some of the major sources of PTEs in the environment include:

I. Atmospheric pollution from motor vehicles: the use of leaded petrol has been responsible for the global dispersion of Pb aerosols. Even with the use of unleaded petrol by many countries of the world, Pb pollution is still a threat to human population particularly those who live in the cities where there are more release of Pb in the environment. It is important to note that in countrieswhere leaded fuel is still in use, the combustion of such fuel in different engines is a potential pathway through which Pb could be released into the environment (Elom *et al*., 2014).

II. The combustion of fossil fuels: This results in the dispersion of many elements in the air over a large area. The disposal of ash is a further source of potentially toxic elements in the atmosphere (Alloway, 2013).

III. Agricultural fertilisers and pesticides: Several of these including phosphatic fertilisers, slags from iron manufacture, pesticides and herbicides contain various combinations of PTEs, either as impurities or active constituents (Zhang *et al.*, 2011)

IV. The disposal of urban and industrial wastes can lead to soil pollution from the deposition of aerosol particles emitted by the incineration of PTE-containing materials. The careless (or unauthorised) dumping or disposal of PTE-containing items, ranging from electronic gadgets, miniature dry-cell batteries(Ni, Cd and Hg) to abandoned cars and car components(e.g. Pb-acid batteries) can give rise to very high PTE concentrations in soils. The disposal of some domestic waste by burning on garden bonfires or burial in the garden can also result in localised anomalously high concentrations of PTEs, such as Pb, in soils used for growing vegetables.

V. Metallurgical industries contribute to soil pollution in several ways e.g. by emissions of fumes and dusts containing PTEs which are transported in the air and eventually deposited onto soils and vegetables, by effluents which may pollute soils when watercourses flood and by the creation of waste dumps (or scrapyards) from which PTEs may be leached and thus pollute underlying or nearby soils.

VI. The mining and smelting of non-ferrous metals (Alloway, 2013).

1.4 TOXICITY OF PTEs

The level of toxicity of elementsin environmental matrices depend on their sources, forms, bioaccessibility, bioavailability as well as underlying human health (Elom *et al.,* 2014).

Toxicity in humansarisewhen PTEs form complexes or "ligands" with organic compounds in the human body system therebyreplacing nutrient minerals in enzyme binding sites (Arora *et al*., 2008). The modified biological molecules (enzymes)therefore lose their ability to function properly, and result in malfunction or death of the affected cells. The most common groups involved in ligand formation are oxygen, sulphur, and nitrogen. When PTEs bind to these groups they make inactive important enzyme systems, or affect protein structure (Lawrence, 2014).These PTEs may also replace other substances in other tissue structures, such as the arteries, joints, bones and muscles which are weakened by the replacement process(Arora *et al*., 2008). For example, As^{3+} can attack –SH groups in glycolyticenzymes to inhibit their bioactivities as illustrated in the reaction in Figure 1.1.

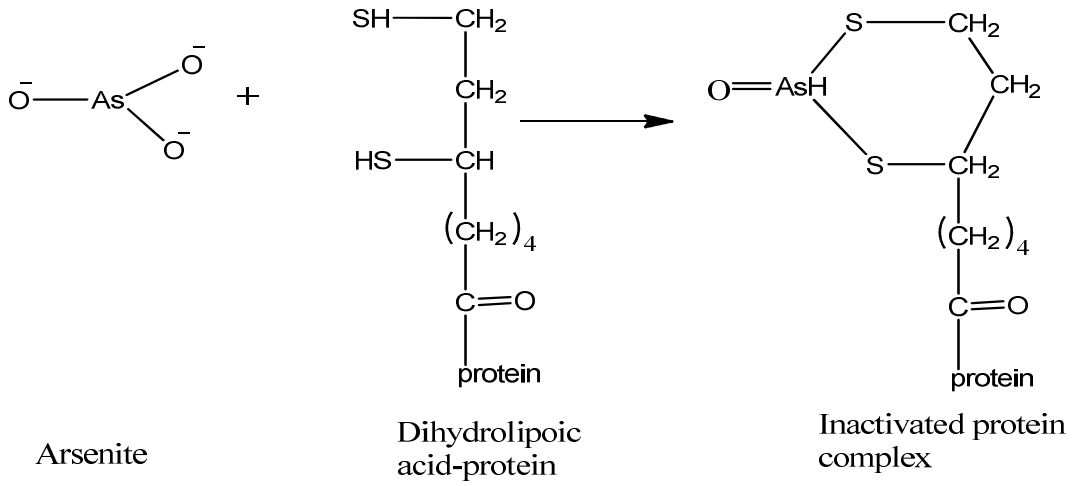


Figure 1.1: As (III) compound attacking –SH group in glycolytic enzyme

1.4.1 Overview ofpotentially toxic elements (PTEs) and their toxicity

The potentially toxic elements of interest in this research study include arsenic, cadmium, chromium, copper, manganese, nickel, lead and zinc. Their sources in the environment and toxicity to humans are discussed below.

1.4.1.1 Arsenic

The US Environmental Protection Agency (EPA) has classified arsenica notoriously poisonous metalloidas the number one toxin. Arsenic is the twentieth most abundant element on earth and its inorganic forms such as arsenite and arsenate compounds are lethal to the environment and living creatures. Humans may encounter arsenic by natural means, industrial source, or from unintended sources (Monisha *et al.,* 2014).Basically the toxicity of arsenic to humans depends on its chemical form (inorganic or organic) and oxidation state, with the inorganic forms being more toxic than the organic forms. Most studies on arsenic centre on arsenite As (III) and arsenate As (V) and have observed that As (III) is more toxic than As (V) (SFEP, 2013).

Sources of arsenic in the environment include: weathering of volcanic rocks, mining and smelting, waste incineration, combustion of coal and petroleum products, the use of As-based wood preservatives, herbicides and pesticides (Watts *et al.,* 2010). Drinking water may get contaminated by use of arsenical pesticides, natural mineral deposits or inappropriate disposal of arsenical chemicals (SFEP, 2013). Deliberate consumption of arsenic in case of suicidal attempts or accidental consumption by children may also result in cases of acute poisoning (Mazumder, 2008).

Soil / dust ingestion and inhalation of industrial emissions are also important exposure pathways. Workers who produce or use arsenic compounds in such occupations as vineyards, ceramics, glass-making, smelting, refining of metallic ores, pesticide manufacturing and application, wood

preservation, semiconductor manufacturing can be exposed to substantially higher levels of arsenic(Tchounwou *et al.,* 2012).

Diet, for most individuals, is the largest source of exposure of arsenic, with an average intake of about 50 µg per day. Intake from air, water and soil are usually much smaller, but exposure from these media may become significant in areas of arsenic contamination.Such exposures result in adverse human health effects including: skin lesions, melanosis (change of pigmentation), cough, chest pain, hypertension and cardiovascular complications (Rahman *et al.*, 2009).

One of the mechanisms by which arsenic exerts its toxic effect is through impairment of cellular respiration by the inhibition of various mitochondrial enzymes (Figure 1.1) and the uncoupling of oxidative phosphorylation (Tchounwou *et al.,* 2012).

1.4.1.2 Cadmium

Cadmium is an extremely toxic element commonly found in industrial workplaces. It is widely distributed in the earth's crust at an average concentration of about 0.1 mg/kg. The major industrial applications of cadmium include the production of alloys, pigments, and batteries. It has no essential biological function in the human body, and is highly toxic to both plants and animals(Tchounwou *et al*., 2012).

Sources of soil contamination by cadmium are the mining and smelting of Cd and Zn, atmospheric pollution from metallurgical industries, the disposal of wastes containing cadmium, such as the incineration of plastic containers and batteries, cement production, sewage sludge application to land and the burning of fossil fuels (SFEP, 2013).

As a result of continuing use of cadmium in industrial applications, the environmental contamination and human exposure to cadmium have dramatically increased during the past century(Tchounwou *et al*., 2012).Food is the main route by which cadmium enters the human body, but tobacco smoking

and occupational exposures to CdO fumes are also very important sources, however, skin absorption is very rare(Tchounwou *et al*., 2012).According to available data, the average weekly intake of cadmium from food in most countries is within the range of 0.7–2.8 μg/kg body weight (UNEP, 2010). Given their smaller size, children may be taking in more cadmium per kilogram of body weight than adults.

Severe exposures to cadmiumcan cause tracheo-bronchitis, pneumonitis, and pulmonary oedema. Inhaling cadmium-laden dust quickly leads to respiratory tract and kidney problems which can be fatal (often from renal failure). Ingestion of any significant amount of cadmium causes immediate poisoning and damage to the liver and the kidneys. Compounds containing cadmium are also carcinogenic. The bones become soft (osteomalacia), lose bone mineral density (osteoporosis) and become weaker i.e itai-itai disease (SFEP, 2013).

Reaction: Cadmium metal burns in air to form cadmium(II) oxide as shown in Equation 1.1

$$2Cd(s) + O_2(g) \rightarrow 2CdO(s) \qquad \text{Equation 1.1}$$

1.4.1.3 CHROMIUM

Chromium is the 22ndmost abundant element in the earth's crust with an average concentration of 100 ppm (Emselm, 2001). It is a naturally occurring element found in rocks, animals, soil, and in volcanic dust and gases. Anthropogenic emissions arise from the use of chromium compounds for metal plating, corrosion inhibitor, plating, wood preservatives, metal finishing, leather tanning, and stainless steel cookware (Faisal and Hasnain, 2006). Chromium is also used to make dyes and pigments for paints, refractory bricks for furnace and as an additive to inhibit corrosion(Martin and Griswold, 2009). It is present in the environment in several different forms, the most common being trivalent chromium Cr (III) and hexavalent chromium Cr (VI).

Chromium(VI) compounds, such as calcium chromate, zinc chromates, strontium chromate and lead chromates, are highly toxic and carcinogenic in nature. Cr (III) compounds are much less toxic and do not appear to cause these problems.Chromium (III) is an essential nutritional supplement for animals and humans and has an important role in glucose metabolism (Tchounwou *et al*., 2012)

The health hazards associated with exposure to chromium are dependent on its oxidation state. In the soil, it exists basically in two oxidation states, Cr (III) and Cr (VI) and its effects both on the environment and humans depend on the oxidation state. Chromium (VI) is about 300 times more toxic than Cr (III) (Stewart *et al*., 2003). In terms of solubility, Cr (VI) is more soluble in soil and its mobility in the soil is more when compared to Cr (III).

Chromium(VI) is mainly toxic to organisms. It can alter genetic materials and cause cancer. Adverse effects on the skin may include ulcerations, dermatitis, and allergic skin reactions. Inhalation of hexavalent chromium compounds can result in ulceration and perforation of the mucous membranes of the nasal septum, irritation of the pharynx and larynx, asthmatic bronchitis, bronchospasms and oedema (Monisha *et al.,* 2014). Respiratory symptoms mainly include coughing and wheezing, shortness of breath, and nasal itch.

Although the mechanisms of biological interaction are uncertain, the variation in toxicity may be related to the ease with which Chromium (VI) can pass through cell membranes and its subsequent intracellular reduction to reactive intermediates. Since Chromium (III) is poorly absorbed by any route, the toxicity of chromium is mainly attributable to the Chromium (VI) form. It can be absorbed by the lung and gastrointestinal tract, and even to a certain extent by intact skin (Faisal and Hasnain, 2006).

1.4.1.4 COPPER

Copper is present in the earth's crust at a concentration of about 50 parts per million (ppm) and is also synthesized in massive stars. The electrical properties of copper are exploited in copper wires and devices such as electromagnets. Integrated circuits and printed circuit boards increasingly feature copper in place of aluminium because of its superior electrical conductivity; heat sinks and heat exchangers use copper as a result of its superior heat dissipation capacity to aluminium (Elom *et al.,* 2014). Vacuum tubes, cathode ray tubes, and the magnetrons in microwave ovens use copper, as do wave guides for microwave radiation. In the agricultural sector, it is used in the manufacturing of fertilizers and fungicides (Puschenreiter and Horak,2003).

In sufficient amounts, it is poisonous to higher organisms but at lower concentrations it is an essential trace nutrient to all higher plant and animal life. In surface water, copper can travel great distances, either suspended on sludge particles or as free ions. When copper ends up in soil it strongly attaches to organic matter and minerals. The absorption of copper is necessary, because copper is a trace element that is essential for human health. Approximately 30 – 50 % of ingested copper is in the form of Cu (II) and is absorbed in the small intestine with a smaller quantity in the stomach (Butterworth, 2010).

Long-term exposure to copper can cause irritation of the nose, mouth and eyes and it causes headaches, stomach aches, dizziness, vomiting and diarrhoea. Intentionally high uptakes of copper may cause liver and kidney damage and even death. Chronic copper poisoning results in Wilson's disease, characterized by a hepatic cirrhosis, brain damage, demyelination, renal disease, and copper deposition in the cornea (Butterworth, 2010).

Reaction: Copper is oxidized by concentrated nitric acid, HNO_3, to produce Cu^{2+} ions; the nitric acid is reduced to nitrogen dioxide, a poisonous brown gas with an irritating odour:The reaction is given in Equation 1.2

$$Cu(s) + 4HNO_3(aq) \longrightarrow Cu(NO_3)_2(aq) + 2NO_2(g) + 2H_2O(l) \qquad \text{Equation 1.2}$$

1.4.1.5 LEAD

Lead is a highly toxic element found in the earth's crust. Because of its abundance, low cost, and physical properties, lead and lead compounds have been used in a wide variety of products including paint, ceramics, pipes, solders, gasoline, batteries, and cosmetics (Flora *et al*., 2006). The major anthropogenic source of lead is automobile exhaust fumes. Tetraethyl-lead has been used as an anti-knock with ordinary petrol. Even with the use of unleaded fuel by many countries of the world, Pb pollution is still a threat to human population especially those who live in the areas where there are more release of Pb in the environment (Alloway, 2013). It is important to note that in countries where leaded fuel is still in use, the combustion of such fuel in different engines is a potential pathway through which Pb could be released into the environment. Lead is also a component of paints, ceramics and pipes used for water supply. (Kumar and Pastore,2007).The main components for lead in the soil are the solution, the adsorption surfaces of the clay-humus exchange complex, precipitated forms, secondary iron and manganese oxides and alkaline earth carbonates, the soil humus and silicate lattices. There is a general agreement that only a small proportion of the lead in soil is actually bioavailability for plants (Alloway, 2013).

Lead could enter the human body either through ingestion or inhalation and affects the development of nervous system and other major organs like the heart, intestine, kidneys and reproductive system (Ahemeda*et al*., 2005). Lead poisoning (also known as plumbism, colica Pictonum, saturnism,

Devon colic, or painter's colic) is a medical condition caused by increased levels of Lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems (Jimoh *et al.,* 2012). It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behaviour disorders. (Liu and Lewis, 2014). Gastrointestinal problems, such as constipation, diarrhea, poor appetite, or weight loss, are common in acute poisoning. Absorption of large amounts of lead over a short time can cause shock (insufficient fluid in the circulatory system) due to loss of water from the gastrointestinal tract. Haemolysis (the rupture of red blood cells) due to acute poisoning can cause anaemia and haemoglobin in the urine (Yedjou *et al*., 2010).

One of the major mechanisms by which lead exerts its toxic effect is through biochemical processes that include lead's ability to inhibit or mimic the actions of calcium and to interact with proteins (Tchounwou *et al*., 2012). Within the skeleton, lead is incorporated into the mineral in place of calcium. Lead binds to biological molecules thereby interfering with their function by a number of mechanisms. Lead binds to sulfhydryl and amide groups of enzymes, altering their configuration and diminishing their activities. Lead may also compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium (Flora and Pachauri, 2010).

1.4.1.6 MANGANESE

Manganese is naturallyubiquitous in the environment, and found in many types of rocks, soil, water, air, and food. It is one out of three toxic essential trace elements required for a variety of biological processes and optimum health necessary for humans to survive. However, both deficiency and excess of manganese have detrimental health effects in the human body (Ferri *et al*., 2015).

Emission of manganese into the urban environment is mainly from metallurgical and chemical industries, combustion of coal and petrol (Röllin and Nogueira, 2011).Manganese compounds have a variety of uses. Manganese dioxide is used in the production of dry-cell batteries, matches, fireworks, and the production of other manganese compounds. Manganese chloride is used as a catalyst in the chlorination of organic compounds, in animal feed, and in dry-cell batteries, while manganese sulfate is used as a fertilizer, livestock nutritional supplement, in glazes and varnishes, and in ceramics. (ATSDR, 2012).

Populations living in close proximity to mining activities and industries using manganese may be exposed by inhalation to high levels of manganese in dust. Workers in these industries are especially vulnerable to exposure to manganese dust. People who smoke tobacco or inhale second-hand smoke are typically exposed to manganese at levels higher than those not exposed to tobacco smoke.The compounds most often encountered in the environment and the workplace are those containing inorganic manganese in the Mn(II), Mn(III), or Mn(IV) oxidation states.

The primary source of manganese intake is through diet. The average manganese levels in various media are as follows: levels in drinking water are approximately 0.004 parts per million (ppm); average air levels are approximately 0.02 ($\mu g/m^3$); levels in soil range from 40 to 900 ppm.Chronic inhalation exposure of humans to high levels may result in a syndrome called manganism which typically begins with feelings of weakness and lethargy and progresses to other symptoms such as gait disturbances, clumsiness, tremors, speech disturbances, a mask-like facial expression, and psychological disturbances (ATSDR, 2012).

1.4.1.7 NICKEL

Nickel is the 24thmost abundant element in the Earth's crust, comprising about 3% of the composition of the earth.Most nickel is used for the production of stainless steel and other nickel

alloys with high corrosion and temperature resistance. Nickel metal and its alloys are used widely in the metallurgical, chemical and food processing industries, especially as catalysts and pigments. It is commonly emitted into the environment through incineration of waste and sewage, combustion of coal and fuel oil (Cempel and Nikel, 2006). Enzymes of some microorganisms and plants contain nickel as an active center, which makes the metal an essential nutrient for them. The bulk of the nickel mined comes from two types of ore deposits. The first are laterites where the principal ore minerals are nickeliferous limonite: (Fe, Ni)O(OH) and garnierite (a hydrous nickel silicate): (Ni, $Mg)_3Si_2O_5(OH)_4$. The second are magmatic sulfide deposits where the principal ore mineral is pentlandite: $(Ni, Fe)_9S_8$ (Alloway, 1995). The larger part of all nickel compounds that are released to the environment will adsorb to sediment or soil particles and become immobile as a result.

Foodstuffs naturally contain small amounts of nickel. Chocolate and fats are known to contain very high nickel content. Plants are known to accumulate some levels of nickel and as a result the nickel uptake from vegetables isinevitable. Nickel and some of its compounds have been listed by the National Toxicology Program (NTP) as being reasonably anticipated to be carcinogens (Andhale and Zambare, 2011). A requirement for nickel has not been conclusively demonstrated in humans. Scattered studies indicate a highly variable dietary intake of nickel but typical daily intake of this metal from food ranges from 100-300 µg/ day in most countries.

1.4.1.8 ZINC

This is one of the most abundanttrace elements in the human body. It is a constituent of all cells and several enzymes depend on it as a cofactor. Zinc is essential for a healthy immune system, production of certain hormones, wound healing, bone formation and clear skin (Walker andRobert,2004).

The principal use of zinc is for the galvanization of other metals to prevent corrosion but in the long run the galvanised materials release zinc in the environment. It is also used in the manufacture of dry cell batteries and dyeing fabrics (Shimin, 2007).

Though zinc is an essential requirement for a healthy body, it is required in very small amounts and is thus known as a trace mineral.Zinc has a rather low toxicity, and a severe impact on human health by intoxication with zinc is a relatively rare event (Plum *et al.,* 2010).

Excessive absorption of zinc can suppress copper and iron absorption, thereby resulting in anaemia. Other symptoms of zinc toxicity include fever, cough, abdominal pain, nausea, vomiting, diarrhea, drowsiness, restlessness and gait abnormalities. In pregnant women, zinc toxicity can result in premature birth and sometimes still birth (Hershfinkel *et al*., 2007).

Reactions: Zinc metal tarnishes in moist air. Zinc metal burns in air to form the white zinc(II) oxide, a material that turns yellow on prolonged heating. The reaction is shown in Equation 1.3

$$2Zn(s) + O_2(g) \rightarrow 2ZnO(s) \qquad \text{Equation 1. 3}$$

Zinc metal dissolves slowly in dilute sulphuric acid to form solutions containing theaquated Zn(II) ion together with hydrogen gas, H_2:The reaction is shown in Equation 1.4

$$Zn(s) + H_2SO_4(aq) \rightarrow Zn^{2+}(aq) + SO_4^{2-}(aq) + H_2(g) \qquad \text{Equation 1.4}$$

- Zinc is oxidized by hydrochloric acid to form zinc chloride. In the process, hydrogen gas is produced. The reaction is shownin Equation.

$$Zn(s) + 2HCl(aq) \rightarrow ZnCl_2(aq) + H_2(g) \qquad \text{Equation 1.5}$$

1.5 POTENTIALLY TOXIC ELEMENTS IN SOIL

The soil is a key component of terrestrial ecosystems, both natural and agricultural; it is essential for the growth of plants and the degradation and recycling of dead biomass. It is also a complex heterogeneous medium comprising minerals and organic solids, aqueous and gaseous components. The minerals present are usually weathering (chemically decomposing) rock fragments and secondary minerals such as clays, hydrous oxides of Fe, Al and Mn and sometimes, carbonates (usually $CaCO_3$). The organic matter comprises living organisms (mesofauna and microorganisms), dead plant material (litter) and colloidal humus formed by the action of microorganisms on plant litter (Alloway, 1995).

Potentially toxic elements contamination of soil is of increasing concern due to food safety issues, potential health risks and detrimental effects on terrestrial ecosystems (Khan *et al.*, 2015). Theaccumulationof PTEsinsurface soils is affected by many environmental variables, including parent material and soil properties, as well as by human activities, such as industrial production, traffic, farming, and irrigation. Large areas of land can be contaminated by PTEs released from smelters, waste incinerators, industrial wastewater, and from the application of sludge or municipal compost, pesticides, and fertilizers. Irrespective of their sources in the soil, bioaccumulation of PTEs can degrade soil quality, reduce crop yield and the quality of agricultural products, and thus negatively impact the health of human, animals, and the ecosystem (Nagajyoti*et al.*, 2010).

1.6 CHEMICAL REACTIONS OF PTEs IN SOIL

A number of chemical reactions control the behaviour and bioavailability of PTEs in soils. The most important chemical processes are those concerned with the adsorption of PTEs from the liquid phase on to the solid phase. These processes control the concentrations of PTEs and complexes in the soil solution and thus exert a major influence on their uptake by plant roots. They include;

- cation exchange,
- specific adsorption,
- organic chelation

1.6.1 Cation exchange

Most heavy metals exist mainly as cations in the soil solution, and their adsorption therefore depends on the density of negative charges on the surfaces of the soil colloids. In order to maintain electroneutrality, the surface negative charge is balanced by an equal quantity of oppositely charged counter-ions. Ion exchange refers to the exchange between counter-ions balancing the surface charge on the colloids and the ions in the soil solution. It is reversible, diffusion controlled, stoichiometric and, in most cases, there is some selectivity or preference for one ion over another by the adsorbent. This selectivity gives rise to a replacing order amongst the cations, determined by their valency and degree of hydration (Tan, 2010).

Adsorption by cation exchange can also be described as the formation of outer-sphere complexes with the surface functional groups to which they are bound electrostatically.The cation exchange capacity of mineral soils can range from a few to 60 meq/100 g, but in organic soils it may exceed 200 meq/100 g (Alloway, 1995).

1.6.2 Specific adsorption

This involves the exchange of heavy metal cations and most anions with surface ligands to form partly covalent bonds with lattice ions. This occurs where metals such as Cd, Cu, Ni, Pb and Zn form complex ions (MOH^+) on surfaces that contain hydroxyl groups. It result in metal ions being adsorbed to a far greater extent than would be expected from the cation exchange capacity of a soil. Specific adsorption is strongly pH dependent and is related to the hydrolysis of the heavy metal ions. The hydrous oxides of Al, Fe and Mn are thought to be the main soil constituents involved in the specific adsorption reaction (Alloway, 1995)

1.6.3 Organic chelation

In addition to being involved in cation exchange reactions, solid-phase humic substances such as humic acids also adsorb metals by forming chelate complexes(Figure 1.2). Low-molecular-weight organic ligands, not necessarily humic in origin, can form soluble complexes with metals and prevent them from being adsorbed or precipitated. Humic compounds with suitable reactive groups, such as hydroxyl, phenoxyl and carboxyl form coordination complexes with metallic ions. The stability constants of chelates with metals tend to be in the following decreasing order: Cu > Fe = Al > Mn = Co > Zn. Carboxyl groups play a predominant role in metal binding in both humic and Fulvic acids. The maximum amount of any given metal that can be bound is found to be approximately equal to the number of carboxyl groups (Alloway, 1995).

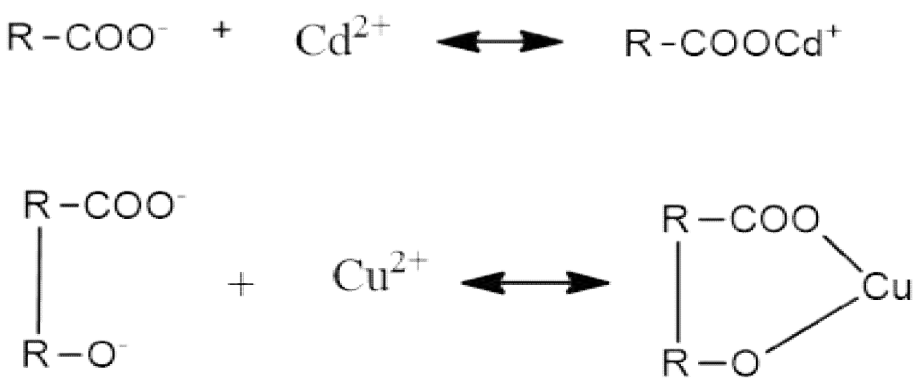


Figure 1.2: Mechanism of Organic Complexation

1.7FACTORS AFFECTING PTEs RETENTION IN SOIL

Some of the factors affecting the retention of potentially toxic elements in the soil include;

1.7.1 The soil pH

Soil pH is a measure of the acidity or basicity in soils. pH is defined as the negative logarithm (base 10) of the activity of hydrogen ions (H^+)in solution. It ranges from 0 to 14, with 0 being most acidic, 14 being highly basic, and 7 being neutral. Soil pH is considered a master variable in soils as it controls many chemical processes that take place. It specifically affects plant nutrient availability by controlling the chemical forms of the nutrient. Acidity in soils comes from H^+ and Al^{3+} ions in the soil solution and absorbed to soil surfaces. While pH is the measure of H^+ in solution, Al^{3+} is important in acid soils because between pH 4 and 6, Al^{3+} reacts with water (H_2O) forming $AlOH^{2+}$, and $Al(OH)_2^+$, releasing extra H^+ ions. Every Al^{3+} ion can create 3 H^+ ions (Tan, 2010).

Many other processes contribute to the formation of acid soils including rainfall, fertilizer use, plant root activity and the weathering of primary and secondary soil minerals. Acid soils can also be caused by pollutants such as acid rain and mine spoilings. Soil with low pH should not be used for agriculture purposes due to high availability of metals in it. Basic soils have a high saturation of base cations (K^+, Ca^{2+}, Mg^{2+} and Na+), this is due to an accumulation of soluble salts. They are also classified as either saline soil, sodic soil, saline-sodic soil or alkaline soil (Sparks and Donald, 2003).

1.7.2. Moisture content

Water content or moisture content is the quantity of water contained in a material, such as soil (called soil moisture), rock, ceramics, fruit, or wood. Water content is used in a wide range of scientific and technical areas, and is expressed as a ratio, which can range from 0 (completely dry) to the value of the materials' porosity at saturation. Methods that determine water content of a sample include

chemical titrations (for example the Karl Fischer titration), determining mass loss on heating (perhaps in the presence of an inert gas), or after freeze drying (Tan, 2010).

1.7.3 Particle size

Soil texture along with other factor is one of the important factors that induces metal availability in soil. Clay contents can significantly affect the availability of heavy metals and their subsequent toxicity to living organisms.(Rashid and Ryan, 2004).Soil-to-plant transfer of heavy metals is strongly influenced by the soil texture.

Treder and Cieslinski (2005) stated that plants cultivated on sandy soil have higher concentrations of heavy metals than those grown on clay loamy soil. The high bioaccumulation in plants is linked with higher mobility of metals in sandy soil as compared to clay soil. Soil sieve analysis or "particle size distribution" is the method used to determine the grain size distribution of soil samples. In other words, the particle size distribution is used for gravel and sand size (coarse) particles, which can be separated into different size ranges with a series of sieves of standard aperture openings. Soil sieving cannot be used for the very much smaller silt and clay (fine) particles. For this reason, the sedimentation procedures are used instead and most common would be the hydrometer test of soil to determine the distribution of the finer particles (AL- Jumaily and AL-Dabbagh, 2013).

Particle size distribution testing can range from a simple sieving test on the clean gravel and sand to elaborate composite tests on clay-silt-sand-gravel mixtures. The test procedures for different types of materials are similar in principle but vary in detail and description in separate manner. As a result of the grain size distribution of soil, it is possible to know whether the soil consists of predominantly gravel, sand, silt or clay sizes (AL- Jumaily and AL-Dabbagh, 2013).

1.8 PLANT UPTAKE OF PTEs IN SOIL

Plant uptake of mobile ions present in the soil solution is largely determined by the total quantity of this ion in the soil but, in the case of strongly adsorbed ions, absorption is more dependent upon the amount of root produced. Roots possess a significant cation exchange capacity, due largely to the presence of carboxyl groups and this may form part of the mechanism of moving ions through the outer part of the root to the plasmalemma where active absorption occurs. Absorption mechanisms can vary for different metal ions, but ions which are absorbed into the root by the same mechanisms are likely to compete with each other. For example, Zn absorption is inhibited by Cu and H^+, but not by Fe and Mn; Cu absorption is inhibited by Zn, NH_4^+, Ca and K (Alloway, 2013).

The bioaccumulation of PTEs is different for different plant species reflected by their growth, reproduction, occurrence and survival in the PTE-contaminated soil. It is notable that different plant species show different toxicity to the same pollutant and in the same environmental condition, because the mechanisms of elemental uptake by plants are not the same for all plant species (Singh *et al*., 2010). Accumulation of PTEs in food plants depends on PTE concentrations as well as phytoavailability and phytovariety, as different plants have different uptake rates (Medina *et al*,. 2005; Yang *et al*., 2009).

The factors affecting the amounts of metal absorbed by a plant are those controlling,

(i) the concentrations and speciation of the metal in the soil solution,

(ii) the movement of the metal from the bulk soil to the root surface,

(iii) the transport of the metal from the root surface into the root and

(iv) its translocation from the root to the shoot.

1.8.1 Translocation of PTEs within plants

Once metal ions have been absorbed through the roots or leaves of a plantand have been transported to the xylem vessels, there is the possibility of movement throughout the whole plant. The rate and extent of movement within plants depends on the metal concerned, the plant organ and the age of the plant. Work on xylem sap has shown that Mn may be largely present as a free ion, although in rice 35% of Mn is organically bound: Ni and Zn may exist as anionic complexes and Cr exists as a trioxalate $–Cr^{3+}$ cation. Copper may exist in organic complexes with amino acids, or in other anionic complexed forms (Alloway, 2013).

1.8.2 Foliar Absorption

In addition to root absorption, plants can also derive significant amounts of some elements through foliar absorption. This is exploited in agriculture as a means of supplying plants with micronutrients, such as Mn and Cu, but can also be a significant route for the entry of atmospheric pollutants, such as Cd into the food chain (Gajbhiye *et al*., 2016).

Foliar absorption of PTEs depends on the plant species, its nutritional status, the thickness of its cuticle, the age of the leaf, the presence of stomata guard cells, the humidity at the leaf surface and the nature of the solutes. Metal antagonisms, such as between Cu and Zn, can occur in foliar absorption as well as in the root, and the accompanying ions also have an effect. Aerosol-deposited Pb particles do not penetrate the cuticle of higher plants, but tend to adhere to the surface of leaves; they can however, be absorbed through the cuticle of some bryophytes (Alloway, 2013).

1.9 EXPOSURE PATHWAYS OFPTEs IN THE ENVIRONMENT

Exposure could be defined as the contact in both space and time of an agent (chemical, physical or biological) and a target organism or a receptor (e.g. humans) such that they come together and interact.

Human exposure to multiple routes can occur simultaneously or at different times. The term "exposure pathway'' refers to the channel an environmental contaminant (example, PTE) takes from its source to exposed populations. It forms a link between environmental release and the potentially exposed populations. This exposure can result from direct contact with the soil, or after transfer of the contaminants to so-called contact media (e.g., vegetables, indoor air) and subsequent exposure to these contact media e.g., due to consumption or inhalation (Figure 1.3**)**. Exposure to soil contaminants can occur by oral, inhalation and dermal routes, thus relating to the pathway within the human body through which the contaminants enter the body (the mouth, gullet, stomach; the nose, trachea and lungs; and the skin; respectively).(Swartjes and Cornelis, 2011).

Humans are exposed to PTEs through various pathways especially the food chain.Loutfy *et al.*(2006)in their report identified food consumption as the major pathway to human exposure accounting for >90% compared to other ways of exposure such as inhalation and dermal contact. According to Suruchi and Khanna (2011), as human activities increase, especially the application of modern technologies, contamination of human food chain becomes inevitable.

The prime route of PTE intake into the human body is through soil–crop system in agriculture area (Liu *et al*.,2007),where the anthropogenic activities are the primary sources of contaminations (Lim *et al*., 2008; Li *et al*., 2008).

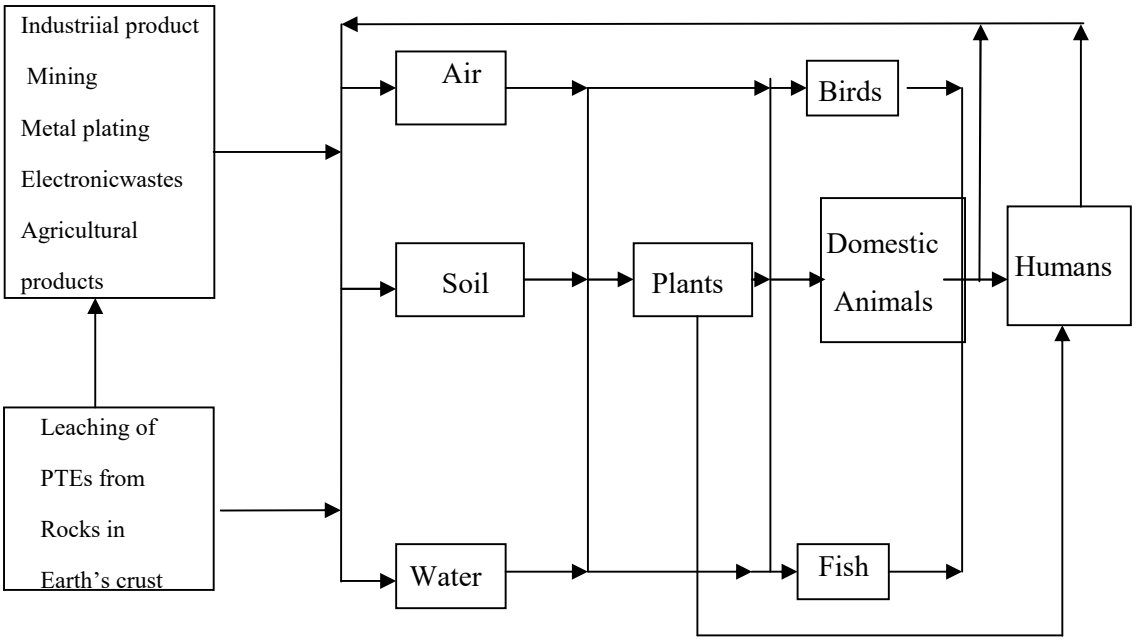


Figure 1.3: Exposure Pathway of PTEs in the environment

Source: Lim *et al*. (2008)

1.9.1. Exposure through soil ingestion

Exposure through soil ingestion is an important pathway especially for immobile contaminants, since exposure through soil ingestion is independent of that part of the contaminant which is in the pore water. Adults ingest soil dust when working indoors or dust from dirt on inappropriately washed plants. Children have specific behaviours: they can ingest soil particles accidentally (by sucking on dirty fingers/hands/toys) or simply by eating dirt. The latter might involve intentional, long-term dirt-eating (the behavioural disorder called "Pica") Exposure rates are generally higher for children. There is some controversy in regard to the signi cance of exposure through soil ingestion for adults. Oral exposure through soil ingestion depends on the soil (or soil particle) ingestion rate, the concentration in the soil (or soil particles) and the availability of contaminants in the human body. Schultz and Biksey(2003) also reported that the primary pathways of human exposure to arsenic in

soil that result in significant health effects are inhalation and oral ingestion leading to both carcinogenic and non-carcinogenic responses whilst dermal adsorption is not thought to be significant.

1.9.2. Exposure through vegetable consumption

The exposure through vegetable consumption is an important pathway, especially for mobile contaminants.Vegetables are an essential part of the human diet because they contain important nutrients such as carbohydrate, protein, vitamins and minerals, as well as essential trace elements, which help in proper growth and development of the body(Doherty *et al.,* 2012). However, vegetables cultivated on contaminated soils can bioaccumulate PTEs in amounts sufficient to cause potential health risks to consumers (Monica *et al*., 2011; Yang *et al.,* 2011; Waqas *et al*., 2015). One of the properties of green leafy vegetable is the accumulation of PTEs in their tissues without exhibiting any toxicity symptoms (Intawongse and Dean, 2006).

Contaminated vegetables can contain toxic elements at a wide range of concentrations and prolonged intake may lead to the bioaccumulation of these in the liver and kidney, resulting in various health disorders such as a weakened immune system, developmental abnormality and cardiovascular diseases (Arora *et al.,* 2008). Several PTEs are known to be easily accumulated in edible parts of leafy vegetables. According to Itanna (2002), leafy vegetables take up higher amounts of PTEs than other vegetables due to their higher translocation and transpiration rates, though the bioaccumulation of PTEs in vegetables are influenced by many factors, suchas climate, atmospheric depositions, the concentration in soil, the nature of soil and the degree of maturity of the plants at harvest (El-Hamiani *et al*., 2015).

1.9.3 Exposure through indoor air inhalation

Exposure through indoor air inhalation is the most important pathway for volatile contaminants. This exposure is dependent on the representative concentration in indoor air and on human characteristics, such as inhalation rate. The representative concentration in indoor air is dependent on advective and diffusive transport of contaminants in pore water and soil air from the groundwater or the soil into a building and, hence, on the soil properties. It also depends on the building characteristics, such as the possibility for intrusion of contaminated air through holes and cracks, the dimensions of the building and the ventilation characteristics of the building. As in the case of the representative vegetable concentration for the calculation of exposure through vegetable consumption, it is important to focus on the most relevant indoor air concentration for the calculation of exposure through indoor air inhalation. Indoor air concentrations often are characterised by a large variation in time and space (height). Therefore, the calculation of indoor air concentrations typically has a relatively limited reliability (Elom *et al.*, 2014).

1.10 HUMAN HEALTH RISK ASSESSMENT OF PTEs

The consistent exposure of humans to environmental PTEs as a result of accidental or intentional ingestion has attracted the attention of international and local organisations as well as agencies such as World Health Organisation (WHO), the United State Environmental Protection Agency (USEPA) and England Department for Food, Environment and Rural Affairs (DEFRA) to the development and implementation of human health risk assessment and encourage studies in that direction (Elom*et al*., 2010).

Risk assessment is a framework that provides the mechanism for a structured review of information relevant to estimating health or environmental outcomes(Sipter *et al*., 2008). It is a science-based process that consists of effects and exposure analyses (Bierkens *et al*., 2009). The aim of risk assessment is to determine if the level of contamination represent an acceptable risk or not for humans (Sipter *et al*., 2008).Decisions in risk assessment include; no risk, moderate risk and significant risk, these indicate the magnitude of the risk and ways to alleviate the potential exposure (Djinovic *et al*., 2008, Santos *et al.,* 2011).

The risk-based approaches for assessing human health risks from contaminated matrices include;

1. Comparison of contaminant concentration with Soil Guideline Values (SGVs).
2. Estimation of daily intake rate and health risk index for determining non-carcinogenic risk
3. Bioavailability / Bioaccessibility studies

1.10.1 Comparison of contaminant concentration with soil guideline values (SGVs)

These are scientifically based generic assessment criteria used to evaluate long-term risks to human health from chemical (PTEs) contamination in soils. These values are given in the form of concentration thresholds of contaminants in soil and act as a check to contamination levels in a site. Contaminants concentration levels below the SGVs implies minimal or no health risks associated with but exceedance signifies risks to human health (Elom *et al*., 2014).

1.10.2 Estimation of the daily intake and health risk index

The risk of exposure can also be expressed in terms of the daily intake and health risk index. Daily intake rate is calculated to averagely estimate the daily PTE loading into the body system of a specified body weight of a person (Tsafe *et al*., 2012).Risk index allows for classi cation of risk quali cations, although this is a subjective process, into classes such as, for example, a 'very high human health risk', when the risk index exceeds a value of 10. Moreover, a risk index offers possibilities for the scaling of human health risks, which is useful in terms of priority setting. It should be noted, however, that in regard to classi cation and ranking of risks, the risk index assumes a linear relationship with seriousness of human health effects (Orisakwe *et al*., 2012).

1.10.3 Bioavailability / Bioaccessibility studies

Consideration of a contaminants oral bioaccessibility is important in understanding exposure associated risk (Intawongseand Dean, 2008).Oral bioaccessibility can be defined as the fraction of a substance that is released from a solid matrix during digestion, thus making it soluble and available for absorption through the gastrointestinal tract (Broadway *et al.,* 2010) while Oral bioavailability is the fraction that reaches the central compartment of the human body system (Ruby *et al*., 1999).

A range of *in-vivo* and *in-vitro* models have been developed to assess bioavailability / bioaccessibility of contaminants in environmental matrices and these are gaining increasing regulatory acceptance worldwide.I*n-vivo* involves the use of animal models in risk assessment while*in-vitro*involves simulation of the human body system (gastrointestinal tract) through the use of laboratory reagents (Oomen *et al*., 2003).

In the absence of human studies or suitable epidemiological data, *in vivo* animal trials have been used to measure the bioavailability of contaminants. Animal species used in bioavailability assessments for contaminants include rabbits, rats, primates and pigs (Drexler and Brattin*.,* 2007). In *in-vivo*studies, one group of animals in the laboratory trial is fed contaminated soil and the other group of animals is fed the contaminant of interest in a (usually more soluble) form which is comparable to that used in the studies to derive the toxicity values. However, this model has some limitation: it is time consuming, expensive, involves labour-intensive experimental protocolsand raise ethical concerns. Moreover, results obtained from *in vivo*investigations cannot be used to represent the real human body system because of the differences in physiologyand behaviour (Intawongse and Dean, 2008).Such problems led to the development of *in-vitro* approaches which mimic the physiological conditions in the human gastro intestinal environment.

In-vitro models provide a means of determining the bioaccessibility of a given contaminant present in environmental matrices (Intawongse and Dean, 2008).They can be simple, rapid, lower in cost and may provide insights which are not achievable in whole animal (*in-vivo*) studies. Apart from that, it reduces the usage of experimental animals. All of these characteristics make it broadly applicable for the human health risk assessment.

Most *in-vitro* digestion models involve a two-step (stomach and small intestine) or three-step (mouth, stomach, small intestine) procedure (Omar *et al.*, 2013). These models have been developed to simulate the dissolution and subsequent absorption of contaminants in the human gastrointestinal tract when soil or food is ingested (Elom *et al.*, 2014). Nevertheless, large intestine is not taken into account because human food digestion and absorption mainly takes place in the small intestine and not in large intestine (Oomen *et al.*, 2003).

1.11 STATEMENT OF THE PROBLEM

Nigeria is confronted with extensive environmental and agricultural challenges which threaten the sustenance of its teeming population especially in urban areas such as Lagos metropolis.Lagos State is Africa's biggest city and the fastest growing metropolis in the world. It is the most heavily industrialised city in Nigeria, with much of the nation's wealth and economic activities located there. The state is reported already to be home to over 70 % of the country's medium and large-scale manufacturing industries. Lagos metropolis has a population of about 18 million people on 3,577 km^2 of land,with water accounting for 30 % of thisarea (Oyeyiola *et al*., 2013), and hence does not have enough land for both residential and agricultural purposes, leading to extensive environmental challenges that threaten the sustenance of the urban population.To meet food demand, urban gardening is therefore practised. Vegetables are now grown on all types of available land such as along roadsides, near dumpsitesand at the bank of polluted rivers (Orubite *et al.,* 2015) especially in urban areas where there is not enough unoccupied land for agricultural purposes (Liu *et. al*., 2013). As a consequence, for residents in close proximity to these contaminated soils, unintentional ingestion of soil (especially by children through hand to mouth behaviour) and consumption of vegetables grown on or near these soil sites expose inhabitantsto significant levels of PTE uptake.

Food safety and security are topics of growing concern regarding human health in recent decades because of the demand for food especially vegetables and health risk associated with consumption of contaminated food has drawn the attention of researchers worldwide (Orisakwe *et al*., 2012). This is because food (including vegetables) can be a major source of human exposure to PTEsand contributeup to 90 % of the total exposure (Gupta *et al*., 2012). For instance, it is estimated that approximately half of human Pb intake is through food, with around half originating from plants (Intawongse and Dean, 2008). The consumption of PTE –contaminated food can seriously cause depletion of some essential nutrients in the body, which in turn leads to a decrease in immunological

defences, intrauterine growth retardation (caused by Cd, Mn and Pb), disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Khan *et al*., 2010).

Due to the unique circumstances found in rapidly developing regions such as Lagos – relatively unregulated urbanisation, widespread informal settlement, and use of any available soil for cultivation – there is a need for studies in such areas.

1.12 AIM AND OBJECTIVES

This research is aimed at assessing and evaluating the potential human health risk associated with ingestion of potentially toxic elements in contaminated soils and vegetables grown on them. The specific objectives of this study include:

1. Optimisation of the operating conditions of instrumental analytical techniques used for the quantification of PTEs.

2. Determination of total PTEs in soils and vegetable samples grown on them and comparison of the bioaccumulation of PTEs in the edible part of vegetables.

3. Utilisation of some risk assessment approaches such as pollution index, daily intake rate and health risk index for estimation of potential human health risk associated with PTEs.

4. Evaluation of the potential health risk associated with oral ingestion of soil and consumption of contaminated vegetables.

1.13 SIGNIFICANCE OF THE STUDY

It has been observed through bibliographic survey that limited information is available in literature on potential human health risk associated with the consumption of vegetables contaminated by PTEs in developing countries. More importantly, very little research has been published on the use of bioaccessibility model as a tool for the evaluation of potential health risk associated with PTEs through involuntary ingestion of soils and none on consumption of contaminated vegetables in Nigeria and other African countries. The majority of researches undertaken in developing countries (such as Nigeria), on human health risk of PTEs were based on the determination of total concentration of PTEs in environmental matrices alone rather than the assessment of their bioavailability and mobilisation. However, experimental evidence has demonstrated that such determinations are not representative of contaminant bioavailability / bioaccessibility in human gastrointestinal tracts i.e. the quantity eventually absorbed into the circulatory system of a human being (Odujebe *et al*., 2016, Okorie *et al*., 2012). Also, to the best of my knowledge, there is no study available on human health risk assessment of arsenic which is one of the most toxic elements (priority pollutant) even at low concentration. Research in this area is therefore important.

1.14 LIST OF ABBREVIATIONS/ACRONYMS

As	Arsenic
ASTDR	Agency for Toxic Substances and Disease Registry
Cd	Cadmium
Cr	Chromium
Cu	Copper
DEFRA	Department for Environment, Food and Rural Affairs
EPA	Environmental Protection Agency
FAAS	Flame atomic absorption spectrophotometer
FAO	Food and Agriculture Organisation
GI	Gastrointestinal tract
HDPE	High-density polyethylene
ICP-MS	Inductively coupled plasma-mass spectrometer
LOD	Limit of detection
Mn	Manganese
Ni	Nickel
Pb	Lead
PBET	Physiologically based extraction test
PTEs	Potentially Toxic Elements
SBET	Simplified bioaccessibility extraction test
SFEP	Science for Environment Policy
SGVs	Soil guideline values
UNEP	United Nations Environment Programme
USEPA	United State Environmental Protection Agency
WHO	World Health Organisation
Zn	Zinc

CHAPTER TWO

LITERATURE REVIEW

2.1 PTEs IN ENVIRONMENTAL MATRICES

As a result of increasing anthropogenic activities, PTEs pollution of soil, water, and air represent a growing environmental problem affecting food quality and human health (Suruchi and Pankaj, 2011).PTEs are chemically reactive in the environment, which results in their mobility and bioavailability to living organisms (Yap *et al*., 2009). Humans may be exposed to high levels of toxic elements by breathing in air, drinking water, or eating food that contains them. As a consequence, PTEs get into the human body by different routes - by inhaling, through skin, and via ingestion of contaminated food (Monica and Marija, 2011).

As a crucial component of the ecosystems, soils generally have elevated concentrations of potentially toxic elements originating from both point and diffuse sources of pollution in cities (Wong *et al.*,2006). These contaminants, which can remain in the soil for a long time may then act as a further source of pollution in the environment and pose a potential threat to both ecological systems and human health (Luo *et al.*, 2011).

PTE concentration in soil typically ranges from less than one to as high as 100,000 mg/kg (Singh *et al.*, 2011a). PTEs are the main group of inorganic contaminants and a considerable large area of land is contaminated with them due to use of sludge or municipal compost, pesticides, fertilizers, and emissions from municipal wastes incinerates, exudates, residues from metalliferous mines and smelting industries. Irrespective of the origin of the metals in the soil, excessive levels of many metals can result in soil quality degradation, crop yield reduction, and poor quality of agricultural products, posing significant hazards to human, animal, and ecosystem health. PTEs may enter the

food chain as a result of their uptake by edible plants, thus, the determination of PTEs in environmental samples is very important (Alirzaveya *et al*., 2006).

PTEs can exist in waters and organisms in a wide variety of chemical forms and in combination with other materials. In aquatic systems, the metals of greatest concerns are cadmium, copper, lead, mercury and zinc. These elements are toxic to organisms above specified threshold concentrations but may also be essential (e.g. copper and zinc) for metabolism at lower concentrations.

The trace elements located in an aquatic environment come from natural and anthropogenic sources. Such sources include geological minerals, windblown silicate dust, volcanic emissions, sea spray and combustion. Human activities such as mining, industry and sewage treatment discharges as well as electronic wastes (computers, printers, photocopy machines, TV sets, mobile phones and toys) and agriculture (agriculture fertlisers) are some of examples of anthropogenic sources contributing to the elevated levels of trace elements.

The bio-accumulation of trace elements in natural waters is related to their ability to cross biological barriers, such as plasma membrane. The bio-uptake of metals include the following steps:

 DIFFUSSION: Trace metals and their complexes must first diffuse from the external medium to the surface of the organism (mass transport);

 COMPLEXATION: Metals may dissociate and re-associate (complexation/dissociation) during diffusion to the biological surface and

 INTERNALIZATION: Metals must react with a sensitive site on the biological membrane (adsorption/desorption) followed by biological transport (internalization).

Most metals are insoluble in water, soluble in fats and resistant to biological and chemical degradation - properties that result in their accumulation in the tissues of organisms and their

biological amplification in food chain and food webs. Aquatic flora and fauna such as algae, invertebrates (oysters, mussels) and fish can accumulate trace metals to several orders of magnitude (thousands to million times) above background levels in the aquatic environment thus human and other predators at the top of the food chain can be at risk from eating trace metal contaminated sea foods. The notable examples of metal accumulation are methyl mercury in fish, and cadmium, copper, lead, zinc and mercury in oysters and mussels.

PTE pollution can harm aquatic organisms through lethal and sub-lethal effects and can reduce or eliminate species from an ecosystem through increased susceptibility to fish disease, mortality and decreased fecundity

2.2 REVIEW OF STUDIES ON PTEs IN SOILS AND VEGETABLES

Contamination of vegetables by PTEs cannot be underestimated as these foodstuffs are important components of human diet (Singh *et al.,* 2011b). Potentially toxic elements have the capability to move from contaminated soil or water and bioaccumulate in vegetables causing human risks.The primary concern with the uptake of contaminants by plants is the presence of the contaminants in produce consumed by humans. Leafy vegetables occupy a very important place in the human diet, but unfortunately are sources of PTE accumulation (Nabulo *et al.,* 2010).

Doherty *et al.* (2012) conducted a study on the determination of the concentration of heavy metals Cu, Zn, Pb and Cd in five vegetables viz; *Cochorus olitorus* (Jew's mallow), *Vernonia amygdalina* (Bitter-leaf), *Talimum triangulare* (Water-leaf), *Telifaria occidentalis* (Fluted pumpkin) and *Spinacia oleracea* (Spinach) and soil collected from selected farm and market sites in industrial, residential and commercial areas of Lagos state. The heavy metals present in the vegetables and soil were analyzed using Atomic Absorption Spectrophotometer. The results revealed that all the heavy metals were detected in both soil and vegetables from the various sites but at concentrations below the World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) safe limit of 40, 60, 5, and 0.2mg/kg for Cu, Zn, Pb and Cd respectively in vegetables. Also, there was no significant difference ($p>0.05$) between the heavy metals found in vegetables collected from industrial, residential and commercial areas.

In another study, Opaluwa *et al.* (2012) investigated the level of PTEs (As, Cd, Co, Cu, Fe, Ni, Pb and Zn) in soils, plant leaves and crops from farmlands around dumpsites using digestion and Atomic absorption spectrophotometer method (AAS). Soil and plant samples were collected from farms around the dump sites and other samples from an area where there were no dump sites, which served as control. The metal concentrations in plant leaves and crops showed high level of Co(0.33

mg/kg) and Fe(0.32 mg/kg) in roselle leaves; Cu(0.71 mg/kg) and As(0.37 mg/kg) in groundnut; Cu(0.48 mg/kg) and As(0.28 mg/kg) in maize grains; As(0.36 mg/kg) and Co(0.32 mg/kg) in spinach leaves; and Cu(0.36 mg/kg) and Co(0.32 mg/kg) in okro. The values of all the PTEs analysed for samples from dumpsites were higher than those from the control site suggesting possible mobility of PTEs from dumpsites to farmlands through leaching and runoffs. However, they were below values recommended by the World Health Organization (WHO).

Concentration of three heavy metals; Zn, Cu and Pb in some leafy vegetables viz., cockscomb (*Celosia argentea*), african spinach (*Amarathus viridis*), , jute plant (*Corchorus olitorus*) and lettuce (*Lactuca capensis*) from four farmlands designated as Idi - araba, Isolo, Owode - Onirin and Badore in Lagos Metropolis was investigated by Babatunde *et al.* (2014). The concentrations of Cu, Zn and Pb in the leaves, stems and roots of cockscomb, african spinach, jute plant and lettuce were found to be 1.542 – 0.125, 88.417 – 17.700, 7.568 – 0.028; 1.633 – 0.125, 82.417 – 18.250, 16.334 – 0.083; 1.583 – 0.028, 17.542 – 8.243, 10.833 – 0.167; 0.046 – 0.235, 0.00, 0.456 – 0.342 mg kg-1 respectively. The concentrations of Pb of the leaves of vegetables at Isolo, *Corchorus olitorus* at Idi – araba, *Celosia argentea* and *Lactuca capensis* at Badore were above the recommended maximum acceptable limits by WHO Expert Committee on Food Additives, whereas, Zn and Cu contents of leaves of all vegetables were below the recommended limits in the four farmlands. This study showed that the vegetables obtained directly from the study sites may not constitute a health hazard for consumers.

In a similar study, the presence of Cr, Mn, Ni, Co, Cu, Cd, Zn and Pb in four of the most commonly consumed vegetables in the Southern part of Nigeriawere also investigated by Kalaghor *et al.* (2014). These vegetables include fluted pumpkin (*Telfairia occidentalis*), Bitter leaf (*Vernonia amygdalina*), Scent leaf (*Ocimum gratissimum*) and Water leaf (*Talinum triangulare*). The vegetables studied

revealed high concentrations of these PTEs which were found to be above the FAO and WHO acceptable limits.

An investigation carried out by Amusan *et al.,* (2005) revealed that soils in municipal waste dump sites commonly serve as fertile ground for the cultivation of a variety of fruits and leafy vegetables and the soils are also used as 'compost' by farmers without regards for the probable health hazards the heavy metal contents of such soils may pose. It was this concern that informed the characterization of soils and crop plants in selected dump sites in Nigeria with particular reference to the heavy metal content, and the assessment of the potential of the crops to mine and deploy heavy metals to their edible portion. The result showed that soils in municipal waste dump sites are higher in heavy metals: Zn, 63.2-102.11; Co, 36.0-132.14; Cu, 36.5-72.99; Pb, 63.58-418.58 and Cd, 17.00-47.06 mg/kg and that crops growing in the dump sites bio-accumulate considerably higher metal contents than those in normal agricultural soils. It was also observed that crops differ in their ability to uptake metals.

Echem and Kabari (2012), carried out studies on the PTE content in Bitter leaf (*Vernonia amygdalina*) grown along heavy traffic routes in Port Harcourt and their result showed that the use of pesticides, insecticides and PTEs in industries has led to widespread environmental contamination and also that some of these compounds are object of study on account of their toxicity and ubiquity.

The concentration of PTEs (Pb Cd Cr) in plants and soils from abandoned mechanic workshops in Umuahia metropolis were determined by Abii (2012), using atomic absorption spectrometer (AAS) to estimate the level of pollution. The mechanic workshops were chosen from the four axis of the metropolis and the soils were collected at the root zone of the plants samples. The results showed that the concentration of those heavy metals in soils and plants were significantly ($p < 0.01$) higher

than the permissible level and correlation analysis showed a strong positive relationships between metal concentration in plants and soil. This study revealed that the consumption of farm products/vegetables grown in the abandoned mechanic workshops pose significant health hazards to human and therefore calls for government action to control the indiscriminate disposal of waste generated in mechanic workshops.

Another study byAdefemi *et al.* (2012) considered level of PTEs in various sections of vegetables harvested from Atikankan, Aba–Egbira and Moshood Road dumpsites in Ekiti State, Nigeria with a view to monitoring the pollutional status of these dumpsites. *Amaranthus cruentus* and *Talinum fruticosum* were the two vegetables used. PTEs concentrations varied among different plants and was found to range from ND - 0.90 mg/kg for Cd, 0.10 - 0.40 mg/kg for Pb, 0.10 - 1.00 mg/kg for Mn, 0.70 - 3.60 mg/kg for Zn, 0.20 - 2.00 mg/kg for Fe, 0.20 - 1.30 mg/kg for Cr, 0 .20 - 2.50 mg/kg for Cu, 0.20 - 2.10 mg/kg for Ni and 0.20 - 1.40 mg/kg for Co respectively in all the dumpsites. Cd was not detected in the root of *Amaranthus creuntus* and leaf of *Talinum fruticosum* (Moshood road dumpsite). The extent of metal contamination in plants samples was greater in Atikankan than other dumpsites. However, concentration of Cd exceeded the WHO/FAO limits for human consumption in *Talinum fruticosum*.

Ogemudia and Mbong (2013) studied the concentration of heavy metals in plants and soil of refuse dumps in Uyo, Nigeria. The soil samples were collected from two dumpsites and digested with concentrated Trioxonitrate (V) acid and Tetraoxochlorate (IV) acid. The heavy metals investigated were: lead, manganese, Iron, chromium Zinc and Cadmium in two vegetables *Ipomea batatas* and *Laportea ovalifolia* . The concentrations of the heavy metals in the leaves of the vegetables were determined using Atomic Absorption Spectrophotometer (AAS). Manganese (54.3±1.4) and Iron (1013.02±8.5) were the most abundant heavy metals while Cadmium (3.7±0.2) was the least

abundant across the two dumpsites. Heavy metals in plants were found to positively correlate with that present in the soil. It was therefore concluded that the consumption of leafy vegetables and crops produced on soils with elevated metal levels pose serious health risk to consumers.

Matured plant samples of Garden Lettuce (*Lactuca sativa L.*) from roadside farm, Badagry expressway, Lagos, Nigeria were assessed by Adu *et al.* (2012). Pressurized digestion with HCl, H_2SO_4 and $HNO_3$was employed to determine the heavy metals. Three composite samples on each bed at each distance were used. The distances were 5m, 10m and15m from the road.The concentration of heavy metals which include, Cu, Zn, Fe, Cr, Cd, and Pb, were determined using Perkin-Elmer analyst 300 Atomic Absorption Spectroscopy (AAS). The mean concentration for each heavy metal in the samples were obtained and compared with the permissible levels set by the FAO and WHO. The results obtained from this analysis revealed that Fe showed the highest concentrations in the stem (14.681±11.621mg/kg), second to Zn in the leaf (0.062±0.047mg/kg) while Cr showed the lowest levels (0.001±0.000mg/kg) in the whole plant organ studied. When compared with standards, heavy metal levels were found to be within safe limit.

In another study, concentrations of heavy metals in and around the largest e-waste dumping site in Nigeria, Alaba International Market in Lagos, was investigated by Olafisoye *et al*., (2013). Concentrations of five heavy metals, namely: cadmium, chromium, lead, nickel, and zinc in soil, water, and plant samples during the wet and dry seasons were measured using an atomic absorption spectrometer. Samples were collected between October 2011 and May 2012 and digested using standard wet digestion methods. Lead recorded the highest values, while the lowest were found for Cdin all the samples during the dry season. Heavy metal concentrations were generally lower during the wet season due to increased aeration and dilution from rainfall. Results showed that the total mean concentrations of the heavy metals decreased with depth in soil samples and also distance from

the dumpsite. A noteworthy observation was that the concentrations of most of the heavy metals under investigation exceeded maximum permissible levels.

The contents of Pb, Cu, Cr, Zn and Cd in various leafy vegetables viz., spinach, coriander, lettuce, radish, cabbage and cauliflower grown in an effluent irrigated fields in the vicinity of an industrial area of Faisalabad, Pakistan were assessed by Muhammed *et al.* (2008) using the Atomic Absorption Spectrophotometer. The concentrations of Pb, Cu, Cr, Zn and Cd in the leaves, stems and roots of spinach, coriander, lettuce, radish, cabbage and cauliflower were found to be 1.1331 2.652, 1.313-2.161, 1.121-2.254; 0.252-0.923, 0.161-0.855, 0.221-0.931; 0.217- 0.546, 0.376-0.495, 0.338-0.511; 0.461-1.893, 0.361-0.874, 0.442-1.637; 0.033-0.073, 0.017-0.061, 0.011-0.052 mg/kg on dry matter basis, respectively. The contents of Cu, Zn, Cr, Pb and Cd were below the recommended maximum acceptable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives. The leaves of spinach, cabbage, cauliflower, radish and coriander contained higher concentrations of Cu (0.923 mg/kg), Cd (0.073 mg/kg), Cr (0.546 mg/kg), Zn (1.893 mg/kg) and Pb (2.652 mg/kg) compared to other parts of each vegetable. High concentrations of heavy metals as analyzed in the present analysis of different parts of the vegetables might be related to their concentration in the soils irrigated with industrial waste water.

Samples of *Amaranthus caudatus* grown on some dump sites at different locations in the Lagos metropolis were analysed for their heavy metal concentrations using the Atomic Absorption Spectrophotometer by Adewuyi *et al.* (2010). The results obtained revealed that the metals were present in the following order of concentration Fe> Cu> Pb> Zn> Mn> Cd. The average values of the heavy metals obtained for vegetable samples at control sites are about 35% less than those at the dumpsite. The pH values of the soil samples at the dumpsites are also relatively higher than the

control. Most of the metals in the dumpsites have impacted the soil environment but copper concentrations were found to be much higher than normal range in the mineral soil environment.

A field study was conducted by Mirecki*et al.*(2015) to investigate translocation and accumulation of four heavy metals: Cd, Pb, Cu and Znin 10 different plants : corn (*Zea mays L.*), bean (*Phaseolus vulgaris L.*), potato (*Solanum tuberosum L.*), onion (*Allium cepa L.*), pepper (*Capsicum annuum L.*), tomato (*Solanum lycopersicum L.*), lettuce (*Lactuca sativa L.*), Swiss chard (*Beta vulgaris subsp.vulgaris L.*), cabbage (*Brassica oleracea var.capitata L.*), plantain (*Plantago major L.*) in samples from 2 sites (unpolluted-Leposavić and polluted-Kosovska Mitrovica, Kosovo province). The results presented here showed that transfer factors -TF (heavy metals from soil to plants) are dependent on each other and comparison of the transfer factor for various species has significance only if all other conditions (especially environments) are equal. Heavy metals were accumulated in plant species with different intensity. The research findings indicated that Cd and Zn accumulated the most with the transfer factor of 1.0 - 10, followed by Cu with TF of 0.1-1.0, while Pb had the lowest accumulation with TF usually 0.01-0.1. TF decreased when the plants were grown in the soil with higher level of heavy metals. When the growing takes place on the same type of soil, the heavy metal accumulation in different species decreased in the following order: grains < root vegetables < fruit vegetables<leaf vegetables.

Dingkwoet*et al.* (2013) conducted a study to analyze some heavy and trace metal contents of selected edible vegetables commonly sold in four local government areas (LGAs) of Plateau State, Nigeria, with a view to unearth their toxicological implications on the populace. The vegetables sampled are *Spinacia oleracea* (spinach), *Lactuca sati* (lettuce), *Cucumis sativus* (cucumber), *Daucus car* (carrot) and *Brassica oleracea* (cabbage), while the metals analysed are Cd, Pb, Fe, Cr and Mn. The Atomic Absorption Spectrophotometer was used to determine the concentration of the

various metals and Tukey-Kramer multiple comparison tests in a one-way analysis of variance was used to compare variations in metal concentrations. Cd and Pb were not detected in all the vegetables sampled from Bassa LGA, but were detected in vegetables of Jos-North, Jos-South and Barkin Ladi LGAs. Cd and Pb concentrations in most samples from Jos-North, Jos-South and Barkin Ladi LGAs were above the WHO maximum permissible limits for vegetables. Cr concentrations in all the vegetables from all the LGAs were far above the WHO maximum permissible limit in the order: Jos-South > Jos-North > Barkin Ladi > Bassa. Fe concentration in most vegetables from all the LGAs is above the WHO maximum permissible limit in the orders: Jos-North – Lettuce > Spinach > cucumber > carrot > cabbage; Jos-South – Lettuce > Spinach > cabbage > carrot > cucumber; Barkin Ladi – Lettuce > cucumber > Spinach > carrot > cabbage; Bassa – Letuce > carrot > spinach > cabbage > cucumber. Mn order of concentrations in the vegetables is: Jos-north – spinach > Lettuce > carrot > cucumber > cabbage; Jos-South – spinach > lettuce > cabbage > carrot > cucumber; Barkin Ladi – Lettuce > cucumber > Spinach > carrot > cabbage; Bassa – Lettuce > spinach > cabbage > carrot > cucumber. The order of lead concentrations in the vegetables are: Jos- North – cabbage > spinach > carrot > lettuce > cucumber; Jos-South – Lettuce > cucumber > carrot > cabbage > spinach; Barkin Ladi – Lettuce > spinach > cucumber > carrot > cabbage. The results indicated that vegetables from Jos-North, Jos-South and Barkin Ladi were contaminated with the metals sampled, while lettuce and spinach were very good bioaccumulators of these metals.

Nanven *et al*. (2015) investigated the concentration of some heavy metals in vegetables grown in a farm treated with dumpsite soils in Kuru Jantar, Nigeria. Soil samples and vegetables from the farm were collected and prepared using standard analytical procedures. The concentrations of metals in both soil and vegetables were determined using the Atomic Absorption Spectrophotometer (AAS). The results showed that the farm was polluted with the metals (Cd, Cr, Cu, Mu, Fe, Pb, Zn, Ni) determined. The Enrichment Factor (EF) showed that some metals had minimal enrichment while Cd

(13.93) had significant enrichment at the farm. The Pollution index (PI) calculations showed that at the farm, the contamination pollution ranged from very slight to very severe. The overall order of the metals at the dumpsite was Fe> Mn> Zn> Cu> Cr> Ni> Pb> Cd while the order of the metal concentrations at the farm was Fe> Mn> Zn> Ni> Cd> Pb. The data obtained in the study were analyzed using Pearson correlation analysis. The results showed perfect positive correlation values above 0.9 between the farm and the dumpsite, which indicated that there was a strong association or similarity between them. The metal concentrations in the vegetables analyzed showed that spinach decreased in the order Fe>Zn>Mn>Cd>Pb while in Cabbage, the order was Mn> Fe> Zn>Cu>Cd; in Radish, the order was Fe>Mn>Cu>Cr>Zn while in pepper, the order was Fe>Cu>Mn>Ni. In general, the metal concentrations were below the recommended limit of USEPA and FEPA standards for agricultural soils and vegetables except for Cd in vegetables. The concentrations were however higher on the farm than in the control. Thus, the farm was polluted with heavy metals from the dumpsite soils.

2.3 ANALYSIS OF PTEs

Sample preparation is the most important step in any analytical process. It is the series of steps required to transform a sample to a form suitable for analysis and involves steps from simple dissolution to partial or total digestion (Altun, 2005). For most methods of analysis, it is required that the analytical sample be in a liquid form, thus, standard procedures to convert solid (or solid containing) samples to solutions prior to detection is required. The methods include wet decomposition and dissolution of the organic and inorganic samples, in open or closed systems, using thermal or radiant (ultraviolet or microwave) energy.

2.3.1 WET DIGESTION METHOD

Wet digestion method involvescomplete dissolution of the organic material in a sample matrice to convert elements of the sample into soluble forms by heating withconcentrated acids. This digestion is produced by supplying energy, such as heat; by using a chemical reagent, such as an acid or a combination of acids. Where a reagent is used, its nature will depend on that of the matrix. The amount of reagent used is dictated by the sample size which, in turn, depends on the sensitivity of the method of determination.

The majority of wet digestion methods (total decomposition and strong attack) involve the use of some combination of oxidising acids (conc. HNO_3, hot conc. $HClO_4$, and conc. H_2SO_4) and non-oxidising acids (HCl, HF, H_3PO_4) and hydrogen peroxide to release the elements of interest from the sample matrix.The acid used in the digestion process depends on the nature of the matrix to be decomposed. Hydrochloric acid (HCl) is useful for salts of carbonates, phosphates, some oxides and some sul des. Nitric acid, HNO_3 (boiling point 122 °C) oxidizes many samples that cannot be dissolved by HCl. Hydrogen peroxide, H_2O_2 (boiling point 150 °C) is a strong oxidizer, which is

used in combination with HNO_3 to dissolve matter that is not fully decomposed by HNO_3 (Ranasinghe*et al.*, 2016).

Wet digestions may be performedeither in open or closed beakers on hot plate, digestion block or using closed vessels, such as polytetra uoroethylene (PTFE)-lined containers in microwave digestion system (Twyman, 2005).

Wet digestion has the advantage of being effective on both organic and inorganic materials. It often destroys or removes the sample matrix, thus helping to reduce or eliminate some types of interference. Most applications of wet digestion involve aqueous or organic matrices such as surface waters, wastewater, biological and clinical samples, food samples, as well as soil, sediments and sewage sludge.The most common oxidising agents used in wet digestion for soils and sediments is a combination of hydrochloric acid and nitric acid in the ratio of 3:1, which is known as aqua regia mixture.Aqua regia digestion is an effective way of digesting under wet digestion.The nitric acid acts as the oxidizing agent, while the hydrochloric acid provides the complexing properties (Ranasinghe*et al.*, 2016).

2.3.1.1 Decomposition of Aqua regiaduring digestion process

Upon mixing of concentrated hydrochloric acid and concentrated nitric acid, chemical reactions occur. These reactions result in the volatile products nitrosyl chloride and chlorine as evidenced by the fuming nature and characteristic yellow color of aqua regia. As the volatile products escape from solution, the *aqua regia* loses its potency.The reaction is shownin Equation 2.1

$$HNO_3(aq) + 3HCl\ (aq) \rightarrow NOCl\ (g) + Cl_2\ (g) + 2H_2O\ (l) \quad \text{Equation 2.1}$$

Nitrosyl Chloride

Nitrosyl chloride further decomposes into nitric oxide and chlorine. This dissociation is equilibrium-limited. Therefore, in addition to nitrosyl chloride and chlorine, the fumes over *aqua regia* contain nitric oxide as shown in Equation 2.2

$$2NOCl\ (g) \rightarrow 2NO\ (g) + Cl_2\ (g) \qquad \text{Equation 2.2}$$

Wet digestion methods can be carried out using either an open or closed vessel digestion system(Muinde *et al*., 2013).

Open vessel acid digestion, one of the oldest techniquesis undoubtedly the most common method of sample decomposition and dissolution of organic and inorganic sample materials in many chemical analysis. Open system reduces the analytical problems consequent to digestion by allowing the analysis of a larger number of samples. These systems also facilitate evaporation of acid and drying of the digests (Arena *et al*., 2013). The system however, is limited by a low maximum digestion temperature which cannot exceed the ambient-pressure boiling point of the corresponding acid or acid mixture. For instance, the oxidising power of nitric acid with respect to many matrices is insufficient at such low temperatures (boiling point 122 ^{o} C). One possible remedy is the addition of sulphuric acid which significantly increases the temperature of the digestion solution. Open digestion systems have been popular in sample analysis over the past decades, but have consistently suffered from the major drawback of their sensitivity against corrosion and subsequent risk of contamination through laboratory air, the use of large amount of required reagent (very often expensive reagents), and the danger of losses of trace elements.

In comparison to open vessel digestion, closed vessel digestion method (microwave assisted digestion system) have many advantages with one disadvantage, which is complex and expensive vessel design.

2.3.2 CONVENTIONAL HEATING (THERMALLY CONVECTIVE WET DIGESTION)

The conventional approach to wet digestion, which has proven its worth over many years, entails a system equipped with heated conventional source (Bunsen, heating plate, send bath, etc.) operating either at a fixed temperature or in response to a temperature program. Acid digestions are often accomplished in any vessel, usually in glass or PTFE (beaker, conical flask, etc.).

2.3.3 MICROWAVE-ASSISTED DIGESTION SYSTEM

Ever since Abu-Samra *et al.*(1975) reported on the application of microwave techniques to wet digestion of biological samples (the first paper published on microwave-assisted digestion), there has been a rapid development in microwave-assisted digestion for elemental analysis.

Microwave energy is a non-ionising type of radiation that causes molecular motion through migration of ions and rotation of dipoles without altering molecular structure. When microwave energy penetrates a sample, the energy is absorbed by the sample and the migration of the analytes out of the matrix (destruction of the macrostructure of the matrix) takes place (Morales-Munoz *et al*., 2003). This process is quite different from the conventional / classical methods, where the solvent diffuses into the matrix and the analytes are removed out of the matrix by solubilisation (Morales-munoz *et al.*, 2003). Microwave radiation is one of the energies more frequently used for accelerating sample preparation by applying it at the appropriate power, mode and during the required time.

The use of microwave heating has numerous advantages over conventional thermal heating in sample extraction and digestion. In conventional heating, the material is heated from an external source outside the digestion vessel whereas in microwave heating the digesting material absorbs the heat inside the vessel directly (Figure 2.1 & 2.2). Heating from an external source requires that the

vessel be heated first and a thermal gradient reached before heating of the sample begins, thus increasing the digestion time and also increasing the energy requirement to heat both the material and the vessel. When using microwave heating the reagent will absorb the energy and heating will begin to occur almost immediately.

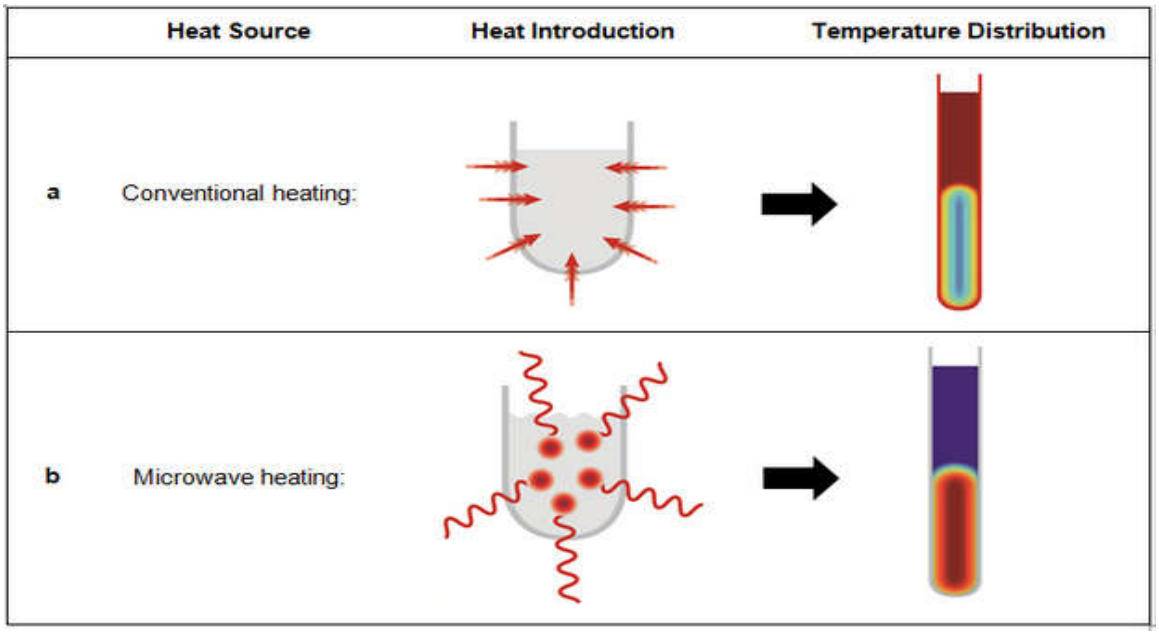


Figure 2.1: Heating Process in Conventional and Microwave Digestion(Morales-munoz *et al.*, 2003)

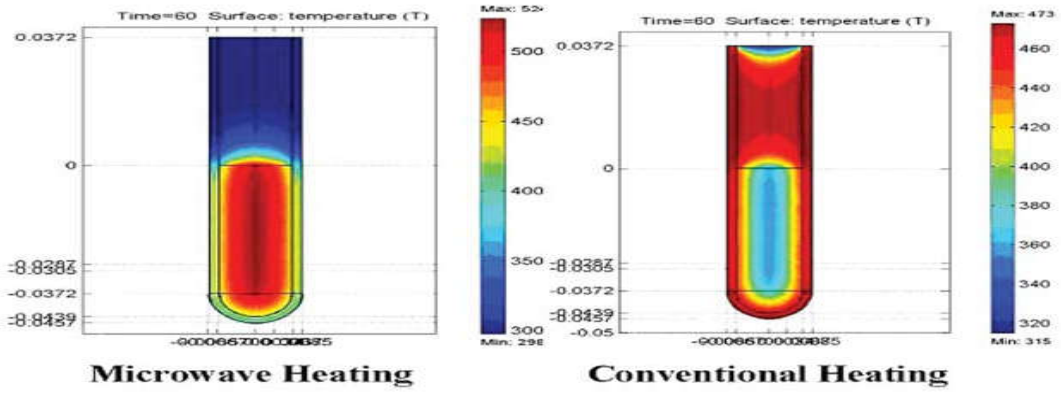


Figure 2.2:Temperature distribution during Microwave and Conventional Heating (Morales-munoz *et al.*, 2003)

The main advantages of microwave assisted digestion over the conventional extraction techniques is that it reduces solvent consumption, it has a shorter operational time, it possess moderately high recoveries, has a good reproducibility and minimal sample manipulation for extraction process (Afoakwah *et al.*, 2012).

Using microwaves, both the speed and the efficiency of digestion for some types of samples considered difficult to solubilize are often improved.

2.3.3.1 THEORY OF MICROWAVE DIGESTION

Microwaves are a form of electromagnetic radiation with wavelengths ranging from 1 mm to 1 m; with frequencies between 300 MHz to 300 GHz. The electromagnetic waves are interconnected electric (E) and magnetic fields (H) (Figure 2.3) which change over time and propagate at the speed of light (c) through space(Anton-Paar, 2006).

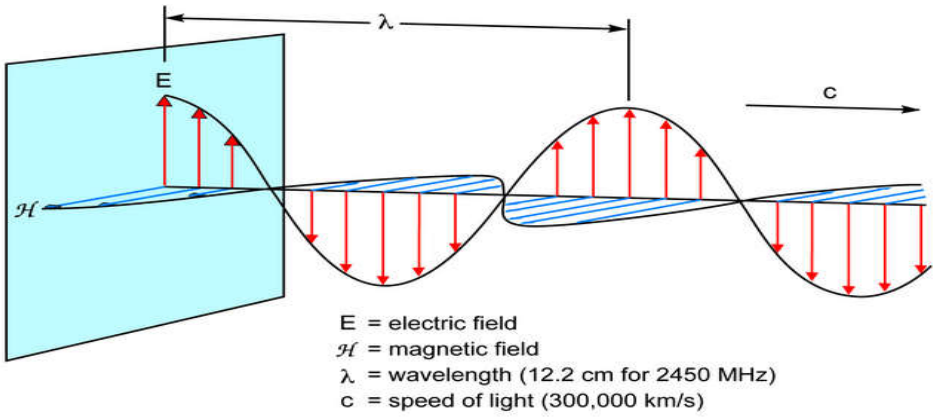


Figure 2.3: Representation of Microwave radiations(Anton-Paar, 2006)

Low microwave frequencies can be produced using electron tubes or transistors, while high and very high frequencies (> 100 MHz) are produced by Klystrons, magnetrons or travelling wave tubes. In decomposition instruments, microwaves are produced using magnetrons.

A magnetron is a metallic vacuum tube, consisting of a cathode and an anode, which is surrounded by a permanent magnet frame. High voltage is applied to a heated rod (cathode) to produce electrons, and these electrons are drawn into a circular path by the applied magnetic field. These electrons hit the outer wall of the tube (anode), which is cut at regular intervals radially to the chamber (resonator). Within these incisions the polarity of the electrical field reverses with the desired frequency and produces microwaves. These are emitted through an antenna, e.g in the microwave oven (Anton-Paar, 2006).

Microwaves are reflected by non-polar metals, they pass through ceramic, glass and porcelain, they are absorbed by organic materials, and they are not an ionizing radiation, neither mutagenic nor carcinogenic.

Microwave assisted extraction consists of heating the solvent in contact with the sample by means of microwave energy. The process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of the ions (ionic conduction), which enhance penetration of the solvent into the matrix, allowing dissolution of the components to be extracted.

2.3.3.1a DIPOLE ROTATION

This is the case with materials which have a pronounced dipole (water, acids, solvents). In the rapidly changing electric field, the molecules try to orient themselves in the direction of the field lines. This sets them in rotation-vibration. To achieve the thermal effect the frequency of microwave is so adjusted that in an alternating electric field, the phase difference between rotating the dipoles and orienting the field causes molecular friction and collisions that give rise to dielectric heating The

closer the resonance frequency of the molecule is to the frequency of microwave, the more intensive the energy absorption from the microwave field (Anwar *et al.,* 2015)

2.3.3.1b IONIC CONDUCTION

In conduction, dissolved charged particles (ions) in a sample oscillate back and forth under the influencing electric force of microwaves creating an electric current. This current faces internal resistance because of collisions of charged species with neighbouring molecules or atoms, which cause materials to heat up (Anwar*et al.*, 2015). Heating in the electromagnetic field also occurs when there are free ions (electrolytes, glassy materials and ceramic materials)

Microwave digestion can be carried out in either closed or open vessels, but closed vessels are more popular because of the higher pressures and temperatures that can be achieved.

Open vessel systems mainly use microwave radiation which is focused on the sample. As the digestion vessel is open the heating temperature is equivalent to the boiling point of the digestion reagents used. One potential benefit over the use of closed vessels is that any gasses evolved from any reactions, occur without build up of pressure. However, as the vessel is open, there is an increased chance of external contamination (Anton-Paar, 2006).

In closed –vessel systems, microwave radiation is dispersed into a cavity where sample vessels are placed. As the vessels are closed, high pressures and therefore temperatures can be obtained. The high pressure and temperature generated in the closed vessel system raises the boiling point of the acid(s) used and substantially increases the dissolution rate of the organic matrices. For example, nitric acid normally boils at 120°C but at 5 atm in a closed container, it boils at 176°C when heated by microwaves (Smita *et al.,* 2013). This is 56°C above its normal boiling point and this results in an increase of its oxidation potential and speeds up its reactions. Under conventional hot-plate

conditions, the organic matrices of biological samples would remain intact due to the low boiling point of nitric acid. Under these circumstances, reagents such as perchloric acid would have to be used for the complete destruction of the organic matter. The closed vessel microwave approach makes it possible to avoid the use of perchloric acid which is potentially explosive when in contact with easily oxidized inorganic or organic materials especially at elevated temperatures.

2.4 BIOAVAILABILITY / BIOACCESSIBILITY OF PTEs

Bioavailability and Bioaccessibility are complex issues that determine whether or not adverse effects are to be expected when plants or organisms are exposed to potentially toxic elements (Ruby *et al.,* 1999).

Risk assessment had over the years considered that the entire quantity of a substance present in an ingested soil or food is bioavailable and will reach the target organs and have a toxic effect. In order to adjust the risk calculations and take into account the fact that only a portion of the ingested dose, variable depending on the compound actually reaches the blood stream, it has been proposed that notions of bioavailability and bioaccessibility be included in risk assessments (USEPA, 2007).

Bioavailabilityis therefore defined as a measure of the proportion of the ingested contaminant that passes through the gastro-intestinal lining into the systemic blood and organs of humans (RECORD, 2012)) while bioaccessibility has been de ned as the fraction of the total amount of the chemical present in ingested food, water, or ingested soil and sediment particles that at maximum can be released during digestion(Figure 2.4). More speci cally, the bioaccessible fraction may also be de ned as the fraction that, after ingestion, may be mobilized into the gut uids (Drexlerand Brattin, 2007). This fraction is considered to represent the maximum amount of contaminant available for intestinal absorption(USEPA,2007) i.e. bioaccessible contaminants can subsequently be absorbed, in other words, transported across the intestinal wall and transferred into the blood or lymph stream and therefore become bioavailable. The compounds may then undergo the first-pass effect in which they are bio-transformed and excreted by the intestinal epithelium or liver. Finally, contaminants that are not metabolized can spread through the body by the systemic circulation, and may exert systemic toxicity. (Oomen, 2003). Hence, oral bioavailability of contaminants is the combined result of ingestion,bioaccessibility, absorption, and the first-pass effect.

Different conditions can affect bioaccessibility of a substance, for instance, the bioaccessibility of PTEs and ionizable contaminants (acids and bases) from soil or sediment is expected to be highly dependent on the pH values in the different compartments of the gastrointestinal (GI) tract. As a consequence, the bioaccessible fraction of ionizable contaminants present in ingested soil or sediment is in general larger for mammals than for soil- dwelling invertebrates, such as earthworms that regulate gut pH at around neutral. Another in uence that may affect bioaccessibility is the presence of food. The presence of food increases the period during which mobilization can take place; thismay be important for compounds for which dissolution is rate limiting. Also, an increased solubilizing capacity of the digestive mixture, due to an augumented ow of the digestive juices or to the presence of food particles, may increase the mobilization of contaminants from soil. Bile is known especially to increase the solubilizing capacity for poorly water-soluble compounds, became bile salts form micelles that have an apolar interior. Furthermore, because bile salts have surfactant properties, they may increase the wetting and thereby the rate of mobilization from soil (Oomen, 2003).

External exposure Exposure to an (external) dose of contaminant in a matrix

⇩

mouth Ingestion of matrix + contaminant

⇩

oesophagus, stomach, small intestine F_B=Fraction of an (external) dose released from matrix (bioaccessible)

⇩

small intestine portal vein F_A = Fraction of F_B absorbed by the small intestine

⇩

liver F_H = Fraction of F_A passing the liver without being metabolised

⇩

Systemic circulation F = Fraction of external dose reaching the systemic circulation

Internal exposure $F = F_B \times F_A \times F_H$

Figure 2.4:Schematic representation of processes determining oral bioavailability.

Source: Oomen *et al*., 2007

2.4.1 Bioavailability / Bioaccessibility Models

Bioavailability of a chemical can be estimated using *in-vivo* tests on animals (sampling and analysing the blood or organs of animals whose gastrointestinal tracts are similar to those of humans) or *in-vitro* tests that simulate physiological conditions in the human digestive tract.

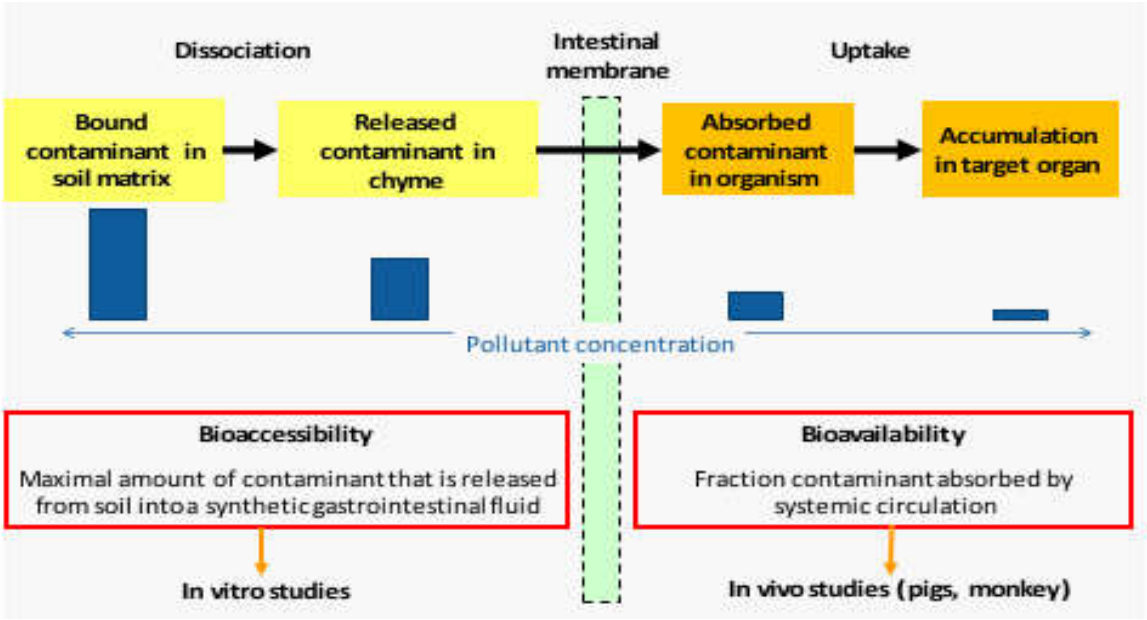


Figure 2.5:Schematic of *In-vivo* and *In-vitro* Models

Source: Kelley *et al.* (2002)

2.4.1.1 *In-Vivo* Models

In the absence of human studies or suitable epidemiological data, *in vivo* animal trials have been used to measure the bioavailability of contaminants. The animals used for these studies must have digestive systems similar to those of humans.Animal species usually used in bioavailability assessments include rabbits, rats, primates and swine (Drexler and Brattin 2007; Kelley *et al*., 2002). Many studies use swine to study the bioavailability of PTEs in soil. Although, there are anatomical differences between pigs' digestive systems and those of humans, the physiology of swine digestion is, for the most part, similar to that of humans. According to Kelley *et al.* (2002) and Juhasz *et al.* (2007), juvenile swine are comparable in size to young children and have similar gastrointestinal physiology. Animal models are, therefore, often used although they can give (relative) bioavailability estimations that are not the same as those of humans.The animals are dosed with the contaminated substrate following which body tissue samples are analysed to determine absorption(Figure 2.5).

In spite of the advantages of using bioavailability in exposure studies, a major drawback to their routine use is the need to carry out *in vivo* studies that are long, costly, hard to set up and liable to entail ethical problems.(Ruby, 2004).

2.4.1.2 *In-Vitro* Models

Mammal dosing trials are time consuming and expensive. To supersede the use of animals in determining the bioavailability of potentially toxic elements for human health risk assessment, or to estimate bioavailability where animal studies are not available, a potential alternative is the use of *in-vitro models.*These particular models are being used as part of a European platform on bioavailability and bioaccessibility, the Bioavailability Research Group Europe (BARGE). The ultimate aim of BARGE is to establish a methodology for estimating more realistic relative bioavailability factors to be used in general and site-specific risk assessments (Oomen *et al.,* 2003). One additional advantage of *in vitro* tests is that they can be designed to simulate the processes and conditions occurring in the human gastrointestinal tract (Broadway *et al*., 2010).

In-vitro models are both rapid and inexpensive, requiring only a day to conduct and costing only a small fraction of what an*in vivo* study would cost. These models make use of simulated gastric and intestinal juices, which are applied to samples in order to predict the availability of a contaminant for human absorption (Intawongse and Dean, 2006).Most models are static gastrointestinal models, which simulate transit through the human digestive tract by sequential exposure of the soil to simulated mouth, gastric, and small intestinal conditions(Figure 2.6). Dynamic gastrointestinal models mimic the gradual transit of ingested compounds through the digestive tract.

In the simplest approach, for the *in-vitro* stomach model, mobilization of the contaminants under gastric pH conditions is simulated. Whereas the more complex models can simulate more aspects of human physiology, the simple models are easy to perform and allow simultaneous determination of largenumbers of samples.

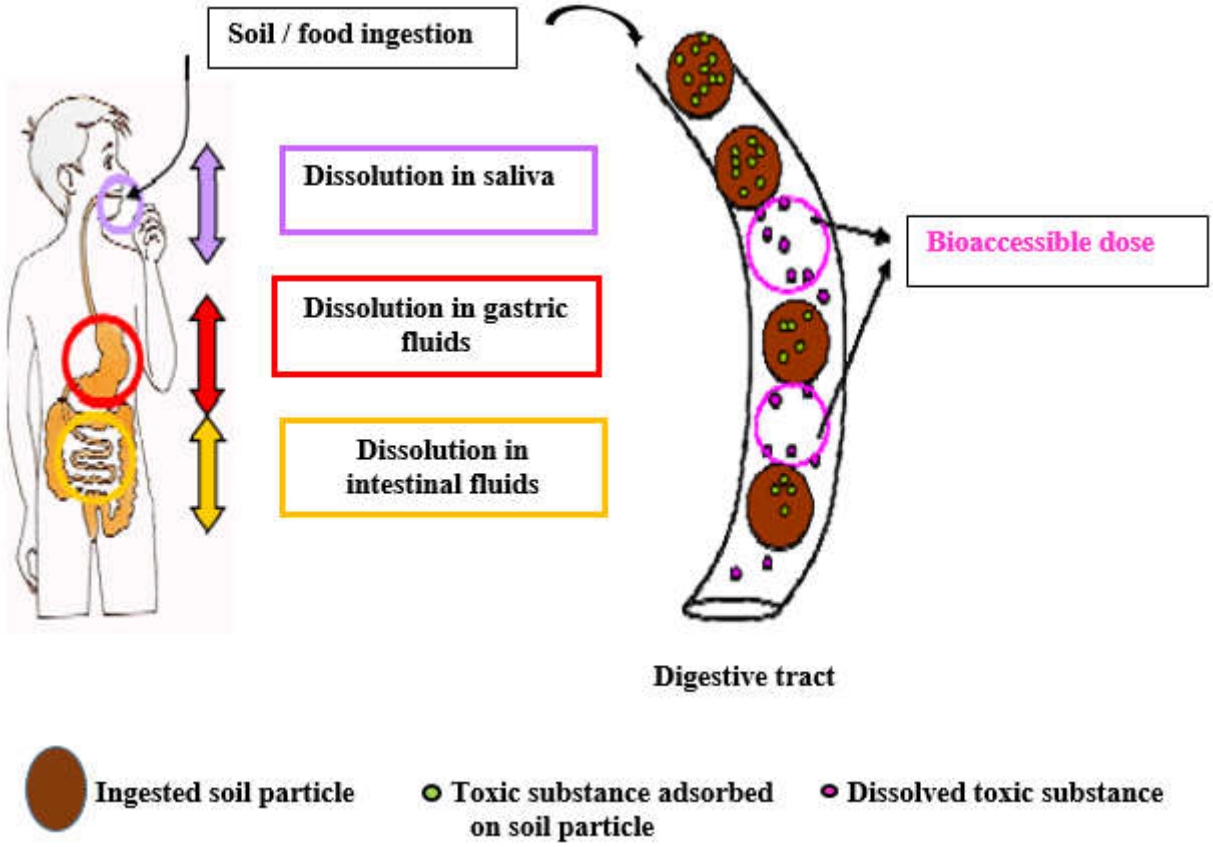


Figure 2.6. Gastrointestinal tract showing dissolution in the saliva, stomach and small intestine

Source: RECORD, (2012)

2.4.1.3 The Composition of the digestive solutions

- **The stomach solutions**

In general, all of the stomach solutions are made up of hydrochloric acid (HCl), like physiological gastric juices. Pepsin, an enzyme that hydrolyses proteins, is also present in the gastric solutions of all of the tests except, those of the Simplified Bioaccessibility Extraction Tests (SBET), which uses glycine (aiming to increase the solubility of the metals by complexation.

- **The intestinal solutions**

All of the intestinal solutions are made up of sodium bicarbonate ($NaHCO_3$), which makes it possible to increase the pH after the stomach phase. Other enzymes are used only in some tests (according to the authors, in order to increase certain hydrolytic capacities. For instance, Lipase (a pancreatic enzyme) is used in the Dutch, National Institute for Health and Environment(RIVM) andunified bioaccessibility method (UBM) tests while trypsin is added in the Standardised German *In vitro* assay (DIN) test.However, the composition of the digestive solutions is specific to each test and is more or less complex (Oomen, 2003)

2.4.2 *In-Vitro* Bioaccessibility Models

Several *in vitro* bioaccessibility models were developed over the decades but only few have been validated and established as standard methods.

2.4.2.1 The Physiologically Based Extraction Test (PBET)

This is a model widely acknowledged and applied as an *in vitro* digestion tests. The PBET model simulates the leaching of a solid matrix in the human gastrointestinal tract and determines the bioaccessibility of a particular element i.e. the total fraction that is available for adsorption during transit through the small intestine (Intawongse and Dean, 2008)). It was designed around the paedriatric gastrointestinal tract for a child of 2-3 years old. This age group was chosen because it is believed to be at greatest risk from accidental soil ingestion (Ruby *et al.*, 1999).

This test is essentially a two stage sequential extraction using various enzymes to simulate both gastric and small intestine compartments, with extractions carried out at 37 ^{o} C. It was proposed by Ruby *et al.* (1996), and is usually composed of successive digestion of gastric and intestinal phase, used to measure the bioaccessibility of Pb and As, and validated by using animal model. After then, PBET has been widely employed to determine the bioaccessibility of metals (Intawongse and Dean 2008; Poggio *et al.,* 2009).

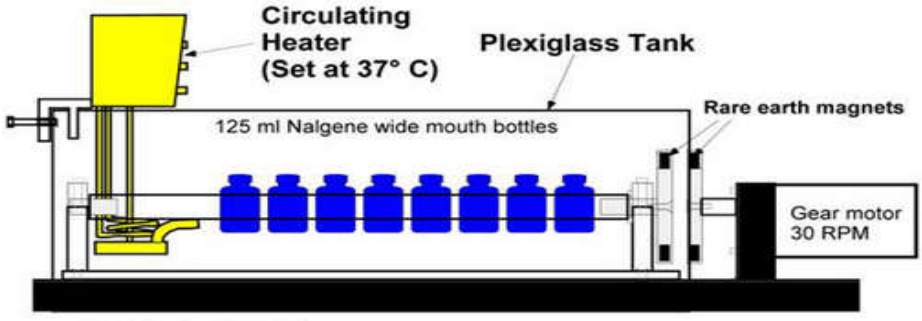


Figure 2.7:Diagram of a typical PBET extraction apparatus

Source: Ruby *et al*.(1996); Intawongse and Dean (2008); Poggio *et al*.(2009).

2.4.2.2 Simplified Bioaccessibility Extraction Test (SBET)

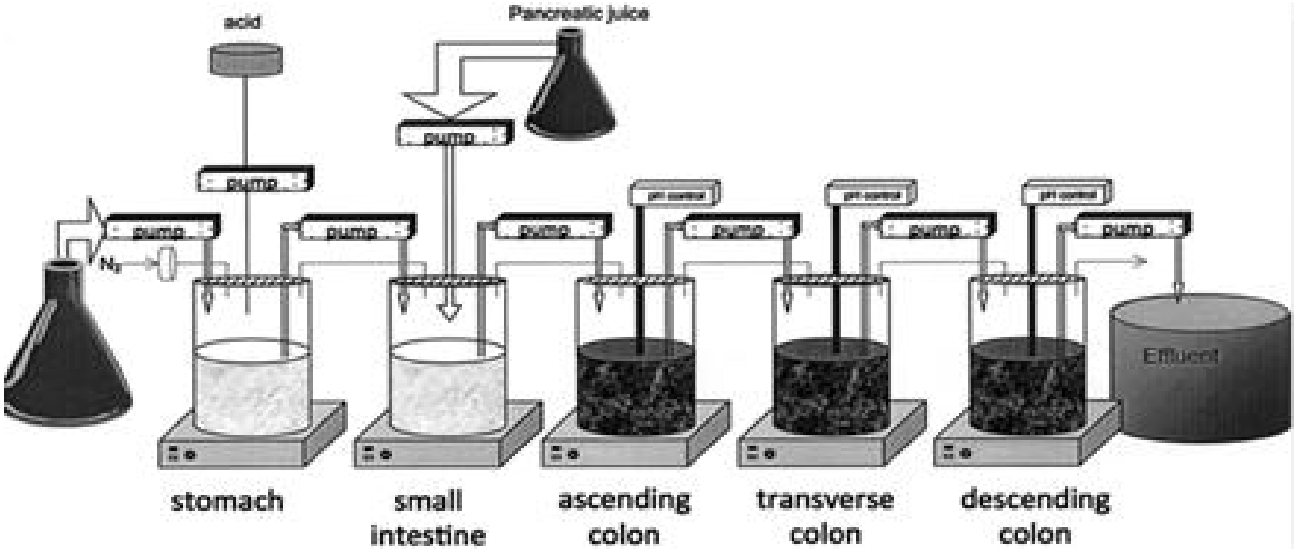
The development of the SBET was in response to a request by the USEPA region III and other US agencies, the need for laboratories to be able to use and apply simple bioaccessibility testing regimes (Sprovieri *et al.,* 2007). It is a simplified form of PBET which uses a single reagent in a single extraction process for a relatively short period of time, making it practically easy to carry out and ideal for large batches of sample (Wragg and Cave, 2011).

The SBET method does not include an intestinal compartment with more neutral pH levels, in contrast to the other in vitro digestion methods.End-over-end agitation is done by vertical rotation of the sample, as opposed to horizontal agitation with shakers, blades or magnetic stirrers. End-over-end rotation also makes it possible to avoid any contamination that might be caused by blades or magnetic stirrers (Drexler and Brattin, 2007).

Juhasz *et al.* (2007) carried out a study using SBET on arsenic and found correlations with *in vivo* studies.

2.4.2.3 The Simulator of human intestinal microbial ecosystem (SHIME) model

The SHIME is a scientifically validated dynamic model of the complete gastrointestinal tract to study physicochemical, enzymatic and microbial parameters in the gastrointestinal tract in a controlled *in vitro* setting(Figure 2.8). The model mimics several segments from the stomach to the colon and makes it possible to create a microbial flora that is representative of the latter since this flora might play a role in the transformation of molecules before their passage through the intestinal membrane. However, it takes 3 weeks to obtain this flora (dynamic SHIME) and the static test is then carried out for approximately 24 hours. This test is of interest because of the use of a bacterial flora that might play a significant role in bioaccessibility due to its ability to degrade and transform molecules (Oomen *et al.*, 2003).



Schematic representation of the SHIME model

Source:Oomen *et al.* (2007)

2.4.2.4 In vitro Gastrointestinal Model (IVG)

This method like PBET, employs simulated gastric solution but unlike the PBET, the simulated gastric solution is prepared in a 0.15 M sodium chloride matrix, it also uses different concentration of reagents and a lower pH i.e. pH 1.8 for the stomach phase and 5.5 for the intestinal phase (Sprovieri *et al.,* 2007). This method was employed to simulate the human gastrointestinal environment and estimate the bioaccessibility of arsenic in soil and soil media. The samples used for this studies were not natural soils but aged (50 year) calcine material, a waste product formed from roasting arsenopyrite ore and an iron ore slag material which was a waste product from smelting lead ores. IVG has also been used by Jiang *et al.* (2009) in the study of As bioavailability.

2.4.2.5 TNO simulated gastrointestinal tract model (TIM)

This is a method developed at the TNO (Toegepast Natuurwetenschappel Onderzoek) Nutrition and Food Research at Zeist in the Netherlands. It was initially developed for the studies of pollutants in soils but has now been used for studies on absorption of minerals and food mutagens in humans; the survival of ingested bacteria and yeasts (Oomen *et al*., 2003). It has also found application in drug industries for studying bioavailability and accessibility of drugs (Barker *et al.,* 2014). The method developed by TNO Nutrition group is a complex *in vitro* test involving a number of gastrointestinal solutions. It mimics the transit through the gut, the pH of the stomach and intestine, secretion of digestive juices over time. It involves mathematical modelling of gastric and intestinal delivery with power exponential on computers for controlling meal transit through the system (Van de Wiele *et al.,* 2007).

2.4.2.6 RIVM *in vitro* digestion model

This is a model designed by the National Institute of Public Health and the Environment (RIVM) in the Netherlands. It is a three stage sequential extraction method using a 5min saliva phase at pH 6.5, followed by a 2 hr stomach extraction at pH 1.07 and a 2 hr small intestine extraction at pH 5.5 (Versantvoort *et al*., 2004).

2.4.3 General principles behind *in vitro*bioaccessibilitymodels

The objective of *in vitro* model is to mimic the physiological conditions in the human body, in particular in children, who are considered to be the population potentially the most exposed to soil contaminants by ingestion. Most of these tests were developed to study the bioaccessibility of contaminants (initially inorganic) in soils. Some, however, were developed within the framework of nutritional studies (RECORD, 2012)

• The potentially contaminated soil, usually after drying and sieving, is put into specifically designed glassware and placed in contact with digestive solutions that are representative of the physiological conditions in the various gastrointestinal segments studied. The composition of these digestive solutions is specific to each test.

• The segments that are most often studied (that are included in most tests) are the stomach and the small intestine (the segments that are most involved in the absorption of dissolved contaminants). Some tests include a salivary phase (RIVM, DIN). Others distinguish between various segments of the small intestine or the colon (TIM, SHIME).

• The pH is adjusted at each step in order to represent the actual pH physiologically encountered. Low pH values (usually between 1 and 2.5, depending on the test) are used for stomach phases and higher pH values (between 5.5 and 7.5) are used for the intestinal phases. Tests that also mimic the salivary phase use a pH value of 5.5 or 6.5.

• Tests are carried out at a temperature of 37 °C.

• The residence time in each segment varies depending on the test. They are short (several minutes) for salivary phases, between 1 and 2.5 h for stomach phases, and 2 to 18 h for intestinal phases. Before concentrations are measured, samples are centrifuged and/or filtered, then stored at 4 °C or are stabilised by adding acid (for metal studies).

• The extracts thus obtained are analysed using suitable methods. Authors generally recommend using standardised methods (variable, depending on the country) that make it possible to attain sufficiently low limits of quantification (RECORD, 2012).

Regardless of which *in vitro*test is used, the bioaccessibility of inorganic micropollutants – cationic micropollutants such as lead and cadmium, in particular – is higher in the gastric compartment than in the intestinal compartment. pH is the parameter that explains the differences observed in bioaccessibility values for gastric and intestinal compartments for inorganic elements.

Some authors recommend using the bioaccessibility results obtained in the gastrointestinal phase, which aim to simulate physiological conditions as realistically as possible in order not to overestimate the fraction of the pollutant that might reach the blood stream. These observations therefore lead researchers to consider that the gastric phase might be sufficient for studying the bioaccessibility of cationic inorganic elements such as lead and cadmium, which would make it possible to limit costs.

2.5REVIEW OF STUDIES ON HUMAN HEALTH RISK ASSESSMENT AND BIOACCESSIBILITY OF PTEsIN MATRICES

Ihediora *et al*., 2016 in their investigation assessed the levels of some heavy metals in soils in the vicinity of a municipal solid waste dumpsite with a view to providing information on the extent of contamination, ecological risk of metals in the soils and human health risk to the residents in Uyo. Soil samples were collected in rainy and dry seasons and analyzed for metals (Pb, Cd, Zn, Mn, Cr, Ni and Fe) using atomic absorption spectrometry. The concentrations of heavy metals (mg/kg) at the dumpsite in rainy season were Pb (9.90), Zn (137), Ni (12.56), Cr (3.60), Cd (9.05) and Mn (94.00), while in dry season, the concentrations were Pb (11.80), Zn (146), Ni (11.82), Cr (4.05), Cd (12.20) and Mn (91.20). The concentrations of metals in the studied sites were higher than that of the control site ($P < 0.05$). Pollution indices studies revealed that soil samples from dumpsite and distances from 10 and 20 m east of the dumpsite were highly polluted with cadmium. Ecological risk assessment carried out showed that cadmium contributed 98-99 % of the total potentially ecological risk. No probable health risk was observed as the total hazard index of all the metals was less than one. However, children were found to be more susceptible to heavy metal contamination than adult.

Kamunda *et al*.(2016) in their study evaluated the health risk caused by heavy metals to the inhabitants of a gold mining area. In this study, 56 soil samples from five mine tailings and 17 from two mine villages were collected and analyzed for Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Nickel (Ni), Copper (Cu) and Zinc (Zn) using ICP-MS. Measured concentrations of these heavy metals were then used to calculate the health risk for adults and children. Their concentrations were such that Cr > Ni > As > Zn > Cu > Co > Pb > Hg > Cd, with As, Cr and Ni higher than permissible levels. For the adult population, the Hazard Index value for all pathways was found to be 2.13, making non-carcinogenic effects significant to the adult

population. For children, the Hazard Index value was 43.80, a value >>1, which poses serious non-carcinogenic effect to children living in the gold mining area.

Vinod *et al.* (2012) also investigated the concentrations of PTE such as Mn, Fe. Cu, Zn and Pb in leafy vegetables spinach samples collected from Industrial area of Bhilai City, Chhattisgarh. The atomic absorption spectrophotometer was used to estimate as well as evaluate the levels of these metals in the vegetables. The study included measurement of hazard quotient (HQ), daily intake of metals (DIM) and health risk index (HRI). It was concluded that the vegetables grown in the region were a health hazard for human consumption.

Similarly, Ogunkunle *et al*. (2013) investigated the non-carcinogenic risk assessment through ingestion pathway for vegetated soil near a cement factory at Sagamu, Nigeria. The vegetables and plants were digested using nitric acid and aqua-regia respectively and elemental analysis was done with flame AAS for heavy metal concentration. The result showed that the adults and children population were seriously at the risk of chronic non carcinogenic health crisis.

Levels of cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni) in vegetables and soils from Ohaji, Umuagwo and Owerri in southern Nigeria were determined and the potential health risks assessed by Orisakwe *et al.* (2012) using the Unicam Atomic Absorption Spectrophotometer (AAS) Model 929. Concentrations of Cd, Ni and Pb in Ohaji exceeded maximum allowable concentrations for agricultural soil. Cadmium, Ni, and Pb in vegetables were highest in *Murraya koenigii*, *Piper guineense* and *Amaranthus viridis* Linn, respectively. The estimated yearly intake of Pb, Cd and Ni in commonly consumed vegetables, Green leaf (*Amaranthus viridis*), fluted pumpkin (*Telfaria occidetalis*) and Curry leaf (*Murraya koenigii*) in Nigeria were calculated to be 1,210, 150 and 456 $mg.kg^{-1}$, respectively.

In another study, Tsafe *et al.* (2012) evaluated the heavy metals uptake of vegetables grown in Yargalma in northern Nigeria and assessed risks involved in consumption of such vegetables were assessed. The results revealed the trend in soil metals concentration is Al > Fe > Mn > Mg > Zn > Pb > Ni > Cr > Co > Cu > Cd and for the plant the trend is Fe > Mn > Mg > Zn > Al > Co > Ni > Pb > Cr > Cu > Cd. The transfer pattern for metals from soil to plant is Co > Cu > Cd > Mg > Ni > Zn > Mn > Fe > Pb > Cr > Al. The trend of the daily intake rate value was Fe > Mn > Mg > Zn > Al > Co > Ni > Pb > Cr > Cu > Cd. The trend in daily dietary intake was found to be slightly different to the one above, such that it followed the trend Al > Fe > Mn > Mg > Zn > Pb > Ni > Cr > Co > Cu > Cd. The result showed very high Health risk index (HRI) values for Cd (65.38), Zn (11.48) and Cu (2.09) while the THQ was similar to the HRI. This confirmed that the soil and vegetables in the area were contaminated with the assayed metals.

Both non-carcinogenic and carcinogenic risks of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn) were characterized by Xiao-San *et al.* (2012) in 40 surface soils (exposed lawns) from 14 urban parks in Xiamen, China. Results based on total metal concentrations was gave an overestimation of the actual risks in comparison with oral bioaccessibility assessment that were estimated by a simplified physiologically based extraction test (SBET). After considering the soil-specific bioaccessibility (Cd > Cu > Pb > Mn > Zn > Co ~ Ni > Cr), the non-cancer hazard of Pb to children via oral ingestion was a consideration though its Hazard Index (HI) was below one. The overall cancer risks to adults exceeded the target value 10^{-6}, mainly contributed by Cr (93.8%) and Pb (6.19%) via dermal contact (68.3%) and oral ingestion (30.4%).

Garg *et al.* (2014) in their study assessed the non-carcinogenic human health risk of heavy metals through the ingestion of locally grown and commonly used vegetables viz. *Raphanus sativus* (root vegetable), *Daucus carota* (root vegetable), *Benincasa hispida* (fruit vegetable) and *Brassica*

*campestris*leaves(leafyvegetable)inasemi-urbanizedareaof Haryana state, India. Heavy metal quantification of soil and vegetable samples was done using flame atomic absorption spectrophotometer. Lead, cadmium and nickel concentration in vegetable samples varied in range of 0.12–6.54 mg kg ^{1}, 0.02–0.67 mg kg ^{1}and <0.05–0.41 mg kg ^{1}, respectively. Cadmium and lead concentration in some vegetable samples exceeded maximum permissible limit given by World Health Organization/Food and Agriculture Organization and Indian standards. Much higher concentrations of Pb (40– 190.5 mg kg ^{1}), Cd (0.56–9.85 mg kg ^{1}) and Ni (3.21 – 45.87 mg kg ^{1}) were reported in corresponding vegetable fields' soils. Correlation analysis revealed the formation of three primary clusters, i.e. Cu–Cd, Cd–Pb and Ni–Zn in vegetable fields' soils further supported by cluster analysis and principal component analysis. Bioconcentration factor revealed that heavy metals' uptake was more by leafy vegetable than root and fruit vegetables. Hazard index of all the vegetables was less than unity; thus, the ingestion of these vegetables was unlikely to pose health risks to the target population.

Poggio *et al.* (2009) studied the diffuse metal contamination in the soils of a municipality in Northern Italy in terms of metal availability and accessibility to the human body and its relationship to soil properties, considering lead, copper, zinc, nickel, and chromium. Soil metal content was measured simulating availability conditions. Human bioaccessibility was derived from a modified physiologically-based extraction test. The human bioaccessible content was then estimated taking into account the relationships between pseudototal content and selected soil parameters. For the case study, the prediction of human bioaccessibility based on pseudototal content, organic matter and soil texture produced statistically significant models, with r^{2}¼ 0.60 for Cu, r^{2}¼ 0.53 for Pb and r^{2}¼ 0.42 for Zn.

The bioaccessibility and human health risks of As and heavy metals (Cu, Pb, Zn, Ni, Co, Cr, Cd and Mn) in total suspended particulates (TSP) and fine particulate matter (PM2.5) in Nanjing, China were investigated by Hu *et al.* (2012). The average mass concentration ratios of PM2.5 to TSP were 0.61 for Gulou sampling site and 0.50 for Pukou sampling site, respectively. Zn, Pb, Mn and Cu were the most abundant elements among the studied metal(loid)s in both TSP and PM2.5. The results of a simple bioaccessibility extraction test of the studied metal(loid)s varied among elements, with Cd, Zn, Mn, Pb and As showing the higher bioaccessibility. The carcinogenic risks of As, Cd, Co, Cr and Ni in both TSP and PM2.5 via dermal contact and inhalation exposure were within the acceptable level ($<1 \times 10^{4}$) for both children and adults, but there was potential carcinogenic risk posed by Pb via ingestion to children and adults. The hazard index values for all of the studied elements suggested no non-carcinogenic health risks via ingestion and dermal contact, but a potential non-carcinogenic health risk via inhalation to adults. Values of hazard quotient and hazard index indicated the non-carcinogenic risks from the studied metal(loid)s to children via ingestion, dermal contact and inhalation pathways in Nanjing given the present air quality.

Potential health risks of heavy metals in soils sampled from an urban environment where high frequency of human exposure may be present were assessed by Lu *et al.* (2011). A bioaccessibility test was used, which is an *in vitro* gastrointestinal (IVG) test of soluble metals under simulated physiological conditions of the human digestion system. Soil samples for assessing the oral bioaccessibility of arsenic (As) and lead (Pb) were collected from a diverse range of different land uses, including urban parks, roadsides, industrial sites and residential areas in Guangzhou City, China. The soil samples contained a wide range of total As (10.2 to 61.0 mg kg^{1}) and Pb (38.4 to 348 mg kg^{1}) concentrations. The bioaccessibility of As and Pb in the soil samples were 11.3 and 39.1% in the stomach phase, and 1.9 and 6.9% in the intestinal phase, respectively. The As and Pb bioaccessibility in the small intestinal phase was significantly lower than those in the gastric phase.

The general risk of As and Pb intake for children from incidental ingestion of soils is low, compared to their maximum doses, without causing negative human health effects. The exposure risk of Pb in the soils ranked in the order of: industrial area/urban parks > residential area/road side. Although the risk of heavy metal exposure from direct ingestion of urban soils was relatively low.

Elom *et al.* (2014) also conducted a study on risk assessment by estimating the total concentrations as well as the oral bioaccessibility of 8 PTEs in 15 urban dust samples collected from Abakaliki, Ebonyi State, Nigeria. The result showed that high concentrations of Pb (ranging from 236 – 1815 mg/kg) were observed in five locations. The Unified Bioaccessibility Method (UBM) was used to investigate the oral bioaccessibility of these PTEs in the urban dust samples. The result revealed that all the PTEs were more bioaccessible in the gastric phase than the gastric + intestinal phase.

Dakane (2012)estimated metals bioaccessibility in soil samples from 15 city parks in Toronto, Ontario, Canada. Total metals concentrations were analyzed to identify contaminants that exceeded the Canadian Council of Ministers of the Environment (CCME) guidelines for residential/parkland use. Arsenic, barium, cadmium, cobalt, copper, lead, nickel and zinc were of particular interest as they have been known to have major effects on human health. Metal concentrations were below the CCME guidelines except for lead at three of the parks. Lead, copper and cadmium bioaccessibility in the soil samples as determined by an in-vitro physiologically based extraction test (PBET) were relatively high. Based on linear regression analyses there were no significant relationships between total metals and soil properties such as pH and total organic carbon (TOC). Generally there was negative correlation between metal bioaccessibility and TOC and positive correlation between bioaccessibility and soil pH.

In another study, Waziri and Julian (2014) investigated the gastric bioaccessibility of Pb in soils and sediments which had been adversely affected by artisanal mining of gold from lead-rich ores from five villages in the Anka area of north-western Nigeria. *In vitro* bioaccessibility experiments were used to determine the extractability of Pb in order to evaluate the human health risk, especially to children below the age of five. The concentration of Pb in the simulated gastric fluids ranges from ~ 198 to 41740 µg.g-1 (mean, 9732 µg.g-1), with corresponding human bioaccessible fraction between ~ 29 and 100% (mean, ~ 60%). Chemical daily intake (CDI, µg/Kg/Day) values of between 111 and 41587 are generally very high compared to the tolerable daily intake (TDI) of ~ 3.6 µg Pb/Kg/Day. The high bioaccessibility was a result of high total concentration of Pb and the presence of highly soluble carbonate and oxide minerals in the ores. These results pointed to the very adverse health effects likely to result from incidental hand-to-mouth ingestion of soils by children in the affected villages. The results further showed that the low pH of gastric juices enhances the extraction of Pb. Given the likelihood of sub-nutrition in the affected communities, characterized by skipping of meals, which can result in lowering of the pH over extended periods, the risk of Pb poisoning is further increased. This study was based on a hand-to-mouth ingestion scenario alone, but as other exposure routes, such as eating improperly washed vegetables and inhalation of dust are possible, the risk might even be higher. This, along with the fate of extracted Pb in the intestinal environment may need to be evaluated in order to fully quantify the bioavailability of Pb in the area.

In vitro digestion and 10 kD centrifugal ultrafiltration device were used by Chen *et al.* (2012) to estimate the oral bioavailability and bioaccessibility of Pb in 5 soil samples. The three soil samples were collected from Guangdong (a paddy field near mining area), Guangxi (a cropland) and Neimenggu (a mining area) of China, and other two soil samples, GSS-1 and GSS-5, were Chinese standard reference soils, which represented the typical soils in north and south China. The 5 soil samples cover a range of Pb concentrations from 98 to 5860 mg/kg. Results showed that not only the

bioaccessibility of Pb, but also the ratio of Pb bioavailability to bioaccessibility varied with the soil samples. The bioaccessible and bioavailable Pb fraction range was 16.10-81.92% and 15.23-80.18% in the gastric phase and 1.69-32.72% and 0.93-20.62% in the small intestinal phase, respectively. The ratio of Pb bioavailability to bioaccessibility ranged from 0.76 to 0.99 and from 0.24 to 0.80 in the gastric and small intestinal phase, respectively. The research study concluded that *In vitro* digestion/centrifugal ultrafiltration was feasible and easy to operate to assess the bioaccessibility and bioavailability of soil heavy metals.

In another study by Li *et al*. (2014), the bioaccessibility and the human health risks of Sb and As in soils from Xikuangshan (XKS) Sb mine, Hunan, China were investigated using two commonly used in vitro extraction methods, Simpli ed Bioaccessibility Extraction Test (SBET) and Physiologically Based Extraction Test (PBET). Soils in the XKS Sb mine area were mainly co-contaminated by Sb (74.2–16,389; mean: 3061 mg/kg) andAs (7.40–596; mean: 216 mg/kg). The bioaccessibility values of Sb and As in most cases were less than 30%, and the average bioaccessibility values of Sb and As were 5.89 % and 2.13 % for the SBET extraction; 7.83 % and 6.62 % for the PBET (Gastric) extraction; and 3.03 % and 2.40 % for the PBET (Intestinal) extraction, respectively. The bioaccessible Sb and As were signi cantly positively correlated with the total concentrations, but negatively correlated with the Fe, Al, Mn and organic matter (OM) contents in soils. Risk assessment results based on total concentrations might overestimate the risk existing in the studied area. The study concluded that considering the bioaccessibility could provide more applicable guidelines for risk assessments and more rational suggestions in the management of the soils contaminated with Sb and As.

2.6 METHODS OF QUANTIFICATION OF POTENTIALLY TOXIC ELEMENTS

Over the years, a number of techniques have been used commercially for quantification of PTEs in environmental matrices. New techniques are also being developed as a results of limitations encountered in previous techniques.

The most common techniques include;

- Atomic spectroscopy i.e. optical (absorption & emission) and mass spectroscopy
- Electroanalytical spectrometry
- X-ray Fluorescence Spectrometry
- Molecular Spectrometry

2.6.1 X-ray Fluorescence Spectroscopy (XRF)

X-ray Fluorescence Spectroscopy is a non-destructive method for the elemental analysis of solids and liquids. This technique is based on the relocation of electrons within the atomic structure of the elements under investigation. When an atom within a sample is irradiated with x-rays, an electron from its inner shell is lost, in order for the atom to resume its most stable energy configuration it requires an electron from a higher energy level to fill the vacancyi.e. lower-energy state (Figure 2.9). This transfer results in the emission of an x-ray characteristic of that element (Mendham*et al*., 2000).

By measuring the energies of x-rays that are emitted from an excited sample and counting the number of x-rays of each energy, XRF allows us to identify which elements are present in a sample, and also determine the relative concentration of these elements within the sample (Brouwer, 2010).

The elements commonly detected range from sodium to uranium. Lighter elements from boron to fluorine may also be detected.

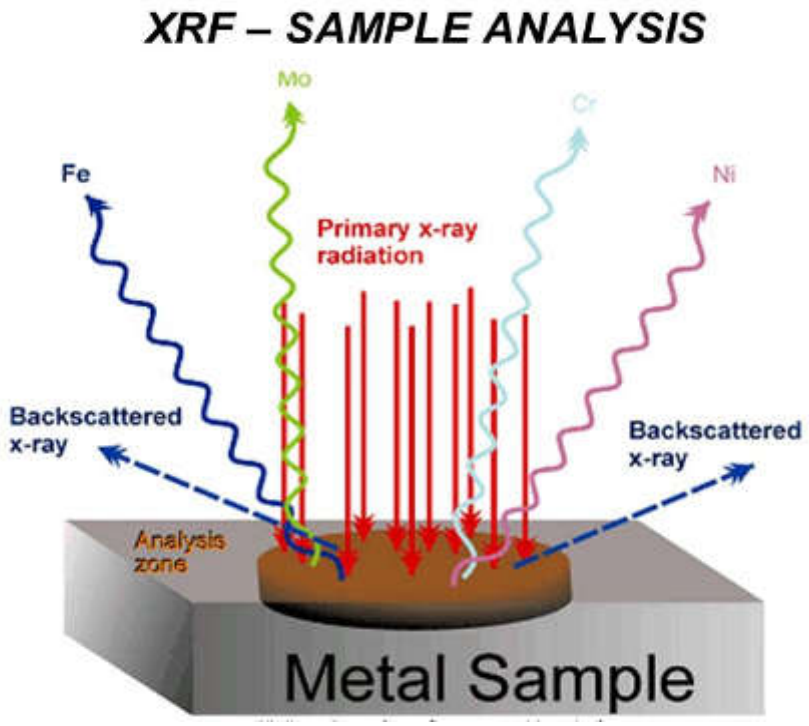


Figure 2.9: Sample analysis in XRF

Source: Brouwer, (2010)

2.6.2 Electroanalytical Techniques

These encompass a group of quantitative analytical methods which are based on the electrical properties of a solution of the analyte when it is made part of an electrochemical cell. Examples of electroanalytical techniques include Potentiometry, Coulometry and Voltammetry. These techniques are specific for a particular oxidation state of a compound and the instrument is relatively inexpensive. The disadvantage of these techniques is that they provide information on the activities of chemical species rather than concentrations. (Skoog *et al.*, 2007). The most common electroanalytical technique used for determination of metals is voltammetry (Stripping voltammetry)

Stripping voltammetry is a two-step technique in which the first step consists of the electrolytic deposition of a chemical species onto an inert electrode surface at a constant potential. This preconcentration step can involve either an anodic or cathodic process (Gupta *et al*., 2013).

The technique of stripping voltammetry has been used in trace analysis with relative ease and success in a variety of analytical applications. With minimal sample preparation, this electrochemical technique is routinely capable of identifying and quantitating trace components from 10^{-5} to 10^{-9} M with excellent sensitivity and selectivity. Stripping analysis has received an unusual degree of interest, since it is the most sensitive electroanalytical technique currently available.

2.6.2.1 Anodic Stripping Voltammetry

Anodic stripping voltammetry is used to determine the concentration of trace metals. ASV consists of a deposition potential that is more negative than the half- wave potential of the metals to be determined and an anodic (positive going) scan to oxidize the reduced metal back into solution (Figure 2.10). During deposition, an amalgam is formed by the elemental metal and the mercury on the electrode. Anodic stripping voltammetry can only be used to determine those metals that exhibit appreciable solubility in mercury (Bento *et al*., 2008).

Deposition: Applied potential more negative than $E_{1/2}$ of Mn^+

$$Mn^+ + ne^- \rightarrow M(Hg)$$

Stripping: Scan in the positive direction, peak current is proportional to the concentration of M

$$M(Hg) \rightarrow Mn^+ + ne^-$$

Figure 2.10: Process of Anodic stripping voltammetry

2.6.2.2 Cathodic Stripping Voltammetry

Cathodic stripping voltammetry is used to determine those materials that form insoluble salts with mercurous ion. In CSV, the mercury working electrode is not inert, but takes an active part in the formation of the deposit (Figure 2.11). The application of a relatively positive potential to a mercury electrode in the presence of such a material will result in the formation of an insoluble film on the surface of the mercury electrode (Bento *et al*., 2008).

Deposition: At a relatively positive potential where Hg^+ ions can be produced.

$$Hg \rightarrow Hg^+ + e^-$$

$$2Hg^+ + 2X^- \rightarrow Hg_2X_2 \text{ (insoluble film)}$$

Stripping: Scan in the negative direction, peak current is proportional to the concentration of X^-

$$Hg_2X_2 + 2e^- \rightarrow 2Hg + 2X^-$$

Figure 2.11: Process of Cathodic stripping voltammetry

2.6.3 Molecular Spectrometry

These include UV/Visible Molecular Absorption spectrometry, Molecular Luminisence spectrometry, Infrared spectrometry, Molecular Emission spectrometry, Raman spectrometry, Nuclear Magnetic Resonance spectroscopy and surface characterization by spectroscopy and Microscopy (such as Laser- Microbe Mass spectrometry, Scanning electron Microscopy, Scanning Tunnelling Microscopy). Of all these, only the UV-Visible can be used for the quantitative analysis of metals either directly or indirectly (Skoog *et al*., 2007).

Molecular Absorption Spectrometry is based on the measurement of transmittance (T) or absorbance of (A) of solutions contained in transparent cells having a path length of b cm. The concentration of an absorbing analyte is linearly related to absorbance as represented by the equation.

$$A = -\log T = \log \frac{P_0}{P} = Ebc$$

UV/ Visible Molecular Absorption Spectrometry is useful in the analysis of compounds that are capable of absorbing ultraviolet or visible radiation. The absorption of this radiation generally results from excitation of bonding electrons; as a consequence, the wavelengths of absorption peaks can be correlated with the types of bonds in the species understudy. Some molecules which absorb ultraviolet radiation include aromatic compounds, organic compounds that are conjugated, Inorganic anions such as nitrate, carbonate, nitrite, phosphate e.t.c, lanthanide and Actinide ions, elements of the first and second row transition metal series (Skoog *et al*., 2007).

For non absorbing species such as lead and copper, reagents can react selectively with them to yield products that absorb strongly in the ultraviolet or visible region. Examples include *o*-phenanthroline for the determination of iron, dimethyl-glyoxime for nickel, diethyldithiocarbamate for copper, and diphenyldithiocarbazone for lead. The disadvantage of this technique over the Atomic spectrometry is its sensitivity, which is not as good (Skoog *et al.,* 2007).

2.6.4 Atomic Spectroscopy

Atomic spectroscopy is the determination of elemental composition by its electromagnetic or mass 6spectrum. It exploits different energetic transitions experienced by atoms that are associated with either the absorption or emission of photons. When these transitions involve the excitation and relaxation of the valence (outer or bonding) shell electrons of metal atoms and ions, the corresponding photons have energies within the ultraviolet and visible regions of the spectrum (Harris, 2007). It is divided into two classes; optical and mass Spectrometry.

Optical spectroscopy is further divided into atomic absorption spectroscopy and atomic emission spectroscopy.

2.6.4.1Atomic emission spectroscopy

This involves the measurement of intensity of radiation emitted by excited state atoms. The excitation method could be the flame, arc and spark, or plasma. The plasma, or the arc and spark emission spectroscopy has several advantages when compared with the flame and electrothermal absorption methods, in that there is less interelement interference, which is due to the higher temperatures used. Furthermore, they permit the determination of low concentration of elements that form refractory compounds, and multielement analysis of very small samples can be done. Despite these advantages, the plasma, or the arc and spark emission spectroscopy cannot completely displace the flame and the electrothermal atomic absorption methods because the spectra obtained from plasma, arc and spark sources are often highly complex, since they are made up of hundreds or even thousands of lines, which consequently require higher resolution and more expensive equipment (Skoog *et al.,* 2007).

2.6.4.2 Atomic absorption spectroscopy

Atomic Absorption Spectroscopy (AAS) is a spectroanalytical procedure for the qualitative and quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples. The modern form of AAS was largely developed during the 1950s by a team of Australian chemists. Atomic absorption spectrometry has been the most widely used method for the determination of single elements in analytical samples (Harris, 2007)

AAS involves the absorption of electromagnetic radiation by ground state atoms, and the intensity of frequency of the electromagnetic radiation is decreased. When atom is irradiated, absorption becomes probable if and only if the energy difference between the ground state and one of the higher energy states of the atom matches exactly the energy of the photon. The extent of absorption is proportional to the number of ground state atoms present (Skoog *et al.,* 2007).

In order to analyze a sample for its atomic constituents, it has to be atomized. The atoms are irradiated by optical radiation (the radiation source could be an element-specific line radiation source or a continuum radiation source). The radiation then passes through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector (Harris, 2007).

2.6.4.3 Atomization devices

There are several methods of atomization used in atomic absorption spectroscopy which includes the flame, electrothermal atomisation, glow discharge atomization, cold-vapour atomization, and hydride atomization. In terms of reproducible behaviour, the flame atomization method is the most superior of all the other atomization methods; however, other atomization methods are better in terms of sampling efficiency and thus sensitivity (Skoog *et al.,* 2007).

Flame Atomiser

There are several types of flame atomizers available. The simplest is a turbulent flow burner that is very similar to conventional Bunsen burner. This type of burner suffers from fluctuations in temperature since there is no good mechanism for homogeneous mixing of fuel and oxidant. The drop size of nebulized sample is also inhomogeneous which adds to fluctuations in signal. The path length of radiation through the flame is small which suggests a lower sensitivity of the technique. Turbulent flow burners are also susceptible to flashback. These drawbacks were overcome using the most widely used laminar flow burner where quite flames and long path length are obtained (Skoog *et al*.,2007). Flashback is avoided and very homogeneous mixing between fuel, oxidant, and droplets take place. Larger droplets are excluded and directed to a waste container.

Liquid or dissolved samples are typically used with flame atomizers. The sample solution is aspirated by a pneumatic analytical nebulizer, transformed into an aerosol, which is introduced into a spray chamber, where it is mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (< 10 μm) enter the flame. This conditioning process is responsible that only about 5% of the aspirated sample solution reaches the flame, but it also guarantees a relatively high freedom from interference.

On top of the spray chamber is a burner head that produces a flame that is laterally long (usually 5–10 cm) and only a few mm deep. The radiation beam passes through this flame at its longest axis, and the flame gas flow-rates may be adjusted to produce the highest concentration of free atoms. The burner height may also be adjusted, so that the radiation beam passes through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity.

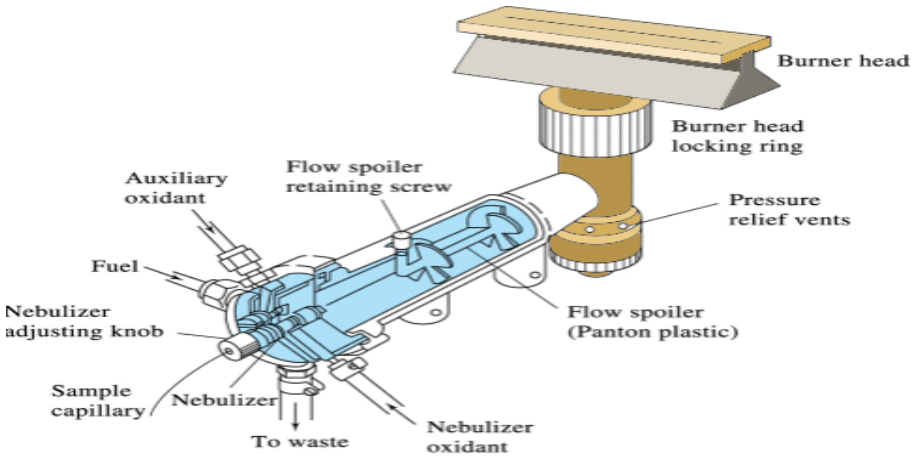


Figure2.12:A Schematic diagram of Nebulizer- Burner used in FAAS

Source: Skoog *et al.(*2007)

The processes in a flame include the following stages:

- Desolvation (drying) – the solvent is evaporated and the dry sample nanoparticles remain;
- Vaporization (transfer to the gaseous phase) – the solid particles are converted into gaseous molecules;
- Atomization – the molecules are dissociated into free atoms;
- Ionization – depending on the ionization potential of the analyte atoms and the energy available in a particular flame, atoms might be in part converted to gaseous ions.

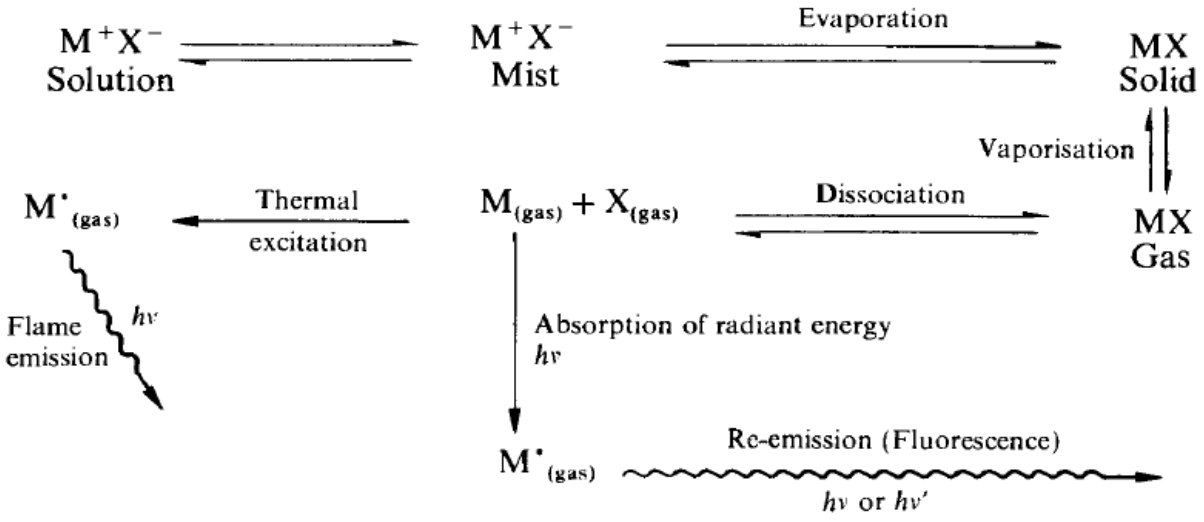


Figure 2.13:Processes occuring during flame atomization in atomic absorption spectrometry.

Source: Mendham *et al.* (2000)

Types of Flame used in AAS

Flames can be classified into several types depending on fuel/oxidant used. Table 2.1 summarizes the features of the most familiar flames.

Table 2.1. Fuel-oxidant used in Flame atomic absorption spectrophotometer

Fuel	**Oxidant**	**Temperature, ^{o}C**	**Maximum Burning Velocity (cm s^{-1})**
Natural Gas	Air	1700-1900	39-43
Natural Gas	Oxygen	2700-2800	370-390
Hydrogen	Air	2000-2100	300-440
Hydrogen	Oxygen	2550-2700	900-1400
Acetylene	Air	2100-2400	158-266
Acetylene	Oxygen	3050-3150	1100-2480
Acetylene	Nitrous Oxide	2600-2800	285

Source: Mendham *et al.* (2000)

It can be clearly seen that significant variations in flame temperatures can be obtained by changing the composition of fuel and oxidant. On the other hand, flames are only stable at certain flow rates and thus the flow rate of the gas is very important where at low flow rates (less than the maximum burning velocity) the flame propagates into the burner body causing flashback and, in some cases, an explosion. As the flow rate is increased, the flame starts to rise above the burner body. Best flames are obtained when the flow rate of the gas is equal to the maximum burning velocity. At this equity

ratio the flame is most stable. At higher ratios, flames will reach a point where they will no longer form and blow off the burner.

The adjustment of the fuel to oxidant ratio and flow rate is undoubtedly very crucial. Although stoichiometric ratios are usually required, optimization is necessary in order to get highest signal. However, in the determination of metals that form stable oxides, a flame with excess fuel is preferred in order to decrease oxide formation.

The two most commonly used flames are the air/acetylene flame and the nitrous oxide/acetylene flame. The nitrous oxide flame is much hotter than the air-based flame, and is essential for the determination of refractory elements such as Al and Si.

Flame Structure

Three well characterized regions can be identified in a conventional flame. A lower region, close to the burner tip, with blue luminescence. This region is called the primary combustion zone which is characterized by existence of some non atomised species and presence of fuel species (C_2 and CH, etc.) that emit in the blue region of the electromagnetic spectrum. The second well defined region is called the interzonal region just above the primary combustion zone. The interzonal region is rich in free atoms and is the region of choice for performing atomic spectroscopy. It also contains the regions of highest temperatures. The third region in the flame is the outer region which is called the secondary combustion region (Figure 2.14). It is characterized by reformation of molecules as the temperature at the edges is much lower than the core (Skoog *et al.,* 2007).

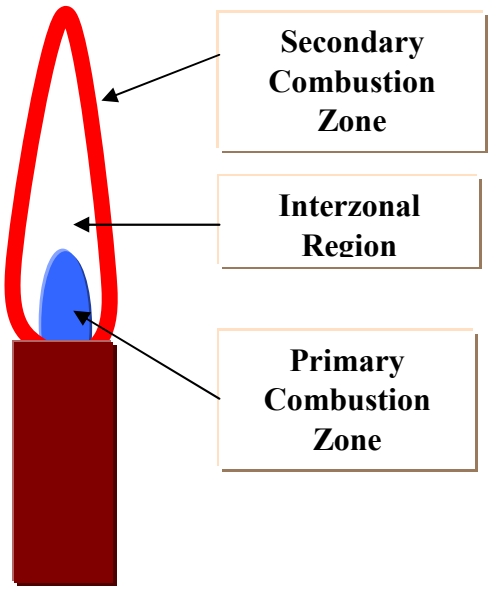


Figure2.14. Regions of a flame

Source: Skoog *et al.*(2007)

2.6.4.3 Non-Flame Atomizers

Electrothermal Atomizer

Electrothermal AAS using graphite tube atomizers was pioneered by Boris V. L'vov at the Saint Petersburg Polytechnical Institute, Russia (L'vov, 2005). With this technique, liquid/dissolved, solid and gaseous samples may be analyzed directly. A measured volume (typically 10–50 µL) or a weighed mass (typically around 1 mg) of a solid sample are introduced into the graphite furnace tube and subject to a temperature program.

The graphite furnace is a small high-temperature furnace. There are several different designs, but basically this furnace is a small cylindrically shaped furnace with a sample injection port at the top. The light to be absorbed enters one end of the cylinder and emerges through the other end. The sample solution (from 1-100 uL) is syringe-injected into the furnace through the injection port. The high temperature of the furnace (about 2500^{o}C) is reached in stages, ultimately resulting in

atomization as in the flame. The atomized metal species then absorbs the light, and the absorption is measured (Skoog *et al.,* 2007).

One obvious difference between the furnace and the flame is that, contrary to the flame, the sample is not continuously fed into the furnace and the sample distribution is neither homogeneous nor reproducible. Thus, a furnace offers greater sensitivity (because more atoms can be placed in the path of the light)with detection limits in the range of 10^{-10} to 10^{-13} g of analyte and requires less sample, but sometimes suffers from lack of accuracy and precision. Thus, the graphite furnace is used only when the sample size is small and/or when the greater sensitivity is needed.

2.6.4.4 Interferences in FAAS

Interferences can be either chemical or instrumental. An instrumental interference is one in which the spectral line of the elements being determined overlaps with a spectral line (or band) from another element present in the sample. In such a case, the effect of the interfering element will also be measured and thus the results will be incorrect. The most common method of solving this problem is to tune the monochromator to a different spectral line for the element of interest so that there is no overlap. So-called secondary lines can be found in the literature (Mendham*et al*., 2000)

Chemical interferences usually result from incomplete atomization caused by an unusually strong ionic bond. An example is in the analysis of a sample for calcium. Calcium chloride completely atomizes, but calcium sulfate does not. Thus if both of these calcium salts are present in a sample, incomplete atomization would lead to useless absorption measurements. Possible solutions would be to

(1) exactly match the sample and the standards in terms of chloride and sulfate content;

(2) use the method of standard additions; or

(3) add a substance to the sample which would free calcium from the sulfate matrix.

Solutions (1) and (2) are not useful if the effect is a concentration effect i.e., if the problem increases with increasing calcium concentration. The Beer's Law plot would not be linear in that case.

2.6.5 Inductively Coupled Plasma-Mass Spectroscopy

This is one of the most recently developed techniques. It is accepted as the most powerful multi-element analytical technique available today, capable of true multi- elemental determinations within minutes. The low detection limits and multi-element capability of inductively coupled plasma-mass spectrometry (ICP-MS) makes it an attractive option in a wide range of environmental, medical, biological, industrial and archaeological applications (Linge and Jarvis, 2009).

The basic principle of ICP-MS is elemental differentiation on the basis of atomic mass, this is achieved by ionising the sample with inductively couple plasma and then using a mass spectrometer to separate and quantify the ions.While atoms of a given element may have different atomic masses, or isotopes, the isotopic composition of each element is well studied and, therefore, is easily predicted (Bohlke *et al.*, 2005).

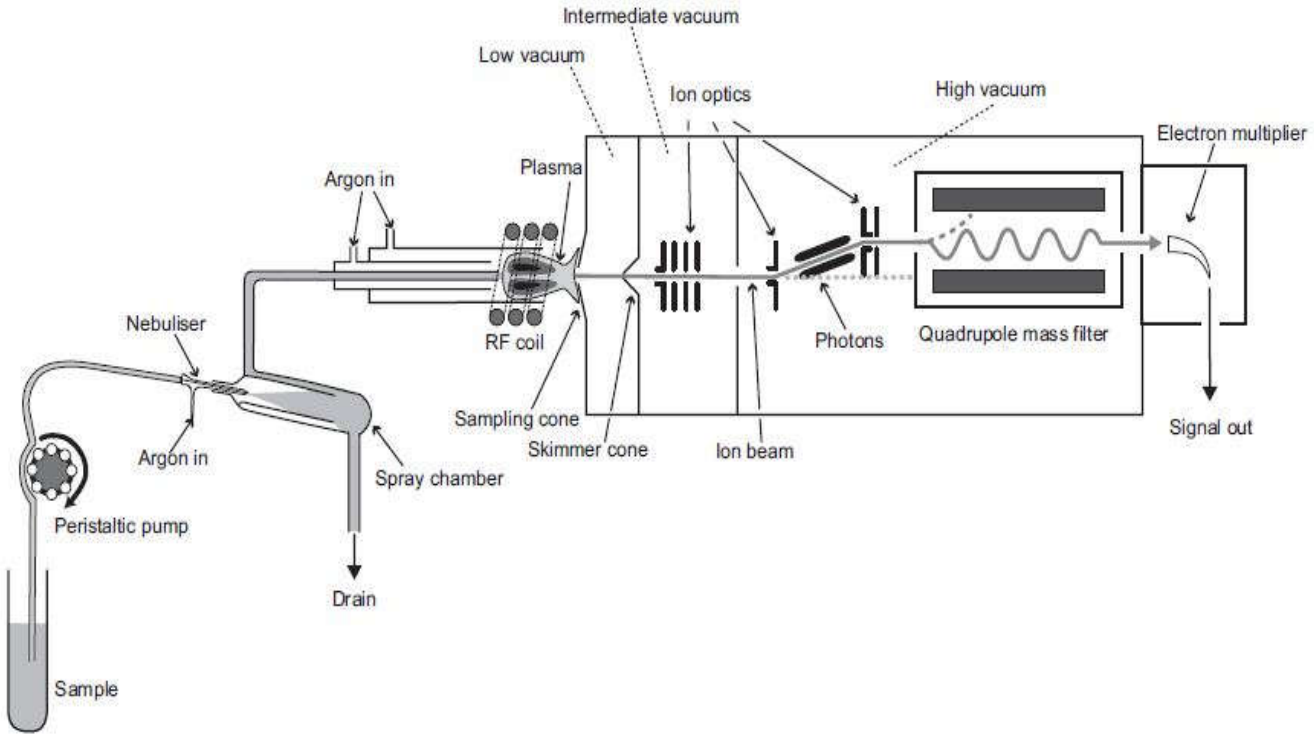


Figure2.15. Schematic of an ICP-MS

Source: Bohlke *et al.*(2005).

2.6.5.1 Overview of ICP-MS Major Components

An ICP-MS instrument consists of several distinct parts, which include;

• Sample introduction

• Ion generation in the ICP

• Plasma/vacuum interface

• Ion focusing

• Ion separation and measurement

2.6.5.1a Sample introduction

The sample introduction system is one of the most important components of the entire ICP-MS system. The sample is typically introduced into the Inductively Coupled Plasma (ICP) as an aerosol, produced by passing the liquid sample through a simple pneumatic nebulizer. Larger aerosol droplets are removed from the gas stream by a spray chamber, and the remaining smaller droplets are swept into the central channel of the argon plasma(Figure 2.16).It is important that samples are introduced to the ICP in small volumes that are finely dispersed as otherwise the plasma may become unstable or even be extinguished ((Linge and Jarvis, 2009).

Normally a nebuliser is used to disperse the solution into a fine, gas-borne aerosol and a spray chamber is used to remove larger droplets (which would not be fully decomposed in the plasma) from the aerosol (Mora *et al.*, 2003). The most commonly used nebulisers are pneumatic, where the aerosol is formed by the action of a high-speed gas jet over a tip of a smallorifice.

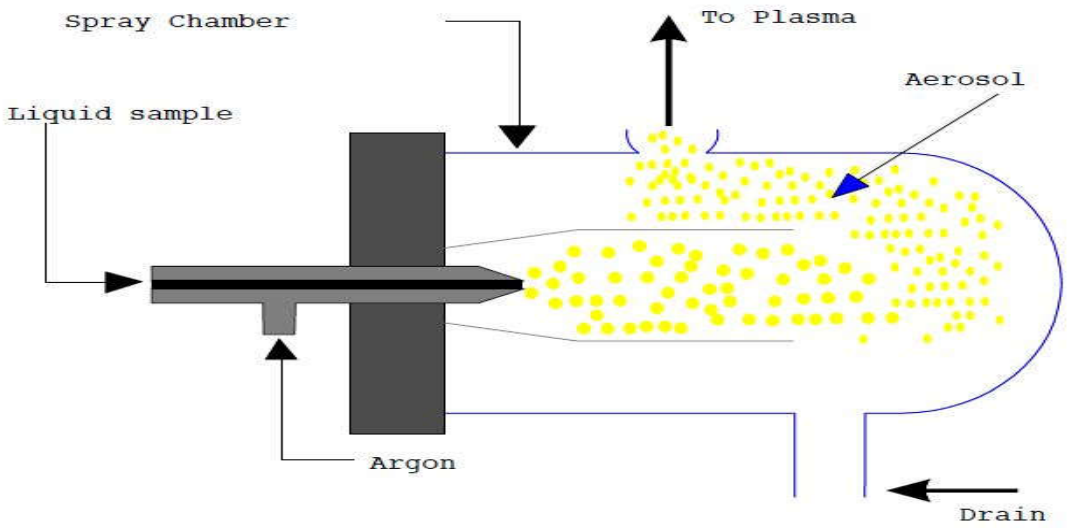


Figure 2.16.Schematic of a Nebulizer-Spray Chamber

Source: Mora *et al.*(2003)

2.6.5.1b Ion generation in the ICP

Once the sample has been converted to a suitable aerosol, it is injected into the ICP for ionisation. The sample aerosolis passed into the plasma, which is generated in a stream of argon (Ar) contained in a quartz tube or "torch". The torch is located in the center of a cooled copper coil, through which a high power, high frequency electric current is passed. The intense magnetic field created by the electric current causes collisions between free electrons and Ar atoms, producing ions and more electrons, until a stable, high temperature plasma is formed (Linge and Jarvis, 2009). The high frequency current is produced by a radio frequency (RF) generator operating at powers up to 1600W. While two RF frequencies are approved for ICPs, 40.68 MHz and 27.12 MHz, the latter has been shown to result in higher plasma temperatures. The very high temperature of the plasma (up to 10,000K maximum and around 7,500K in the central channel) means that the aerosol droplets are rapidly dried, decomposed, vaporized and atomized, then ionized by the removal of one electron from each atom. The resulting ions, which are formed within about 10ms of the original aerosol droplet entering the back of the plasma, are present at the highest level at about 7mm from the end of the load coil, which is where the spectrometer interface is positioned (Kathryn and Kym, 2009)

Inductively Coupled Plasma

A plasma is a highly ionised gas composed of ions, electrons, and neutral particles, usually at high temperature. An Inductively coupled plasma (ICP) is a plasma in which the transfer of energy to create and maintain the ionised gas is carried out via electromagnetic induction – that is, by using time- varying magnetic fields (Warra and Jimoh, 2011).The plasma is twice as hot as a conventional flame, and the residence time of analyte in the flame is about twice as long. Therefore, atomization is more complete and signal is enhanced (Harris, 2007).

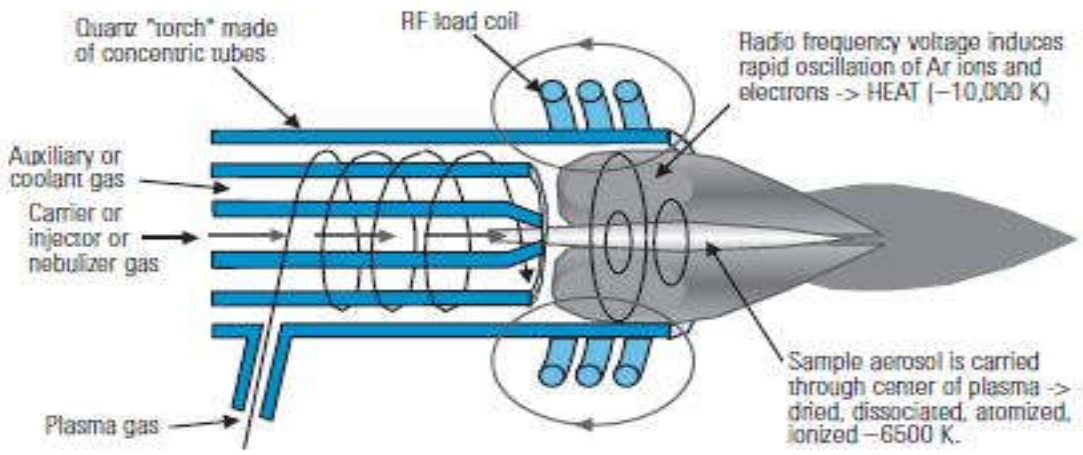


Figure 2.17.Schematic of an Inductively coupled plasma

Source: Harris(2007)

The purpose of the plasma is to form positively charged ions from the sample aerosol. To ensure good results from samples with high or varying matrices, plasma loading is optimized to maintain high ionization temperatures while retaining good sensitivity. The goal is to achieve as high a degree of matrix decomposition and analyte ionization as possible. Efficient matrix decomposition reduces deposition on the interface and contamination of the expansion stage pump oil. A well optimized and high temperature plasma greatly improves sensitivity for elements such as Hg, Be and As which have high ionization potentials.

Although many gases can be used to produce a plasma, argon is the most appropriate gas for ICP-MS because it is relatively inert and does not form stable compounds. The ionisation energy of argon (15.2 eV) ensures that most elements will be ionised in an argon-based plasma, though the degree of ionisation decreases as the ionisation energy of an element becomes closer to that of argon (Warra and Jimoh, 2011).

2.6.5.1(c) The ion extraction Interface

Placing a plasma, operating at 6000 °C, near an ion focusing device operating near room temperature is a bit like placing the earth about a half-mile away from the sun. In addition to a large temperature difference, the plasma operates at a pressure that is much higher than the vacuum required by the ion lens and mass spectrometer portions of the instrument.

Hence, the positively charged ions that are produced in the plasma are extracted into the ICP-MS interface which comprises of a step-down vacuum stage, located between a pair of conical metal plates, known as interface cones, in which small orifices have been drilled(Figure 2.18). The term "interface" is applied to the cones and the enclosed space (interface or “expansion” vacuum chamber) formed between them. In common terminology, the first and second cones are referred to as the samplerand skimmer cone, respectively (Warra and Jimoh, 2011).

After passing through the sampler cone, the plasma gas expands as a supersonic jet into a lower pressure region of between 1 x 10^{-2} and 1 x 10^{-1} kPa. The central section of the jet then flows through a second skimmer cone, located directly behind the sampler cone(Figure 2.18).

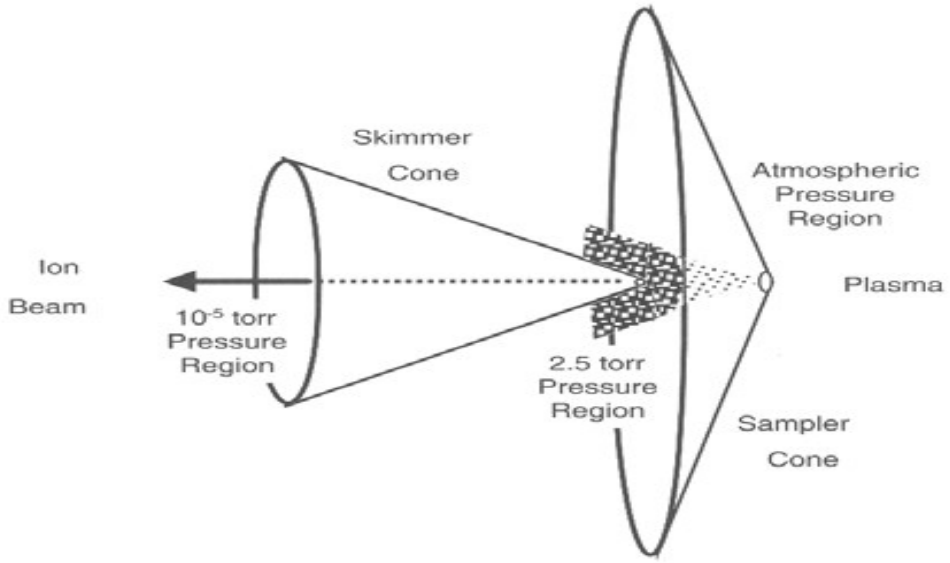


Figure2.18. ICP interface cones, showing the ion beam

Source: Warra and Jimoh, (2011)

The cones are essentially metal plates with central orifices through which the ions pass. Small orifices are used, typically 1mm diameter or less, to maintain the high vacuum in the mass spectrometer region. The orifice size and shape of the interface cones is critical and influences many aspects of instrument performance including sensitivity, mass response, oxide and doubly charged formation and robustness to high matrix samples (Kathryn and Kym, 2009). Skimmer cones are usually made of nickel or platinum and have a smaller orifice than the sampler cone (~ 0.4-0.7 mm).

2.6.5.1(d) Ion focusing

Electrostatic lenses keep the ions focused in a compact "ion beam" as they pass through the vacuum system to the final chamber, where the mass spectrometer (MS) and detector are housed. The ion lenses perform a second, essential, function of separating the ions from the photons and residual neutral material.Electrons are diffused out of the ion beam, leaving a positively charged beam, whose ions are focused back towards the center of the ion beam (Dean, 2005).

2.6.5.1(e) Ion Separation and Measurement

To quantify each element, the ions must be separated from the ion beam and counted. A mass spectrometer is able to distinguish between different ions based on mass-to-charge ratio (m/z).

Three different types of mass spectrometer have been used with ICP-MS; these are quadrupole, magnetic sector, and time-of-flight analyzers. By far the most common mass analyzer used in ICP-MS is the quadrupole (Warra and Jimoh, 2011).

Quadrupole

The quadrupole is a sequential mass filter, which separates ions based on their mass to charge ratio (m/z). It comprises of two pairs of parallel cylindrical rods, arranged in a square, on the axis of the ion beam(Figure 2.19). A varying or AC (alternating current) voltage, operating at high frequency, plus a DC (direct current) voltage is applied to the two pairs of rods. The AC (same voltage but out of phase between the 2 pairs of rods) and DC (positive on one pair and negative on the other) voltages give a dynamic hyperbolic electric field, in which any ion above or below the set mass of the quadrupole enters an unstable trajectory and is lost from the ion beam (Kathryn and Kym, 2009).

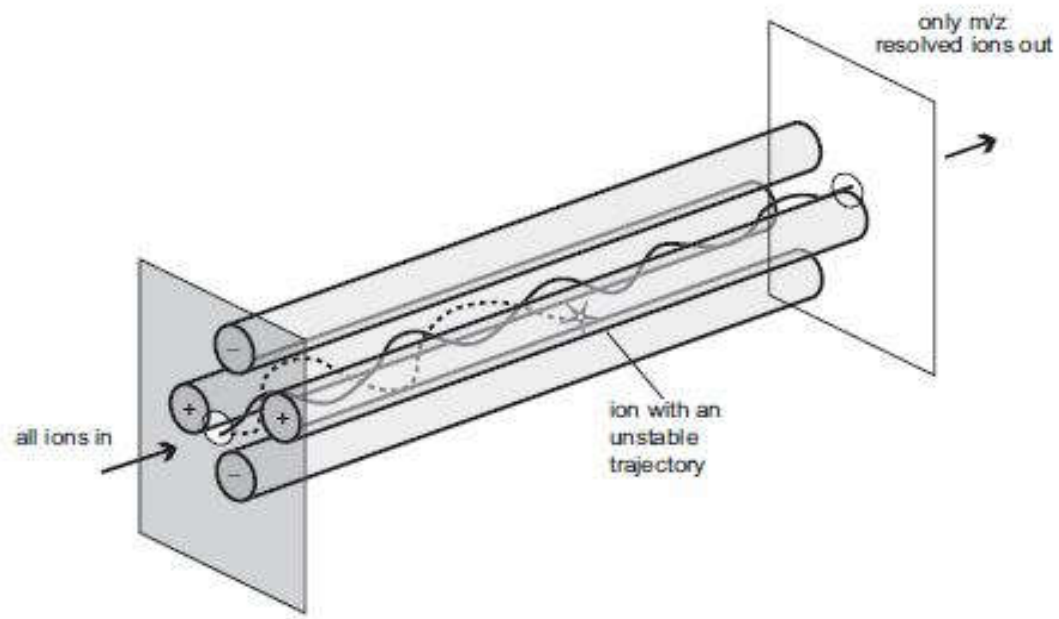


Figure 2.19.Schematic of a quadrupole mass filter

Source: Kathryn and Kym, (2009)

Combining the AC and DC components produces a narrow bandpass filter that allows only a narrow range of masses to be transmitted. By varying the AC and DC fields, but keeping the ratio between them constant, different masses can be selectively allowed to pass through the filter. Since these voltages can be adjusted very rapidly, the elemental mass range from 2 to 260 amu can be scanned very quickly, giving a mass spectrum for all elements and their isotopes (Li to U), which is acquired virtually simultaneously. The full mass range is normally scanned for qualitative measurements, but the quadrupole can also be set to acquire only masses of interest, jumping between each measured mass to reduce measurement time.The quadrupole is always maintained at a high vacuum to limit interferences with the mass filtering process (Kathryn and Kym, 2009).

2.6.5.1(f) Ion detection:

The electron multiplier detects each ion as it exits the quadrupole. The detector electronics count and store the total signal for each mass (m/z), creating a mass spectrum. The spectrum that is produced provides a simple and accurate qualitative representation of the sample. The magnitude of each peak is directly proportional to the concentration of an element in a sample; quantitative results are produced by comparing signal intensities to those generated by calibration standards (Dean, 2005)

2.6.5.2 Interferences in ICP-MS

An inductively coupled argon plasma eliminates many common interferences (Harris, 2007). However, interferences in ICP-MS are classed as either spectroscopic or non-spectroscopic.

Spectroscopic interferences

Spectroscopic interferences arise when different ions with the same m/z are counted together. In such cases attributing the entire signal only to the analyte of interest results in an overestimation in concentration. In most cases, spectroscopic interferences are simple to predict when the operator is familiar with typical mass spectra and they do not vary with time.

The most problematic spectroscopic interference is an isobaric overlap, when two elements have isotopes nominally of the same mass. For example Cr and Fe both have an isotope of 54 amu. While the exact masses differ (54Fe = 53.939612 amu compared to 54Cr = 53.938882 amu), the quadrupole mass analyzer cannot resolve this tiny difference in mass (0.00073 amu) and ions counted at m/z = 54 may be either Fe or Cr. Counting at a mass free from isobaric overlap (e.g., 52Cr or 57Fe) will give a measurement free from this interference (Linge and Jarvis, 2010).

Polyatomic ions, or adduct ions, are formed by the combination of two or more atoms and, again, the quadrupole mass analyser cannot distinguish between a polyatomic, and an atomic ion if they have the same nominal m/z. The most significant polyatomic ions are formed from the most abundant isotopes of argon, atmospheric gases, and the solvents or acids used during sample preparation (Jarvis *et al.,* 2003). Despite the combinations possible, polyatomic ion formation is generally insignificant above m/z = 80 (Linge and Jarvis, 2010).

The other major type of polyatomic interferences is caused by refractory oxides, which result from incomplete dissociation, or recombination in cooler plasma regions, particularly in the boundary layer around the sampler cone (Linge and Jarvis 2010).

Attenuating spectroscopic interferences

In principle, any isobaric overlap can be corrected by calculating the relative contribution of the interfering analyte, based on signal of another isotope at an interference free m/z (Raut *et al.,*2005).

Adding another gas such as nitrogen, oxygen, air, helium, and hydrogen to the argon plasma gas feed can significantly change the fundamental properties of the ICP, altering or minimising inherent polyatomic interferences. Nitrogen in particular has been found useful for increasing signal and decreasing Ar and O- based interferences. Nitrogen- argon plasmas are more energetic and hotter than argon-only plasmas, attributed to the higher thermal conductivity of nitrogen leading to more efficient energy transfer within the plasma.

Non-spectroscopic interferences

Unlike spectroscopic interferences, non-spectroscopic interferences usually affect whole sections of the mass range and can be broadly categorised as suppression/enhancement effects and signal drift.

Suppression or enhancement effects arise from changes in sample transport to the plasma, ionisation in the plasma, or transmission of the ion beam, all of which directly affect the number of ions reaching the detector. Suppression is often seen in samples with a high level of Total Dissolved Solids. All of the elements present in solution are ionised by the ICP, regardless of whether they require determination. Excessive matrix may result in an offset between the signal from samples and synthetic calibration solutions and can also result in a loss of light ions from the ion beam. Most matrix effects can be minimised by matrix matching calibration solutions to samples, dilution or, in more complex cases, by chemically separating the analytes from the matrix before analysis (Jarvis *et al.,* 2003,).

In the late 1990s, a new technique for interference reduction emerged - the collision or reaction cell (Tanner *et al.,* 2002). In "cell" ICP-MS, the ion beam passes through a cell filled with a specifically chosen gas before entering the mass spectrometer. Interfering species are removed through interactions with the gas, whilst analyte ions pass through to the mass spectrometer to be detected.

While cell designs vary, they all comprise a multipole ion guide inside a small chamber. The ion guide is made up of a fixed number of parallel rods equidistant from the ion beam, similar to the quadrupole mass analyser itself. Multipole guides currently in use include the quadrupole (four rods), the hexapole (six rods) or the octopole (eight rods). The cell is generally positioned after the ion lenses and just before the mass analyser. Without a gas flow into the chamber, cell instruments operate in normal ICP-MS mode with the cell acting as an ion guide only (Linge and Jarvis, 2009)

2.8 CHEMOMETRIC / STATISTICAL ANALYSIS

The use of mathematical and statistical analytical tools for data analysis is becoming increasingly widespread in analytical chemistry. Chemometrics is the art of extracting chemically relevant information from data produced in chemical experiments. The conversion of multivariate data into useful information is one of the most important areas of Chemometrics (Mendham *et al.,* 2000).

In recent years, chemometric evaluation has increasingly been used in food research. Most applications of chemometric methods focus on establishing correlations between different foods and their composition determining geographical origin, quality of environment, modelling of heavy metals contamination of fruits and vegetables (Konstadinovic *et al.,* 2010). Some of the chemometric analysis used in this research study include significant test, correlation analysis, principal component analysis (PCA) and cluster analysis (CA).

Correlation analysis: helps to study the strength of the relationship between two variables. The statistical process produce a correlation coefficient tell the information about the strength of the relationship. The correlation coefficient is a measure of linear association between two variables.

Significant test: One of the most important properties of an analytical method is that it should be free from systematic error, which then means that the value given for the amount of analyte should be the true value. This property of an analytical method may be tested by applying the method to a standard test portion containing a known amount of analyte. In order to decide whether the difference between the measured value and standard amount can be accounted for by only random error, a significance test is employed (Miller and Miller, 2010). A statistical t-value is calculated and compared with a tabulated value for the given number of tests at the desired confidence level. If the calculated t exceeds the tabulated t-value, then there is a significant difference between the results by the two

methods at that confidence level. If it does not exceed the tabulated value, then we can predict that there is no significant difference between the two methods which then implies that that the two results are identical (Skoog *et al*.,2007).

Principal Component and Cluster Analysis: PCA and Cluster analysis (CA) are two unsupervised methods that allow us to deduce how certain variables (metals concentration, other parameters of the soil or plants) that characterize objects (soil, plant) determine their association (Abad-Garcia *et al.,* 2012) If the CA method is used for samples grouping original variables, PCA estimates the correlation structure of the variables by finding hypothetical new variables (principal components - PC) that account for as much as possible of the variance (or correlation) in a multidimensional data set. These new variables are linear combinations of the original variables (Natsheh *et al.,* 2013). This method helps us to identify groups of variables (i.e. heavy metals concentrations or other soil or plant parameters) based on the loadings and groups of samples (soil or vegetable species) based on the scores (Gergen *et al*., 2012)

CHAPTER THREE

MATERIALS AND METHODS

This chapter describes the methodologies and instrumental techniques used in this study. The chapter entails discussions onthe methods of sample collection, preparation and pre-treatment, the physicochemical analysis of the soils samples,digestion of soil and vegetable samples as well asthe risk assessment and bioaccessibility approaches used for evaluation of the risk associated with ingestion of the contaminated soils and vegetables. The last part of this chapterdiscusses the optimisation of the instrumentation techniques employed for the quantification of the elements of interest.

3.1 SOIL SAMPLING AND PREPARATION

Soil samples were collected randomly from seven locations around Lagos, Nigeria. Sixof the locations were contaminated sites mostly dumpsites at a distance of 60-100 m from residential and commercial areas while the seventh sample was a control site devoid of anthropogenic activities.

From each of the locations, several grab samples were taken at a depth of 0-10 cm, each grab sample was collected a distance of 5 m apart and mixed together to obtain a composite sample. The soil samples were placed in polyethylene containers and taken to the laboratory where they were homogenized.

Sub samples weretaken, air dried, and sieved usinga 2 mmEndocett stainless steel sieve. The samples were kept in a polyethylene bag and labelled accordinglyprior to further analysis. The remaining (unsieved) portionof the soils were used to growvegetable samples.

Table 3.1: Description of Sampling location and Anthropogenic Activities on sites

Sampling Location	Sample Site	Sample Identifier	Activities	Location Coordinates
Iwaya	MFM Park dumpsite	MFM	Dumpsite at a mechanic shop beside 2nd gate University of Lagos.	N 06 30' 42.3" E 003 23' 16.7"
Orile	Smelting & Welding workshop dumpsite	ORL	Welding, Smelting and sale of metal rods	N 06 31' 13.9" E 003 23' 49.3"
Abule Egba	Katangua dumpsite	KATANG	A massive dumpsite for electronic wastes, metal scrap, plastics, types e.t.c. disposal	N 06 38' 43.4" E 003 18' 16.5"
Ikorodu	Owode Onirin Market dumpsite	OWD	Metal scarps, iron rods and battery dumpsite	N 06 44' 09.7" E 003 25' 08.7"
Ibafo	Ibafo trailer park roadside	IBF	Dumpsite at trailer park along the roadside	N 06 43' 44.4" E 003 24'57.2 "
University of Lagos	FSS dumpsite	FSS	A big dumpsite situated across the Faculty of Social Sciences within University of Lagos	N 06 30' 52.3" E 003 23' 32.2"
	Botanical Garden	CONTROL	Botanical garden within University of Lagos	N 06 31' 32.3" E 003 24' 10.1"

MFM- Mountain of fire and ministry park dumpsite; ORL- Orile dumpsite; KATANG- Katangua dumpsite; OWD- Owode onirin dumpsite; IBF- Ibafo roadside dump; FSS- Faculty of social sciences dumpsite, Unilag; CONTROL- Unilag botanical garden soil.

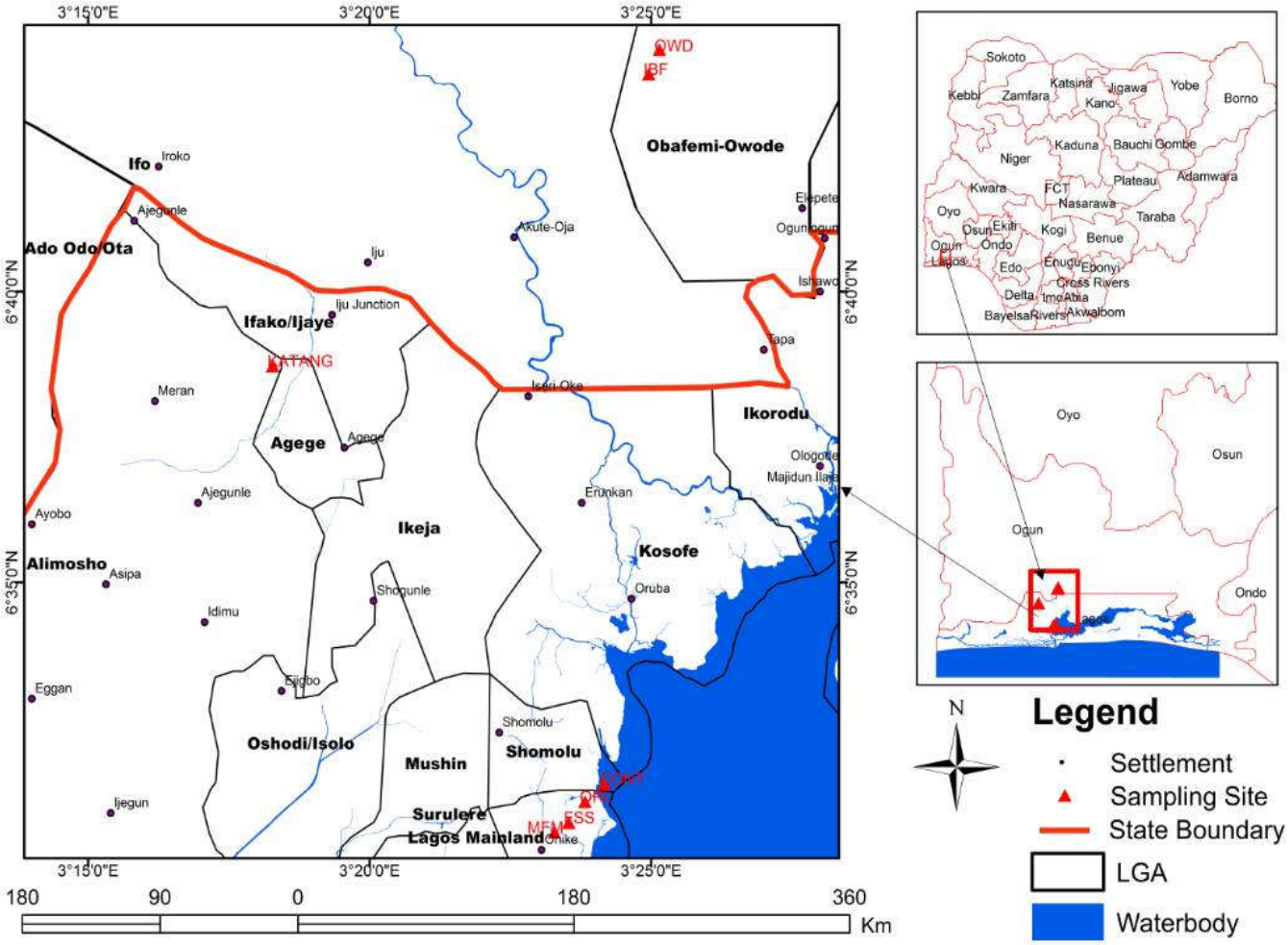


Fig 3.1 Map of Soil Sampling sites

3.2 PLANTING OF VEGETABLES

Seeds of tropical leafy vegetables were purchased from the National Horticultural Research Institute (NiHORT) Ibadan, Oyo state, Nigeria. The vegetables include waterleaf (*Talinum triangulare*), spinach (*Basella alba),* pumpkin leaf (*Telfairia occidentalis),* okro (*Abelmolschus esculentus)*, cockscomb (*Celosia argentea)* and green leaf (*Amaranthus viridis*).

The seeds were pre-germinated in a nursery in pots of uncontaminated agricultural soils in a greenhouse at the Botanical garden of the University of Lagos (Plate 1 & 2).After two weeks of germination, some of the seedlings were taken for proper specie identification at the Department of Botany, University of Lagos.

The seedling in the pots of soils were then transplanted into individual plastic pots.These pots contained different contaminated soil samples already mixed with inorganic fertilizer (pink granularNPK 15-15-15).After about 6 - 8 weeks of planting with periodic watering and monitoring, matured vegetables were harvested, rinsed thoroughly with tap and deionised water to wash off soil particles and debris underneath. The cleaned vegetable samples were taken to the laboratory where they were cut into small pieces using a stainless steel knife. The shredded samples were oven dried at $60 \pm 2\ ^{o}C$ for 48 hours, pulverised and stored in the refrigerator at $4^{o}C$ until pending analysis.

Table 3.2:Description of vegetables examined in this study

English Name	Botanical Name	Authority / Family	Vernacular Name
Waterleaf	*Talinum triangulare*	(Jacq.) Wild/ Magnoliopsida	Gbure
Pumpkin leaf	*Telfairia occidentalis*	Hook. f / Cucurbita	Ugwu
Spinach	*Basella alba*	Linnaeus / Basellaceae	Amunututu
Okro (Lady's finger)	*Abelmolschus esculentus*	(L.) Moench / Malvaceae	Ila
Cockscomb	*Celosia argentea*	(L.) Kuntze / Amaranthaceae	Efo Soko
Green leaf	*Amaranthus viridis*	Linnaeus / Amaranthaceae	Efo tete

Plate 1: Transplanted Seedlings in pots of soil

Plate 2: Matured vegetables in the greenhouse

3.3 PHYSICOCHEMICAL ANALYSIS OF SOIL SAMPLES

3.3.1 Determination of pH

The pH of the soil samples were determined according to British Standard ISO (2005). Approximately5.0 g of soil sample was weighed into a beaker and 25 ml 0.01M solution of $CaCl_2$ was added. The suspension was shaken at 150 rpm for 15 mins on a mechanical shaker, allowed to stand for another 15 mins to equilibrate and left to stand for about 30 mins before measurement. The FiveEasy FE 20 Mettler ToledopH meter which had beencalibrated with buffers 4.0 and 9.0 respectively was used to measure the pH of the suspension. The procedure wascarried out in triplicate for each soil samples under investigation.

3.3.2Determination of total organic content

0.5 grams to 1.0 grams of each soil sample was accurately weighed and transferred into a 250mL conical flask. 10mL of 0.1M potassium dichromate ($K_2Cr_2O_7$)solution was added to the soil using a pipette and the flask swirled gently until the soil solution was dispersed. 20mL concentrated sulphuric acid (H_2SO_4) was quickly added directing the steam into the suspension. The flask was again swirled vigorously and allowed to stand on an insulator for 30 mins.

The mixture was diluted with 100mL of deionised water, 3 drops of ferroin indicator was added and the mixture was titrated with 0.5N Ferrous sulphatesolution, first to green and later to dark brown end point. This procedure was carried out in triplicate on each soil and the total organic content determined. Blank titration was also performedusing the same process standardize the ferrous sulphate solution.

$$\%\ Total\ Organic\ Content = \frac{A \quad B \times M \times 0.003 \times f \times 100}{Mass\ of\ Sample\ (g)}$$

A = Titre value of blank

B = Titre value of sample

M = Molarity of $FeSO_4$

f = Correction factor (1.33)

3.3.3 Determination of moisture content

An aliquot of 10g of soil sample was measured into a cleanpre-weighed dry petri dish and placed in an oven to dry at 103-105^{0}C for 2hours, after which the dish was removed and weighed. This process was repeated until a constant weight was obtained. The percent moisture content was then calculated from the sample weight before and after drying. This procedure was performed in triplicate.

$$\%\ Moisture\ Content = \frac{W2 \quad W3}{W2 \quad W1}\ X\ 100$$

W_1 = Initial weight of empty dish

W_2 = Weight of dish + sample before drying

W_3 = Final weight of dish + sample after drying

3.3.4 Determination of particle size distribution in Soils

The particle size distribution of soil samples were determined using the wet sieving method described by the British Standard Method (Lorenzi, 2011). This was carried out by drying the soil samples at 105 ^{o} C for 24 hours.

About 200g of each oven dried soil was weighed and transferred in to a dish. 100 ml sodium hexa meta phosphate solutionand 200 mL water was added to cover the soil mixture, and allowed to stand for 30 mins. The sample was then transferred into a 75 mm sieve and carefully washedwith water through the sieve until nothing passed through it.The residue was poured using back washing into a pan and allowed to sit for a short period of time until the water on top of the soil became clear.The clear top water was poured off as much as possible, the remaining soil-water suspension was placed in the oven to dry for 24 hours. The dried soil residue was passed through fitted test sieves of aperture sizes of 50 mm, 37.5 mm, 28 mm, 20 mm, 14 mm, 10 mm, 6.3 mm, 5mm, 3.35 mm, 2mm, 1.18 mm, 600 µm, 425 µm, 300 µm, 212 µm, 150 µm, 63 µm. The material retained in the sieve tray were weighed, the percentage retained and percentage that passed through the various sieve sizes calculated. The values obtained were plotted on a particle size distribution chart and classified into gravel, sand, silt and clay.

3.4 CHEMICAL ANALYSIS OF SOILS AND VEGETABLES

3.4.1 Hot plate digestion of samples

Approximately 1.0 g each of soil and vegetable samples were weighed into 50mL beaker and 20mL of *aqua regia*(HCl: HNO_3, 3: 1 v/v) and covered with a watch glass. The beaker with the sample mixture was allowed to digest on aStuart CB300 hot plate for 2 to 3 hrs till the brown fumes disappeared and the solution was evaporated to near dryness (Olayinka *et al*., 2011). The resulting solution was then removed from the hotplate, allowed to cool, filtered through a whatman 11cm filter paper into a 50 mL volumetric flask and made up to mark with deionised water. The filtrate was then transferred to cleaned dried plastic bottles prior to analysis.Digestion was carried out in triplicate for all the samples.

3.4.2 Microwave digestion of samples

Approximately 1.0 g of sample was weighed into a 50 mLteflon vesselpre-cleaned with 5 % concentrated nitric acid, 10mL of *aqua regia* (HCl: HNO_3, 3: 1 v/v)and nitric acid for soil and vegetable samples respectively were added, each solution was gently swirled to homogenize the sample with the reagents and the mixture left overnight in order to avoid any violent reaction which may lead to explosion in the microwave. The teflonvessels with the solution werethen introduced into the carousel and then placed on the drive lug in the oven. All the vessels containing samples were properly sealed and arranged prior to starting the microwave digestion process.The microwave oven was operated according to the digestion program presented in Table 3.3. After cooling, the resulting solution was filtered using a whatman filter paper (11 cm) into 50 mL volumetric flask. The filtrate was diluted to the mark with deionised water, transferred into a 50mL and stored in the refrigerator at 4^{o}C prior to ICP-MS analysis.

Table 3.3: Microwave digestion program

No of sample vessels	Ramp time (min)	Hold (Cooling) time (min)	Temp (^{o}C)	Power (watt)
5-15	20	20	122	800
16-30	20	20	128	1600

3.5 RISK ASSESSMENT OF PTEs IN SOILS AND VEGETABLES

3.5.1 Pollution index

The assessment of the soil contamination was carried out using the pollution index (PI) which is calculated as the ratio between the PTE concentration in a soil sample and its reference value (Hu *et al.,* 2013)

Pollution index (PI_i) = C_i / S_i

...where PIi is the single pollution index; Ci represents the mean concentrations of PTEs of soil and Si indicates the evaluation criteria values. The adopted evaluation criteria for this study was the South Africa recommended soil guideline values for residential landwhichare presented in Appendix I, as Nigeria has no stipulated guideline for PTEs in soil.

Liang*et al.,* 2011gave the following interpretation for the pollution index: 0 < PI< 1 = unpolluted to moderately polluted; 1 <PI< 2 = moderately polluted, 2<PI< 3 = moderately to strongly polluted;3<PI<4=strongly polluted;4<PI <5= strongly to extremely polluted; and PI>5 = extremely polluted.

3.5.2 Estimation of daily intake rate

Daily intake rate (DIR) is calculated to averagely estimate the daily PTE loading into the body system of a specified body weight of a consumer using the equation:

$$DIR = \frac{Cm \times Di}{Bw},$$

Where Cm, Di and Bw, represent the concentration of PTE in vegetables (mg/kg), average daily vegetable intake and average body weight respectively.

The average daily vegetable intake for adults was considered to be 0.345 kg person^{-1} day^{-1}, and 0.232 kg person^{-1} day^{-1} for children while the average adult and child body weight were considered as 70 kg and 32.7 kg respectively. (USEPA, 2007)

3.5.3Estimation of health risk index

It is used to assess the non-carcinogenic health risks associated with the ingestion of individual PTE via dietary intake of vegetables using the equation:

$$\text{EHRI} = \frac{DIR}{RfD} ;$$

Where, DIR is Estimated daily intake rate and R_fD is oral reference dose. If the value of EHRI is less than 1 then the exposed population is said to be safe.

Table 3.4: Oral reference dose for potentially toxic elements

PTEs (mg/kg)	As	Cd	Cr	Cu	Ni	Pb	Zn
RfD (USEPA, 2007)	0.0003	0.001	1.5	0.04	0.02	0.004	0.3

PTEs- potentially toxic elements; RfD- oral reference dose; USEPA- United State Environmental Protection Agency.

3.6 *IN-VITRO*BIOACCESSIBILITY STUDIES

Bioaccessibility studies of potentially toxic elements to human through ingestion of contaminated vegetables and accidental/involuntary ingestion of soil was carried out using the physiologically based extraction test (PBET) and Simplified bioaccessibility extraction test (SBET) as described by Navarro *et al.,* (2008) and and Drexler *et al.,* (2007) respectively.

3.6.1Physiologically based extraction test (PBET)

This method consists of two sequential extraction processes; the gastric and intestinal digestion with each one carried out employing simulated human conditions i.e., enzymes, pH and temperature (Navarro *et al*., 2008)

3.6.1 (a) Preparation of simulated gastric solution

The gastric juice was prepared based on the method of Navarro *et al*., (2008) with a little modification which involved adding 1.25 g of pepsin, 0.5 g of sodium malate, 0.5 g of sodium citrate, 0.5 ml of acetic acid and 0.42 mL of lactic acid to 1 Litre of deionised water. The solution was mixed and the pH of the resulting solution measured and adjusted to 1.8 ± 0.2 with the dropwise addition of 12 M hydrochloric acid.

Gastric phase extraction

Approximately 0.5 g of vegetable sample was added toa 50 mL HDPE screw top tubecontaining the gastric solution and the mixture was agitated end over endat 100rpm in a thermostatic water bath maintained at 37^{o} C 1 h. At the end of the extraction,the sample tubes were removed from the shaker and the pH of each of the suspension was measured to ensure that they were within the pH range. 1.2 – 1.7, thencentrifuged at 3000 rpm for 10 min and the supernatant removedand filtered using a

cellulose acetate membrane. The filterate was stored at 4^{o} C in the refrigerator prior to instrumental analysis.The pH value of the sample solution was taken at the beginning and the end of the extraction to ensure it was within required range.

3.6.1 (b) Preparation of simulated intestinal solution

The intestinal juice was prepared by adding 15mg of Pancreatin, 0.1 g of amylase and 52.5 g of bile salts in Saline solution. The solution pH was measured and adjusted to 7.0 ± 0.1 using sodium bicarbonate.

Intestinal phase extraction

To the residue from the gastric extraction, 50 mL of intestinal juice was added. The mixture isagitated end over end for 4 hrs at 100 rpm in a thermostatic water bath maintained at 37^{o} C. The resulting solution was then centrifuged at 3000 rpm for 10 min and the supernatant removed and filtered using a cellulose acetate membrane. The filterate was stored at 4^{o} C in the refrigerator prior to instrumental analysis.The pH value of the sample solution was taken at the beginning and the end of the extraction to ensure it was within required range.

3.6.2Simplified bioaccessibility extraction test (SBET)

This is a one phase extraction process carried out by adding gastric juice containing 0.4 M glycine solution (prepared by adding 30.3 g of glycine to 1Litre deionised water) adjusted with 12 M HCl to pH 1.5 ± 0.2. (Drexler *et al*., 2007).

Approximately 0.5 g of sample was placed in a HDPE screw top tube and 50 mL gastric solution was added. The mixture was agitated in an end-over-end orbital shaker maintained at 37^{o} C and 100 rpm for 1hr. After extraction was completed, some aliquot was removed with a disposable syringe attached to a 0.45 μm cellulose acetate membrane filter, the filterate was transferred into a centrifuge

tube and stored in a refrigerator at 4 ^{0}C prior to instrumental analysis.For each sample extraction performed, pH value of the sample solution was taken at the beginning and the end of the extraction to ensure it was within required range (pH 1.3 -1.7) before filteration.The extraction was carried out in triplicate for each sample.

3.7 INSTRUMENTAL ANALYSIS OF PTEs

Quantification of the levels of PTEs in the soil and vegetable samples was done using both Flame atomic absorption spectrophotometer (FAAS) and Inductively coupled plasma - mass spectrometer (ICP-MS)

3.7.1Optimisation of FAAS

(a) Assessment of the fuel flow rates

The flame fuel flow of the FAAS employed for this study was optimised for the analyte of interest using a low, non-zero standard solutions for each analyte. The optimisation was achieved by analysing the calibration standards at flow rates of between 2.0 and 4.0 L min^{-1}.

(b) Linearity of PTE calibration

The dynamic working linear range of each PTE of interest was investigated. Calibration curves were constructed for each element by preparing standard solutions in 5% HNO_3, and illustrated as in Figures4.1-4.2. The linear least squares regression analysis was performed on the results to obtain the lines of best fit and the equations of the best fit lines were used to calculate the detection limits of the instrument.

(c) Analysis of PTEs using FAAS

For the analysis of solutions for the pseudototal concentration of PTEs in soil, calibration standards were prepared by serial dilution of 1,000 mg/l of stock solutions. Calibration standards of 0, 0.2, 0.4, 0.6, and 0.8 mg/L were used for Cd, 0, 2.5, 5.0, 10 and 20.0 mg/L for Cr, 0, 2.0, 4.0, 8.0 and 10.0 mg/L for Cu, 0, 5.0, 10.0, 20.0 and 40.0 mg/L for Pb and 0, 0.2, 0.4, 0.6 and 0.8 mg/L for Zn. The standards were prepared in 5 % HNO_3. This was done in order to ensure standard and sample solutions were of similar bulk composition so that matrix effects can be eliminated. The samples were then analysed after the calibration by reading triplicate measurement of each PTE under investigation.

As a quality control measure, for every analysis carried out on each day, freshly prepared calibration standards were used.

(d) Limit of detection

The limits of detection of the instrument ($LOD_{inst.}$) for each PTE under investigation i.e. Cd, Cr, Cu, Pb and Zn was determined by using ten replicate measurements of the lowest calibrant solutions and the standard deviation of the mean value. The limits of detection were also calculated taking into consideration the digestion methods to obtain the procedural detection limits ($LOD_{pro.}$).

The equations used for the calculation of $LOD_{inst.}$ and $LOD_{pro.}$ are given as

$$LOD_{instrument} = \frac{3s}{b}$$

Where s and b represent the standard deviation of replicate measurements of calibrant and minimum concentration of calibrant respectively.

$LOD_{prol} = LOD_{inst} \times$ dilution factor

3.7.2 Optimisation of ICP-MS

The operating conditions employed for determination of PTEs using ICP-MS are presented in Appendix III, operating conditions such as nebulizer gas flow, RF power, sample uptake rate as well as lens voltage were optimised to produce good sensitivity of the instrument. The calibration standards were prepared by diluting the stock multi-elemental standard solution (1000 mg/L) in 2 % (v/v) nitric acid. The concentration of calibration standards for all the studied elements were in the range of 0.0 to 2.0 mg/L. An internal standard (Indium) was also added to all samples, blanks and calibration solutions in a final concentration of 2.0 mg/L to compensate for electronic drift induced by instrument and sample variation.Samples to be analysed by ICP-MS were prepared in triplicate by measuring 1 mL of either the filtrate, GLA URM or blank into a 10 mL centrifuge tube

(a) Analysis of PTEs using ICP-MS

Sample solutions to be analysed were prepared in triplicate by measuring 1 mL of either the filtrate, or blank into a 10 mL centrifuge tube. A series of calibration standards over the range 0.0-2000µg / L were prepared from a 1000 mg / L multi-element standard. This was used to calibrate the instrument and also to construct the calibration curves.Regression coefficients and detection limits were also obtained from the curves (Figures 4.6-4.7). During sample analysis, an internal standard (Indium) was added through the peristaltic pump of the ICP-MS to each sample to be analysed. The internal standardization method was used to effectively correct for changes in element sensitivity as a result of the variation of matrix components in the sample and the instrument drift (Jayawardene*et al.*, 2011)., As a quality control measure, calibration standard solutions were also determined after every tenth sample measurement to check for instrument consistency, this was carried out for every batch of analysis performed each day.

3.8 QUALITY CONTROL

During processing and analysis of samples, quality assurance and control measures were adopted to ensure accuracy and reliability of results. All glass and non-glassware was cleaned by soaking in 5% nitric acid overnight and then rinsed thoroughly with deionised water before use in order to avoid sample contamination. All chemicals, especially enzymes used for bioaccessibility studies were stored at recommended storage temperatures before and after use to prevent denature. De-ionised water were used throughout sample preparation and analysis. Reagent blanks were analysed to identify any possible contamination by reagents, digestion chemicals and digestion vessels. The data were subsequently blank corrected to remove analytical bias. Replicate measurements of samples were carried out to determine the reproducibility of results. The quality control of the measurement was carried out by measuring the standard solutions after every ten samples. To check the analytical performance of the laboratory, analyst and the procedures a Certified Secondary Reference material (GLA URM) was used (Davidson *et al.,* 2006).

3.9 STATISTICAL ANALYSIS OF DATA

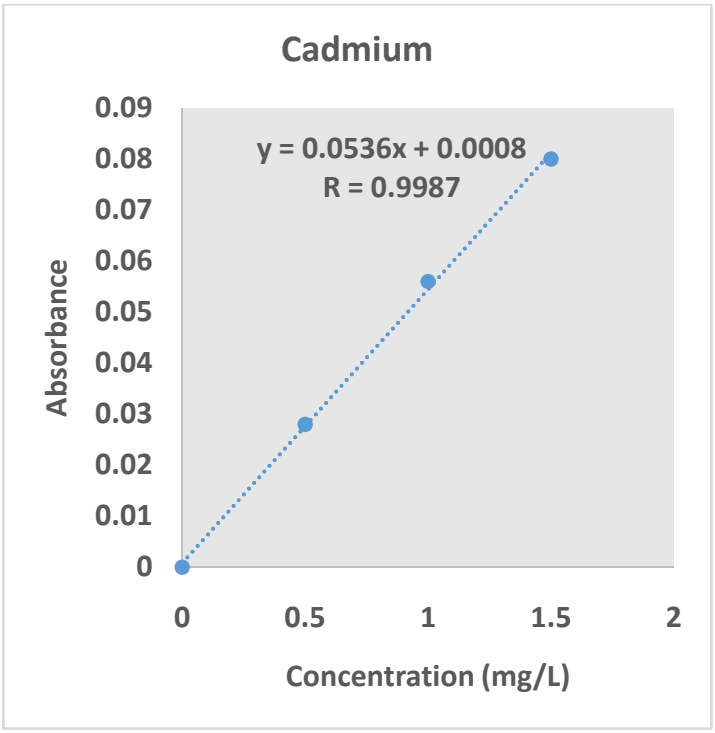
The Statsgraphics Centurion XVI statistical software package was employed for data analysis. Correlation analysis and t-test were performed to ascertain relationship between soil parameters and determine any difference or similarity in the instrumental techniques used for this study. In order to reduce the large number of variables to smaller number of orthogonal factors, the original data obtained was processed using multivariate methods. Source groupings and the association of sample parameters were determined using Cluster and Principal Component Analysis. Principal components having eigenvalues > 1 of the complete data set were retained.

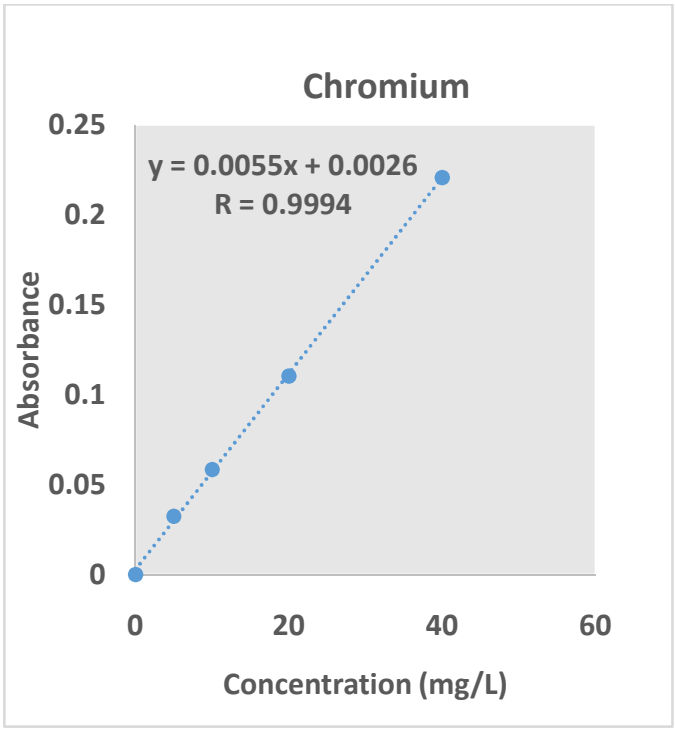
CHAPTER FOUR

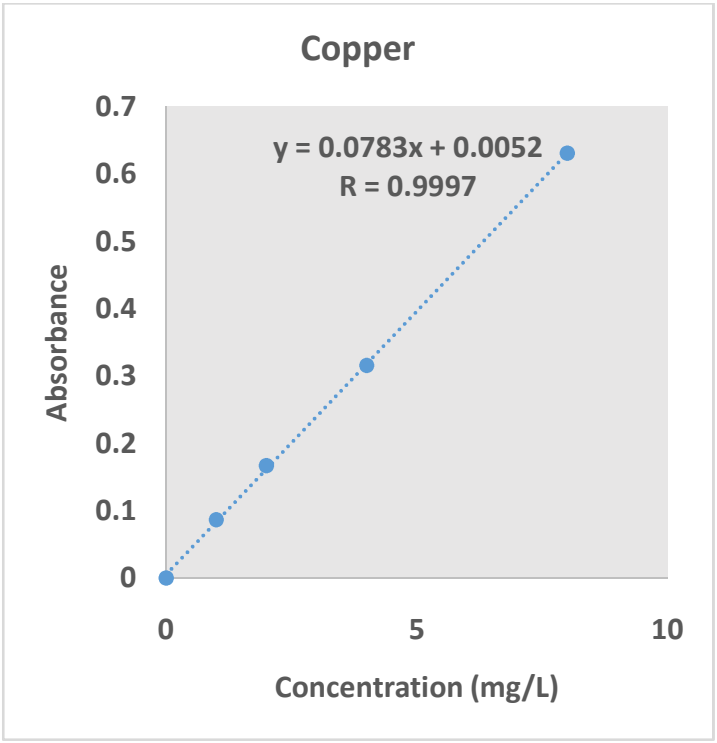
RESULTS AND DISCUSSION

4.1 Optimisation of FAAS

The optimisation of the working conditions of flame atomic absorption spectrophotometer such as the linear working range, optimal absorbance, lamp current and energy are presented in Appendix II. Calibration standards for each element was prepared by serial dilution of 1,000 mg/l of stock solutionsand the best result of the calibration curve fit was obtained for the standard concentrations for each element under determination.The calibration curve with the correlation coefficient (R> 0.998) for each element (Cd, Cr, Cu, Pb, Zn) determined are as illustrated in Figures 4.1 - 4.2. Precautions were taken to ensure that linearity in the experimental response was maintained over a wide range of concentrations.







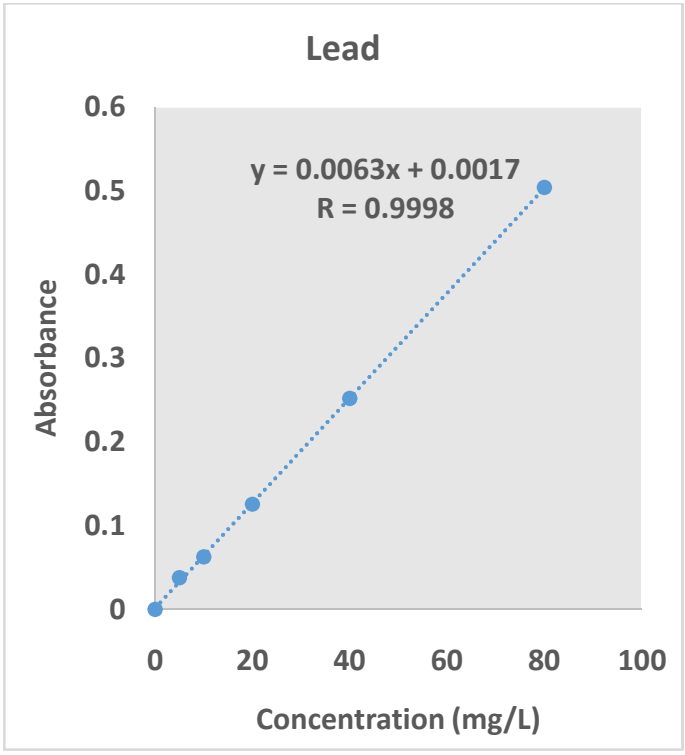


Figure 4.1: Calibration curve of Cd, Cr, Cu and Pb using FAAS

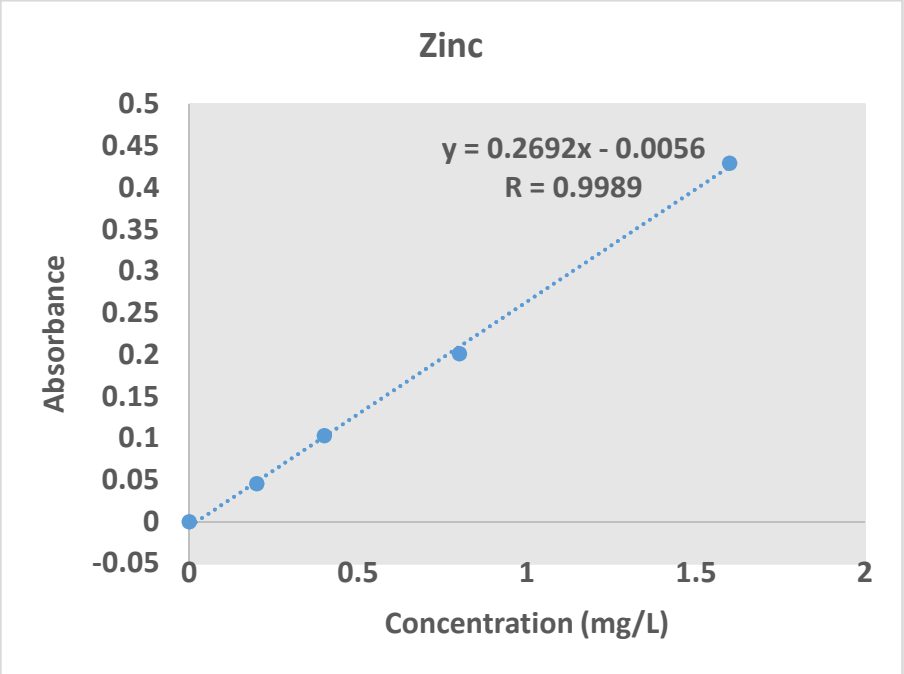


Figure 4.2: Calibration curve of Zn using FAAS

4.2 Optimisation of ICP-MS

Prior to determination of potentially toxic elements concentration in the study samples (soils and vegetables), the operating conditions of the ICP-MS were optimised. Typical optimised operating conditions are presented in Appendix III. The calibration curve obtained from the series of multi-element standard solutionsshowing the regression coefficient as well as the instrument limit of detection for each element determined are illustrated in Figures 4.6 – 4.7.Calibration curves for PTEs based on a concentration range of 0-2000 ppb with six calibration data points were done on ICP-MS and the regression coefficient (R) obtained for each element was 0.999. The internal standard stability graphillustrated in Figure 4.8 was used to effectively correct for changes in analyte sensitivity caused by the variation of matrix components in the sample and the instrument drift. The percent recovery for the internal standard used for this study was between 80–110 % which indicate very good sensitivity of the ICP-MS instrument employed for analyte measurement.

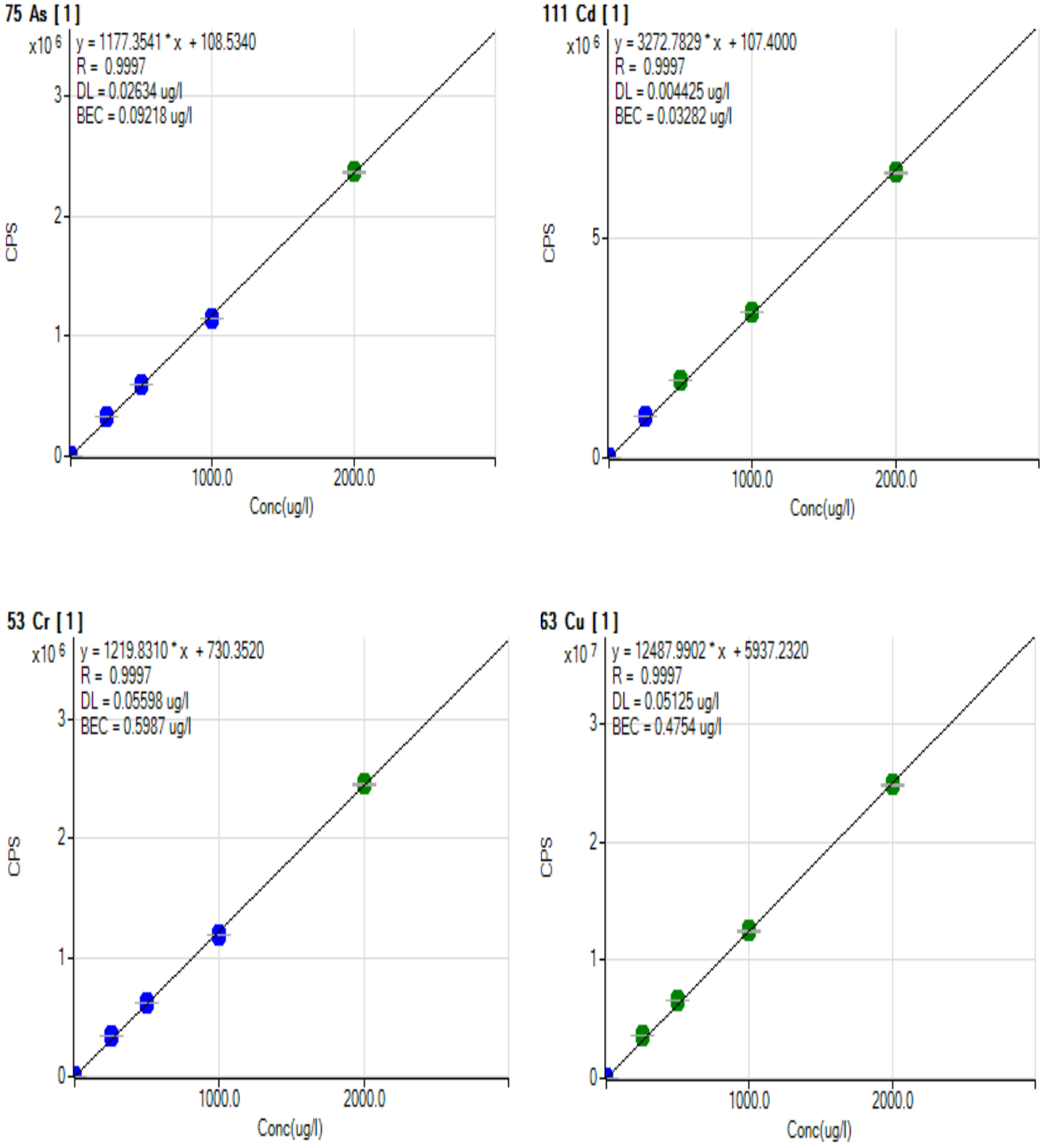


Figure 4.6:ICP-MS Calibration curve for As, Cd, Cr and Cu

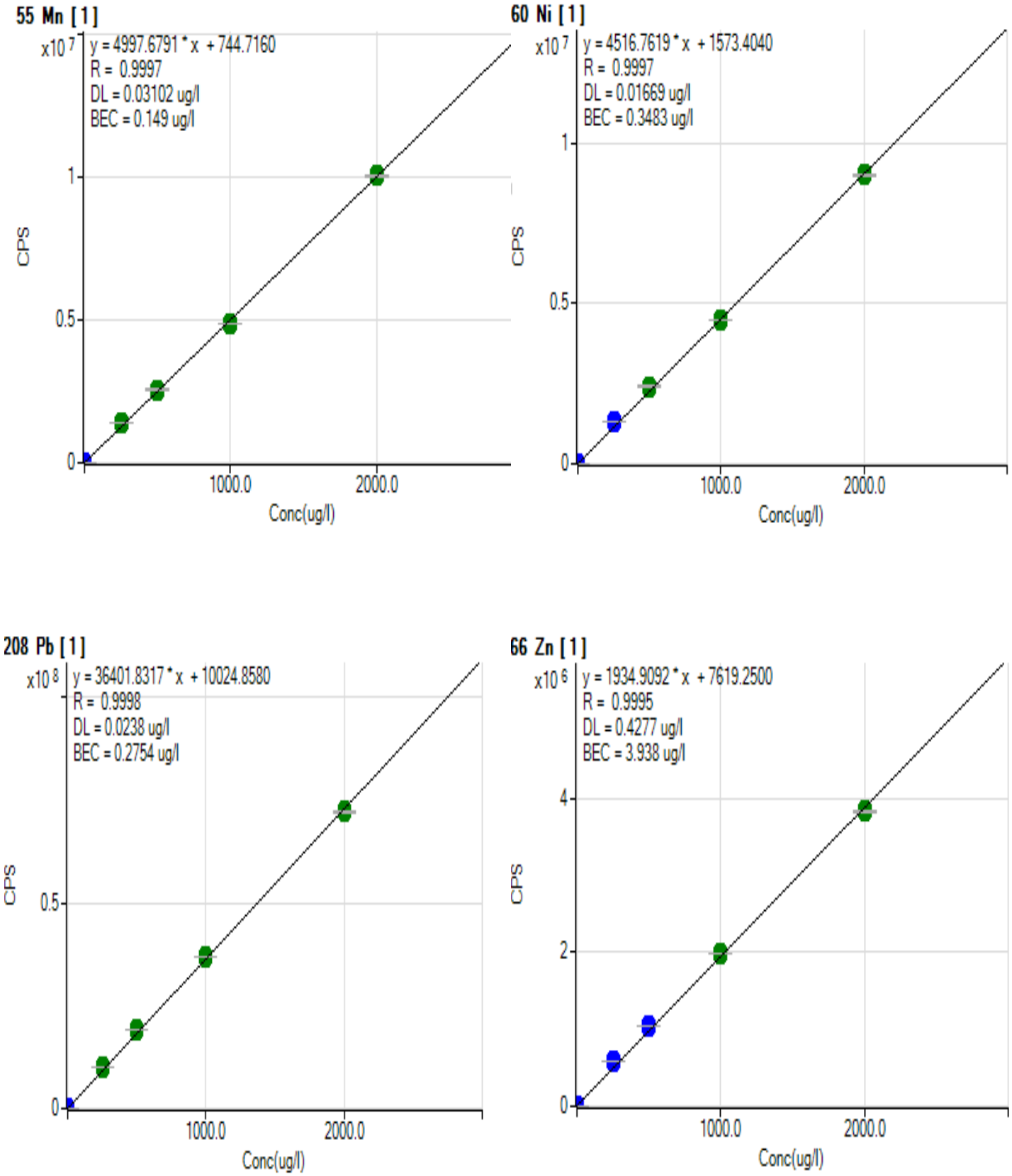


Figure 4.7:ICP-MS Calibration curve for Mn, Ni, Pb and Zn

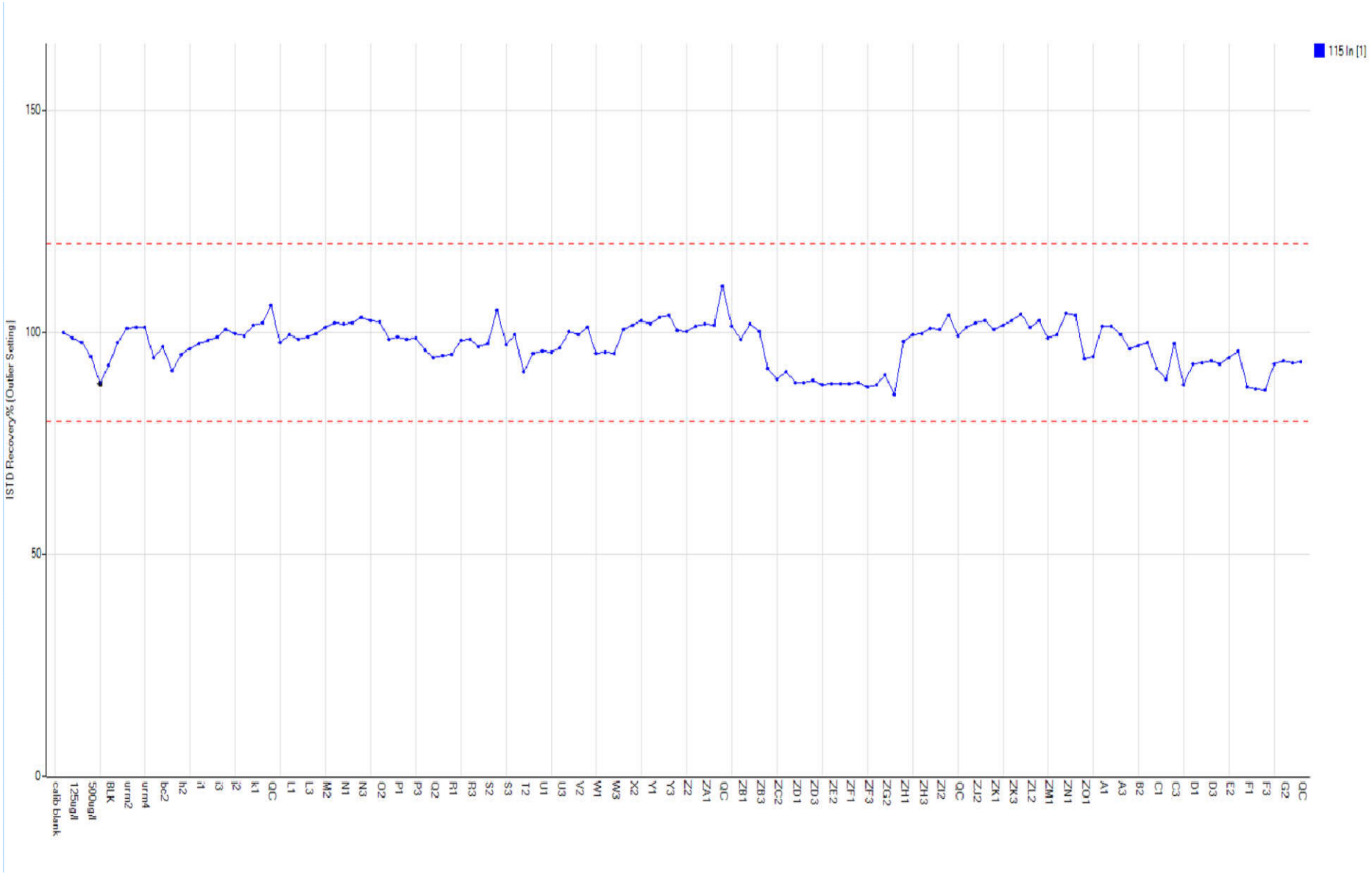


Figure 4.8: Internal standard stability graph

4.3QUALITY CONTROL OF TOTAL PTEs IN SOILS

The analytical performance of the laboratory and the method used in this study waschecked using a reference material GLA URM - an urban soil secondary material prepared by participants in the EU URBSOIL project for use as internal quality control sample (Davidson *et al*., 2006).

The results for the pseudototalPTE concentration and the recoveries relative to target values of the secondary reference material- GLAURM is shown in Table 4.1.Excellent results were obtained for the total PTE determination when compared with certificate/ target values.The percent recovery of the elements was found to be in the range of 92%-120%.The relative standard deviation was less than 10% for all the elements and recoveries were 100 ± 12% except for Cd. The closeness of the measured value to the certified /target values of the reference material and excellent % recovery is an indication that the method used for the digestion is good and accurate and the small standard deviation of replicate analysis of the reference material showed good precision.

Table 4.1: Results of soil reference material GLA-URM in mg/kg

GLAURM	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Measured value	19 ± 1	< 0.5	52 ± 9	118 ± 4	455 ± 16	54.9 ± 6	359 ± 19	184 ± 12
Target /Certified value	N/A	< 0.74	43.2± 3.0	111 ± 5	442 ± 18	48.8± 7.0	389 ± 25	177 ± 11
% Recovery			120	106	102	112	92	103

Results are means of replicate analysis ± standard deviation (n=20), ''n'' denotes number of determinations, N/A denotes Not Available, ''<'' denotes values below detection limit

Figure 4.9 gives the representation (J- Chart) of the results obtained for the digestion of each subsample of the secondary reference material GLA-URM. The J-Chart showed that the values obtained for almost all the PTEs from digestion carried out on twenty batches of the secondary reference material were within three standard deviations of the certified values, most of the values fell within the lower and upper limits of the J-Chart,with the exception of Cr in only one of the subsample which fall a little above the upper limit, which could probably be due to dilution errors.The results of this chart indicated a good analytical performance of the digestion procedures and the laboratory.

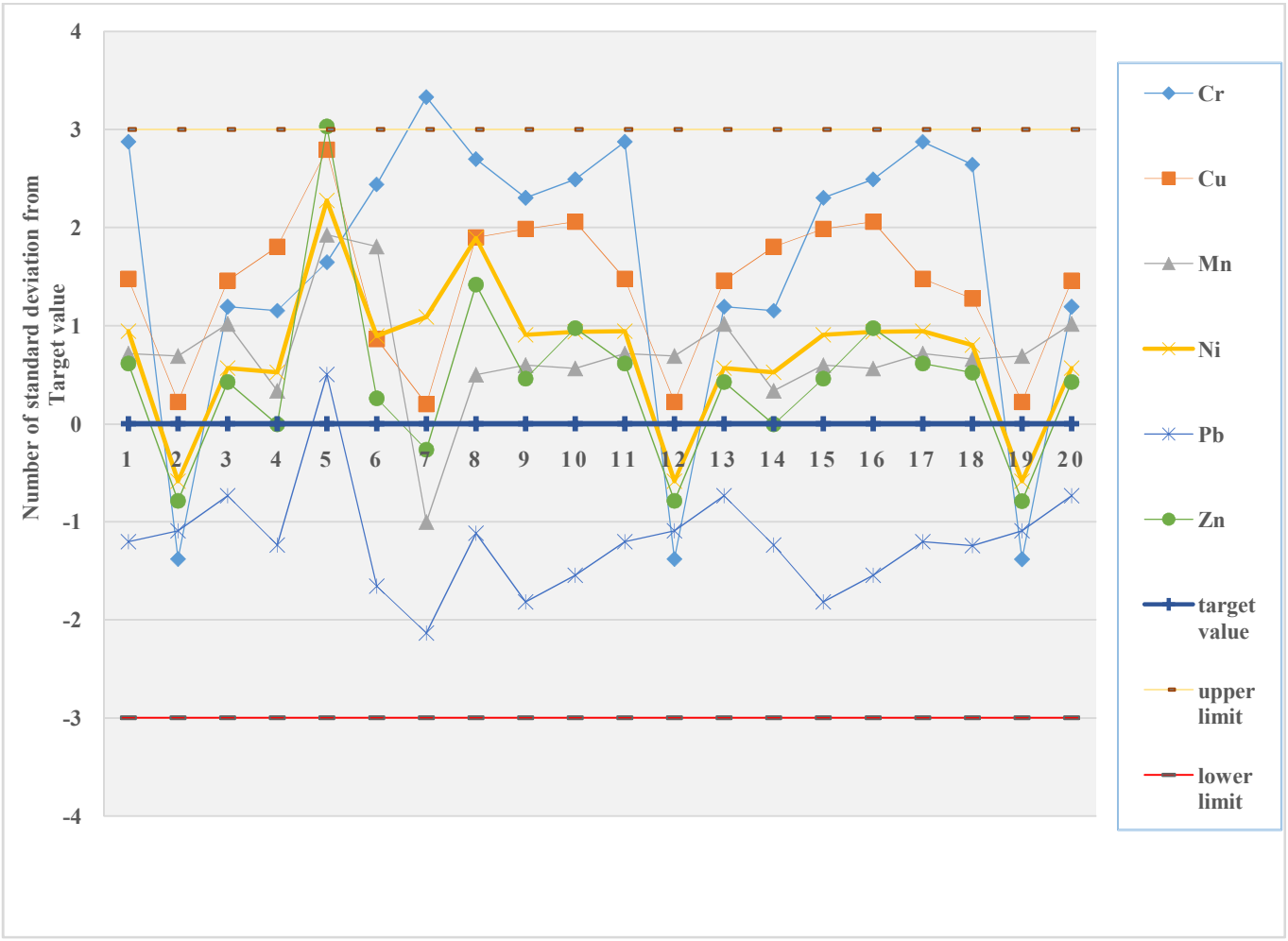


Figure 4.9: Control chart showing results obtained from the digestion of subsamples of the secondary reference material- GLAURM

4.4POTENTIALLY TOXIC ELEMENTS IN SOILS

4.4.1 Physicochemical analysis of soils

The mobility and bioavailability of PTEs in contaminated soil is affected by a number of biological processes and physiochemical properties like soil pH, organic matter (OM) and soil texture (Ahmad and Goni 2010). Soil pH has significant effects on the bioavailability and accumulation of potentially toxic elements in the edible parts of plants (Hu *et al.,* 2013; Wang *et al.,* 2013).

Table 4.4 gives the results of physicochemical parameters of the different soil samples investigated.The values obtained for the pH in the different soils ranged from 6.6 to 7.1, indicating that the soils were all slightly acidic to neutral in nature. The results obtained for percent total organic content of the soilswere rather low and rangedbetween1.2 %to 5.4 %, Owode soil had the highest organic content (5.4%) followed by Katangua soil with 4.2 % while the other soils had between 1.2 % and 3.8 % organic content. Moisture content of the soils ranged from 0.8 % to 2.9 %. The particle size distribution showed that the soils were all sandy soils with very low clay content. The values rangedfrom 69 % to 88 % sand, 2 % to 19 % silt, 2 % - 15 % clay and 0 % - 2 % gravel.

Table 4.2: Physicochemical parameters of soil samples

Soil Sample	pH	TOC (%)	Moisture content (%)	Particle size distribution (%)				
				Gravel	Sand	Silt	Clay	Gravel
MFM	6.6	2.7	0.8	0	70	18	12	0
ORL	6.8	1.2	1.0	2	85	7	6	2
KATANG	7.1	4.2	2.9	0	77	8	15	0
OWD	6.7	5.4	1.2	0	88	2	0	0
IBF	6.8	3.3	0.8	0	69	19	12	0
FSS	7.3	3.8	2.7	0	82	16	2	0

4.4.2Pseudo total concentration of PTEs in Soils

The results obtained for PTE concentration in the soil samples investigated are presented in Tables 4.5 and 4.6. Table 4.5 gives the results obtained when FAAS was employed for PTE quantification while Table 4.6 gives the values obtained when an ICP-MS was used. Only five (Cd, Cr, Cu, Pb and Zn) of the eight PTEsof interest in this study were determined using FAAS, this was as a result of the non-availability of Hollow cathode lamps for Mn and Ni as well as Hydride generation AAS for the determination of As during that period of the research.The mean concentration of the PTEs in the soil samples using FAAS and ICP-MS are summarized in Table 4.5 and 4.6 respectively. Concentrations varied greatly between soils from different locations, with Katangua soil being the most contaminated soil when the two techniques were employed for quantification of PTEs.

Paired t-test was performed on the results obtained using the two instrumental techniques (FAAS and ICP-MS) and it indicated no significant difference observed ($P > 0.05$) in the trend of results obtained for PTEs in the different soil samples but paired t-test wascarried out by comparing individual values of PTEs using the two techniques, result obtained showed there was a significant difference ($P < 0.05$) between the values obtained for the PTEs in the soil samples. This is due to the greater sensitivity and wider linear dynamic range of the ICP-MS than the FAAS as indicated by the difference in values obtained by the two methods. Hence, the results of PTEs concentration employing ICP-MS for determination was used in further discussions.

A wide range of PTE concentrations were observed for the different soil samples investigated in this study with values obtainedin the following range; As 0.7– 20 mg/kg, Cd <0.04 -20 mg/kg, Cr 2.4 – 2410 mg/kg, Cu 14.3– 14900 mg/kg, Mn 131 – 3020 mg/kg, Ni 7.0 – 1050 mg/kg, Pb 17.2 – 6200 mg/kg and Zn 71 – 2760 mg/kg respectively. These values were compared withbackground and soil guideline valuesfrom several countries. Moreso, since, as at the time of this studyno SGVs existed for Nigeria, it was considered necessary to compare the result obtained in this study with soil

background values(Squirts, 2008) and more specifically South African SGVs (the only country in the African continent with SGVs). (FWMCL, 2010).When compared withSouth African recommended soil guideline values (Appendix 1), it was observedthat the levels of some PTEs such as As, Cd, Cr, andNiwere below the SGVs while other PTEs such as Cu, Mn, Pb and Zn were found to be higher, especially in the soils from Katangua, Orile and Owode sites, which may be as a results of the anthropogenic activities that take place on those sites.Katangua site handles mostly electronic wastes and this may explain the elevated concentration of the PTEs since they are constituents of electronic devices such as computer/TV screens, printed circuit boards, batteries and wiring. Orile site is associated with welding, smelting and sale of metal rods and car battery– which may account for the high levels of Cr, Mn and Ni observed in the soil. Owode site on the other hand is a battery and metal scrap market dumpsite, and batteries have been found to have high concentrations of PTEs (Adelakun and Abegunde, 2011) which may also account for the high level of PTEs in the soils.HoweverMFM and Ibafo soils hadvery low concentrations of PTEs, which is because theyare newly sited small dumpsites which handle mostlydomestic wastes, some car batteries andmetal scraps. The concentrations of PTEs from the control site wasas expected, relatively low, because the area is devoid of vehicular movements or anthropogenic activity that could result in soil pollution.

Theresults of PTEs concentration in the soils investigated in this study compared with those obtained in other similar published works in Nigeria (Adewuyi *et al.,* 2010; Ogbemudia and Mbong, 2013; Olafisoye *et al*., 2013,Amos-Tautau *et al.*,2014; Abidemi and Onwordi, 2015).

The mean concentration of As in the different soils ranged from 0.7 – 20 mg/kg, with the highest concentration found in Katangua and Owode soils with concentrationsof 20 mg/kg and 19 mg/kg respectively, these high levels may be attributed to anthropogenic factor at the sites. while the lowest concentration (1.0 mg/kg) was found in Ibafo soil rather than Control soil which was used as the control site with concentration of 2.0 mg/kg,, the presence of As in the Control soil may be as a

result of the use of pesticideon some of the plants grown at the botanical garden which may have inadvertently leached into surrounding soil in the garden.

Cadmium is a very toxic element with no beneficial physiological function in the human body even at very low concentrations and was found in all the soil samples except the Control soil. Cd concentration ranged from <0.09 mg/kg -20 mg/kg, with highest concentration (20 mg/kg) found in Katangua soil, an indication of the gross pollution of the soiland greater risk from Cd in the soil whereas the lowest concentration was found in Control soil (< 0.09 mg/kg). The very high concentration of Cd in Katanguacould be attributed to amount of polyvinylchloride (PVC) plastics, nickel-cadmium batteries, motor oil and disposal sludge on the soil in the dismantling operation at the dumpsite. Cd is relatively soluble and capable of being retained in the soil at any pH for a long time, hence they can easily be transported in the form of its ion in solution into plants cultivated on the soil (Ebong *et al*., 2008). Similarly, Balkhair and Ashraf (2016) reported that Cd had a greater exchangeable capacity, thus easily accumulating and bioavailable in the edible parts of plants. The Cd levels obtained in this study were consistent with similar studies by Ogbemudia and Mbong (2013) and Olafisoye *et al.*, (2013).

The mean concentration of Cr in the soil samples ranged from 2.4 – 2410 mg/kg with ORL having the highest concentration (2410 mg/kg) followed by Owode soil (1360 mg/kg), and the lowest concentration was found in Control soil. It was also observed that Katangua soil which had been pronounced as the most grossly polluted site due to its very high levels of other PTEs had a relatively lower concentration of Cr (188 mg/kg), which suggest that the presence of some major anthropogenic input of Cr to the environment were not prevalent in Katangua soil, but were rather found to be very predominantin orile soil (2410 mg/kg) and owode soils (1360 mg/kg). The anthropogenic factors contributing Cr in the environment include panel beating of vehicular parts,

use of chrome- steel alloys, plastics, metallic colour coats amongst many others. Chromium and its oxides are also widely used because of their high conductivity and anti-corrosive properties (Kalaghor *et al.,* (2014). Although Cr toxicity is relatively rare, there are still some level of risk associated to human health, this is because while some forms of Cr are non-toxic, Cr(VI) is easily absorbed in the human body and can produce various toxic effects within cells (Olafisoye *et al*., 2013). The levels of Cr found in this study were higher than the results of similar study by Olafisoye *et al*., (2013); Ogbemudia and Mbong (2013); Amos-Tautau *et al.* (2014); Abidemi and Onwordi,(2015) for soils from dumpsites.

Copperis one of the essential elements found on the earth crust. At high concentration, Cu can become toxic causing serious diseases such as Wilson's disease amongst many others (Abdel-Satar *et al.,* 2017). The concentration in the studied soils ranged from 14 – 14900 mg/kg. Amongst the soils, Katangua soil was found to have the highest concentration 14,900 mg/kg while the lowest concentration was found in Control soil. The high concentration of Cu is most likely due to the substantial amount of electrical materials such as copper conductors, wires, solders, tubes and myriads of other items made from copper dumped at this site. The levels of Cu in most of the soils investigated in this study (i.e. orile, owode, FSS and Katangua soils)were found to be much higher than what was reported by Amos-Tautau *et al.* (2014) and Abidemi and Onwordi, (2015) on heavy metals of dumpsites in Yenagoa and Ido-Osun, Osogbo respectively.

Similarly, Mn is one of the essential mineral and it was present in substantial amount in all thestudied soil samples including the Control soil. The values ranged between 131 – 3020 mg/kg, with Orile soil having the highest concentration.

Nickel is found mainly from natural sources (Abdel-Satar *et al*., 2017), the concentration found in the soil samples investigated ranged from7.0 – 1050 mg/kg. Though Ni was present in all the soil sample investigated, the highest concentration was found in Orile soil while Control soil had the lowest concentration. The high Ni content in Orile soil may be attributed to the disposal of spent automobile batteries from nearby auto battery charger workshops around the dumpsite. This level of Ni found in orile soil when compared with results fromsimilar studies by Olafisoye *et al.,* 2013, Abidemi and Onwordi, 2015 was higher but for Ni levels found in the other soils in this study (MFM, Katangua, Owode, Ibafo, FSS)the results were in agreementwith other reports.

Lead is one of the most important pollutants in soils because of its potentially deleterious effect on soil and food quality (Wei and Yang, 2010). For the seven soil sites investigatedin this study, Pb concentrations were in the range of 17.2 – 6200 mg/kg. The highest concentration (6200 mg/kg) was found in Katangua soil, which was far beyond soil guideline values (Appendix 1) while the least was found in Control soil (17.2 mg/kg). The very high Pb level present in Katangua dumpsite soil may be attributed to itssubstantial contribution from the disposals of electronic wastes such as cathode ray tubes, computer monitor glass,printed wiring boards, lead-acid batteries and metal scraps on these dumpsite over a long period of time(Olafisoye *et al*., 2013). The findingsin this study (with the exception of Pb level in Katangua soil) compared well with what was obtained in similar reports by Olayinka *et al.,* (2011); Olafisoye *et al*., (2013); Ogbemudia and Mbong, 2013; Abidemi and Onwordi, (2015).

Zinc also is another element which covers the class of essential elementswasfound in substantial amount in the soil samples investigated in this study. Its concentration ranged between 71- 4760 mg/kg, with Katangua soil having the highest concentration (4760 mg/kg), followed by Orile soil (2760 mg/kg) , these values were found to be more than soil guideline values (Appendix 1) while the lowest concentration was found in MFM and Control soil with values obtained lower than the soil guideline values. Meanwhile, the high Zn concentration observed in Katangua, Orile and Owode soil may be attributed to dumping of zinc containing solid waste materials at the sites.Kalaghor *et al*., (2014) attributed the source of Zn to domestic refuse, construction materials, motor vehicle emissions and motor vehicle wear.

Table 4.3: FAAS Results of pseudo total concentration of PTE in soils (mg/kg)

Soil Sample	Cd	Cr	Cu	Pb	Zn
MFM	<0.5	14.2 ± 0.8	85.2 ± 9	51.1 ± 12	67.9 ± 15
ORL	2.0 ± 0.4	1150 ± 56	185 ± 64	64.9 ± 5	730 ± 32
KATANG	19.8 ± 2	198 ± 42	4960 ± 510	1940 ± 180	1600 ± 130
OWD	6.05 ± 2.2	515 ± 58	2630 ± 430	336 ± 100	1130 ± 130
IBF	0.71 ± 0.2	52.1 ± 24	102 ± 65	63.2 ± 13	47.1 ± 12
FSS	3.0 ± 0.4	112 ± 48	1410 ± 72	412 ± 20	1280 ± 120
CONT	<0.5	< 3.5	< 0.37	< 12.5	18.7 ± 8

Results are expressed as the mean of triplicates ± Standard deviation,
< denotes below detection limits

Table 4.4: Results of Pseudo total concentration of PTE in Soilsusing ICP-MS

Soil Sample	Potentially Toxic Elements (mg/kg)							
	As	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**
MFM	1.20 ± 0.2	0.20± 0.01	24.2 ± 3	19.9 ± 1	131 ± 28	8.21 ± 1.0	37.1 ± 3	172 ± 5
ORL	17.2 ± 2	0.51 ± 0.1	2410± 680	577 ± 150	3020 ± 430	1050 ± 200	164 ± 25	2760 ±120
KATANG	19.8 ± 2	20.1 ± 2	188 ± 12	14910±680	924 ± 25	136 ± 25	6200 ± 170	4760 ± 190
OWD	19.2 ± 1	2.10 ± 0.3	1360 ± 52	4070 ± 180	2320 ± 77	265 ± 48	230 ± 12	2600 ± 130
IBF	1.0 ± 0.1	0.22 ± 0.02	28.8 ± 1	58.2 ± 10	294 ± 67	10.1 ± 1.2	58.8 ± 1.0	430 ± 25
FSS	3.88 ± 1	7.10 ± 0.4	117 ± 31	524 ± 90	620 ± 120	83.2 ± 19	437 ± 39	2640 ± 130
CONT	2.0 ± 0.1	<0.09	24.2 ± 2.0	14.3 ± 2.1	231 ± 14	7.10 ± 1.1	17.2 ± 1.2	71.2 ± 8
SGVs	48	32	(Cr^{3+}) 9600 (Cr^{6+}) 13	2300	1500	1200	230	1900
Background values*	5.2	N/A	< 37	17	330	13	16	48

Results are expressed as the mean of triplicate measurement ± Standard deviation, "<": values below detection limits, SGVs: South African soil guideline values, N/A: Not available, *SQuiRTs (2008)

4.4.3 Statistical analysis of PTEs in soils

4.4.3.1 Pearson's correlation analysis of soil parameters

The relationship between some physico-chemical properties and PTEs concentrations were analysed using Pearson's correlation coefficient. Table 4.5 shows the Pearson product moment correlations between each pair of variables (soil parameters). These correlation coefficients measure the strength of the linear relationship between the variables. P-value tests the statistical significance of the estimated correlations. P-values below 0.05 indicate statistically significant non-zero correlations at the 95.0 % confidence level. The results obtained in this study revealed thatAs was strongly correlated with Mn and Zn with r values of 0.78 and 0.85 respectively,Cd was also observed to be strongly correlated with Cu, Pb and Zn with r values of 0.92, 0.96 and 0.81 respectively. Similarly, Cr was strongly correlated with Mn and Ni with r values of 0.97 and 0.95. The high correlation coefficients observed means very good relationship existed between the variables. On the other hand, moderate correlation was observed to exist between the pHand TOC while weak correlation was found between pH and individual PTEs with r values less than 0.5, as well as TOC and individual PTEs with r values less than 0.45.

Negative correlations washowever observed between Cd and Cr, Cd and Mn, Cd and Ni. Similarly, Cu had negative correlation with Cr and Ni, while Pb had negative correlation with Cr, Mn, Ni, which means no relationship exist between the variables.

Table 4.5: Pearson's correlation analysis of soil parameters

	pH	TOC	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
pH		0.5510	0.3403	0.5383	0.1044	0.3817	0.2037	0.1659	0.4065	0.6645
P-value		0.1999	0.4551	0.2126	0.8237	0.3982	0.6613	0.7222	0.3655	0.1035
TOC	0.5510		0.3770	0.4372	-0.1390	0.4829	0.0535	-0.3357	0.3391	0.4652
P-value	0.1999		0.4045	0.3267	0.7663	0.2724	0.9093	0.4616	0.4568	0.2929
As	0.3403	0.3770		0.5050	0.6561	0.6836	0.7850	0.5759	0.5478	0.8445
P-value	0.4551	0.4045		0.2477	0.1095	0.0904	**0.0365***	0.1761	0.2031	**0.0168***
Cd	0.5383	0.4372	0.5050		-0.2248	0.9224	-0.0646	-0.1394	0.9602	0.8114
P-value	0.2126	0.3267	0.2477		0.6280	**0.0031***	0.8906	0.7656	**0.0006***	**0.0267***
Cr	0.1044	-0.1390	0.6561	-0.2248		-0.0706	0.9747	0.9480	-0.1797	0.3502
P-value	0.8237	0.7663	0.1095	0.6280		0.8805	**0.0002***	**0.0011***	0.6999	0.4413
Cu	0.3817	0.4829	0.6836	0.9224	-0.0706		0.0950	-0.0590	0.9686	0.7926
P-value	0.3982	0.2724	0.0904	**0.0031***	0.8805		0.8395	0.9000	**0.0003***	**0.0335***
Mn	0.2037	0.0535	0.7850	-0.0646	0.9747	0.0950		0.8911	-0.0408	0.5094
P-value	0.6613	0.9093	**0.0365***	0.8906	**0.0002***	0.8395		**0.0071***	0.9308	0.2430
Ni	0.1659	-0.3357	0.5759	-0.1394	0.9480	-0.0590	0.8911		-0.0921	0.3712
P-value	0.7222	0.4616	0.1761	0.7656	**0.0011***	0.9000	**0.0071***		0.8442	0.4123
Pb	0.4065	0.3391	0.5478	0.9602	-0.1797	0.9686	-0.0408	-0.0921		0.7493
P-value	0.3655	0.4568	0.2031	**0.0006***	0.6999	**0.0003***	0.9308	0.8442		0.0525
Zn	0.6645	0.4652	0.8445	0.8114	0.3502	0.7926	0.5094	0.3712	0.7493	
P-value	0.1035	0.2929	**0.0168***	**0.0267***	0.4413	**0.0335***	0.2430	0.4123	0.0525	

*** - values significant at 95% confidence level**

4.4.3.2Cluster analysis of soils

Cluster analysis (CA) grouped the studied soil samples into clusters (called groups in this study) on the basis of similarities within a group and dissimilarities between different groups. It was performed on the data using squared Euclidean distance which agglomerated the seven soil samples into four group depicted by a dendrogramas shown in Figure 4.10, where the vertical axis represents the degree of association between groups of variables (soils), that is, the lower the value on the axis the more significant the association. As seen on the dendrogram, MFM, Control and Ibafo soils were grouped into one cluster, which confirms the similarities in the level of PTEs and degree of pollution of these sites. FSS was grouped into another cluster alone, Orile and Owode soils were grouped into one cluster, confirming their association in terms of levels of PTEs this is because they were observed to have values within the same range for most of the PTEs studied (i.e. As, Cr, Mn, Pband Zn). However, Katanguasoil was also isolated in one cluster which showed its extreme difference from the other soil investigated because of its very high concentration for all the PTEs.

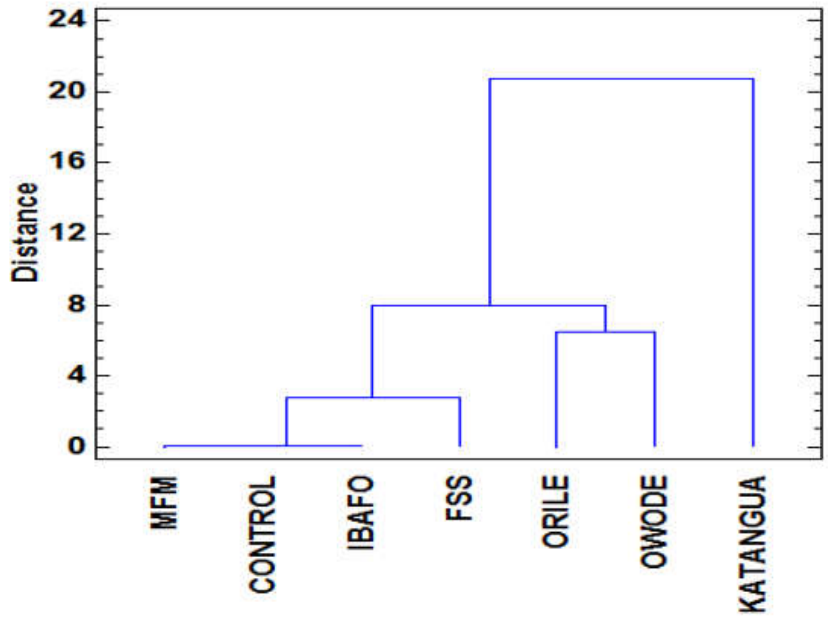


Figure 4.10: Dendrogram showing cluster analysis of soil sites

4.3.3.3Principal Component Analysis of PTEs

While cluster analysis only gives similarities between soil samples investigated, principal component analysis (PCA) was further performed with the purpose of reducing the relatively large number of variables to a smaller number of orthogonal latent variables to further investigate the relationship between potentially toxic elements and some soil parameters (pH and total organic content) as shown in Figure 4.11 and 4.12.

Corresponding components, variable loadings, and the variances are presented in Table 4.6. Only PCs with eigenvalues greater than one were considered during this analysis. Based on the eigen values obtained, the first three components with values greater than one were retained and used. Together they account for 93 % of the variability in the original data. The first two principal components (PC1 and PC2) togetherexplained 83% of the variance in the data, the first component PC1 which explained 49.5 % of the total variance is positively correlated with As, Cd, Cu, Pb and Zn .Theassociation of the PTEsin PC1 suggests anthropogenic influencefrom the contamination sources which may includemetal scraps, battery and electronic gadgets such as phones, radio and television sets, laptops, torches, printers e.t.c. at the dumpsites.

The second component PC2 was positively correlated withCr, Mn and Ni accounting for 33.5 % of the variance. All these PTEsare essential minerals which, however, pose high risks when consumed in large doses which implied that though they are mainly due to geogenic influence but high concentrations are usually from anthropogenic influence.The third component PC3 however was correlated with pH and TOC accounting for 10 % of the total variance.

Table 4.6: Eigen values of principal components

Component Number	Eigenvalue	Percent of Variance	Cumulative Percentage
1	**4.9471**	**49.471**	**49.471**
2	**3.34787**	**33.479**	**82.950**
3	**1.01053**	**10.105**	**93.055**
4	0.615622	6.156	99.211
5	0.078009	0.780	99.991
6	0.000877009	0.009	100.000
7	2.59258E-17	0.000	100.000
8	0.0	0.000	100.000

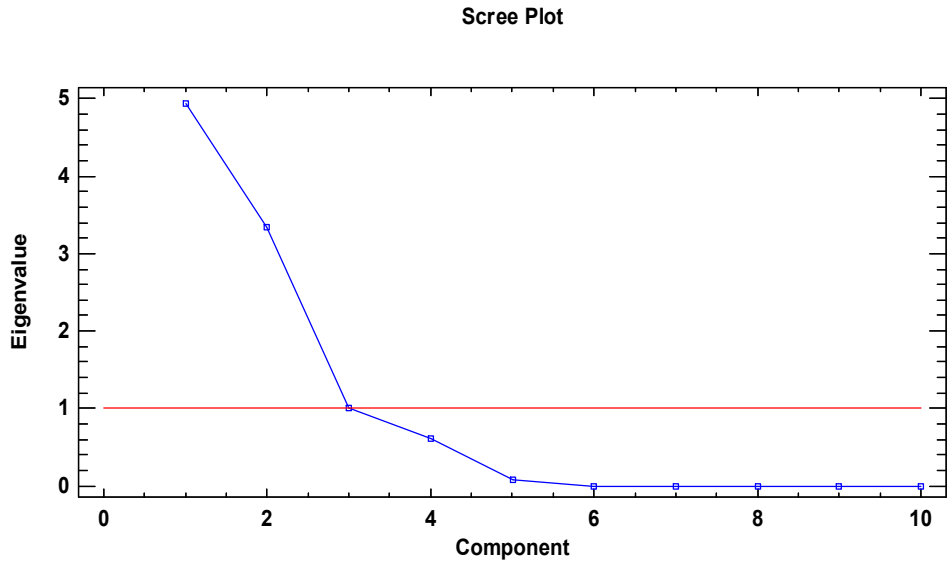


Figure 4.11: Plot of loadings of principal components

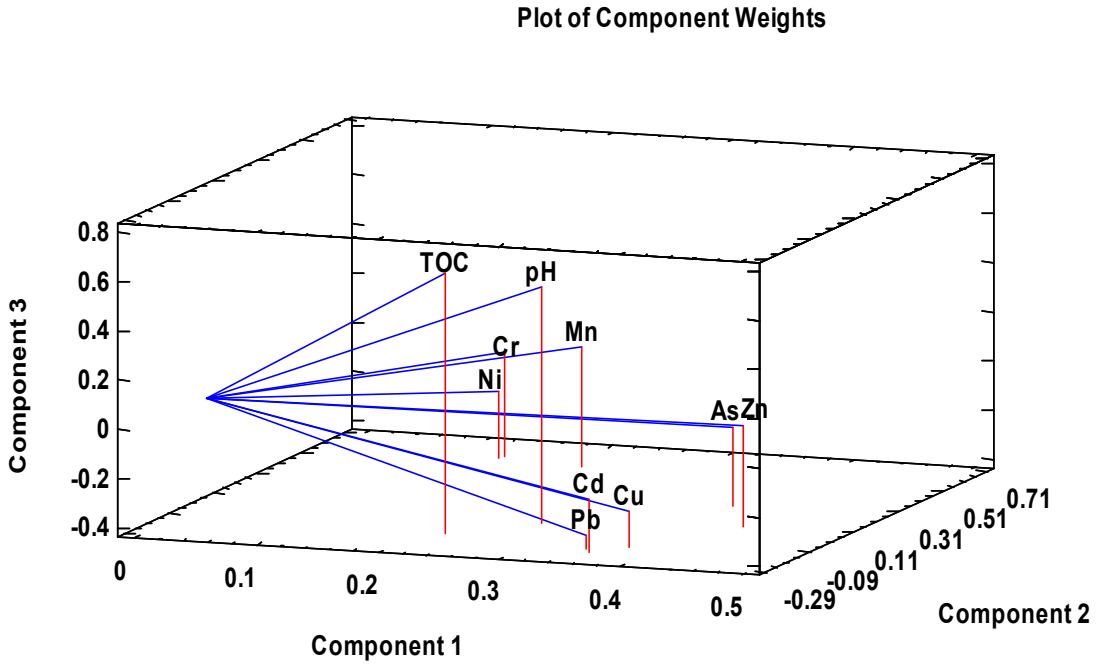


Figure 4.12: Principal component analysis of Soil Parameters

4.5RISK ASSESSMENT OF PTEsIN SOILS

4.5.1 Pollution indices (PI) of soils

It was essential to estimate the pollution degree of PTEs in the different soils using the pollution index method. Pollution index gives the pollution status of the soil with respect to individual PTE(Hu *et al.,* 2013). The pollution indices of the various PTEs investigated in this study are shown in Table 4.7,it was observed from the results obtained that there was a moderate to extreme level of PTE pollution in the study areas.According to the values obtained, Cd and Pb contributed to the high pollution level of Katangua soil with pollution index of 10.3 and 14.2 respectively. Also, Cr and Ni were observed to cause the very extreme pollution level in Orile and Owode soils as indicated by their pollution indices of 18.7 and 10.5 for Cr and 8.15 and 2.1 for Ni respectively , an indication of very extreme pollution degree at both soil sites. Also, Cdwas observed to be the PTE contributing the strong pollution level in FSS soil with pollution index of 3.4, the PTEs are most likely from used electronic gadgets, batteries, plastics and metal scraps dumped on these sites.Futhermore, the maximum pollution index of Cu (PI = 6.6) was found in Katangua soil while that of Mn (PI =2.0) was found in Orile soil. However, the other PTEs (i.e. As and Zn) had relatively very low pollution indices ($PI \leq 1$) for all the soils.Only MFM, Ibafo and Control soils had PI values less than one for all the PTEs studied, suggesting the very low level of pollution at the soil sites, an indication of no risk associated with the soils.

Therefore, theresults obtained for the pollution indices of the soils investigatedgenerally suggest the existence of some health risks at the sampling sites (especially KATANG, OWD, ORL, FSS soils) particularly for children through their hand to mouth activities and people who work at or near the dumpsites. Based on these findings, there was a need for further evaluation of the potential risk posed by the PTEs in these soils to humansusingthe oral bioaccessibility protocols.

Table 4.7: Pollution indices of PTEs in soils (mg/kg)

	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	0.03	0.10	0.19	0.01	0.09	0.06	0.08	0.05
ORL	0.52	0.25	18.69	0.25	2.03	8.15	0.37	0.74
KATANG	0.62	10.25	1.48	6.59	0.63	1.08	14.18	1.31
OWD	0.59	1.10	10.54	1.77	1.56	2.06	0.52	0.70
IBF	0.03	0.10	0.23	0.02	0.20	0.08	0.13	0.11
FSS	0.13	3.35	0.92	0.23	0.42	0.65	1.00	0.72
CONT	0.07	0.00	0.19	0.01	0.15	0.05	0.04	0.02

Table 4.8: Pollution indices of PTEs in soils and their classification

Pollution Index	**Degree of pollution**
0<PI<1	Unpolluted to moderately polluted
1<PI<2	Moderately polluted
2<PI<3	Moderately to strongly polluted
3<PI<4	Strongly polluted
4<PI<5	Strongly to extremely polluted
PI>5	Extremely polluted

4.5.2SBET Bioaccessible concentration of PTEs in soils

Another way of assessing the potential health risk of accidental ingestion of soil especially by children (through pica behaviour) is to investigate the level of PTEs in the human gastrointestinal tracts.This is done by the use of oral bioaccessibility study (Elom *et al.*, 2014).The health risk of the PTEs in soil samples in this studyto humans was therefore investigated using the simplified bioaccessibility extraction test (SBET) and the results obtained are as presented in Table 4.9.

The levels of PTEs found to be bioacessible to humans through oral ingestion of soil using SBET were found to be relatively low, the values obtained weremostly below the USEPArecommended oral ingestion rate of soil for some of the PTEs (Cr, Mn, Ni) studied with the exception of As, Pb, Cu and Zn especially from KATANG and OWD soils which had levelsbeyond USEPA recommmended ingestion rate, an indication that very high risk exist for humans in close proximity to KATANG and OWD soils, more importantly children who play with soil around the sites as well as workers or scavengers who usually frequent these dumpsites to pick some of the solid wastes materials to resell to people or companies.

The SBET results showed that the PTEs bioaccessible in the following range: 0.10 – 1.91 mg/kg for As, 0.10 – 7.88 mg/kg for Cd, 0.12 – 4.4 mg/kg for Cr, 4.50 – 2990 mg/kg for Cu, 32.4 – 193 mg/kg for Mn, 0.0 – 24.9 mg/kg for Ni, 3.30 – 874 mg/kg for Pb and 68.2 – 1960 mg/kg for Zn. It was observed that the soil with the highest bioaccessible concentration of PTEs was Katangua soil, followed by Orile and Owode soils which may be primarily due to their very high Total PTE concentrations.

Subsequently,the percent bioaccessibility of each PTE was calculated in order to evaluate the fraction of the PTEs bioaccessible from the total PTE concnetrations. As shown in table 4.10, percent bioaccessibility varied across the PTEs studied.The percent bioaccessible from total concentration

for the PTEs are as follows; 0.4- 24.5 % for As, 20- 94 % for Cd, 1.1-2.7 % for Cr, 4.3- 33.7 % for Cu, 2.8-24.8 % for Mn, 0.0- 17.7 % for Ni, 8.0- 41.3 % for Pb and 16.4- 93 % for Zn. This findingsshowed that Cd and Zn had the highest percent bioaccessibility which means they were the most solubilised in the human body. On the otherhand, Pb and Mn were observed to be moderately bioaccessible while As, Cr and Ni were the PTEs with the lowest percent bioaccessibility which means that Cr and Ni were not easily solubilised in thegastrointestinal environment of the body, hence could not be absorbed into the blood stream and get into other organs of the body to cause any health hazard but retained in the human body and ejected eventually. Juhasz *et al.,* (2007) following the same SBET protocol in their study observed the bioaccessibility rates of As-contaminated soils along railway corridor in Australia to be very low.

Furthermore, it wasobserved in Table 4.9that although the percent bioaccesibility of some PTEs (e.g. As and Pb) weremoderately low. However, their bioaccessible concentrations were found to be very high when compared to USEPA tolerable oral ingestion rate. This is an indication that when using oral bioaccessibility studies for evaluation of risk, the level of potentialhealth risk of the PTEs cannot bedetermined by the percent bioaccessibilitybut rather the bioaccessible concentration of the PTEs in the soils.

Table 4.9: Total and Bioaccessible concentration of PTEs in soils (mg/kg)

Soil	PTEs	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	**Totalconc.**	1.1	0.2	24.2	20.3	131	8.3	36.9	172
	Bioaccess. conc	0.1	0.2	0.3	4.5	32.4	0.0	12.4	101
	%Bioaccessible	13.1	94.8	1.4	22.0	24.6	0.0	33.5	58.8
ORL	**Total conc.**	16.7	0.5	2430	583	3050	1060	165	2790
	Bioaccess conc.	0.1	0.1	2.30	25.0	193	3.3	13.3	456
	%Bioaccessible	0.4	20.4	0.1	4.3	6.3	0.3	8.0	16.4
KATANG	**Total conc.**	19.7	20.5	193	15300	951	140	6380	4900
	Bioaccess conc.	1.9	7.9	4.40	2990	185	24.9	874	1960
	%Bioaccessible	9.7	38.3	2.3	19.5	19.5	17.7	13.7	40.0
OWD	**Total conc.**	18.9	2.2	1370	4120	2340	268	235	2630
	Bioaccess conc	0.2	1.3	1.60	328	65.0	2.7	57.0	1120
	%Bioaccessible	1.1	58.1	0.1	8.0	2.8	1.0	24.2	42.5
IBF	**Totalconc.**	1.0	0.2	29.7	58.1	296	9.8	59.9	428
	Bioaccess conc.	0.1	0.2	0.40	19.6	64.3	0.0	23.9	270
	%Bioaccessible	11.0	86.0	1.4	33.7	21.7	0.0	40.0	62.8
FSS	**Totalconc.**	4.3	6.7	120	538	636	84.9	448	2710
	Bioaccess conc.	1.1	4.2	3.20	95.0	130	2.8	185	1320
	%Bioaccessible	24.5	62.9	2.7	17.6	20.5	3.3	41.3	48.8
CONT	**Totalconc.**	2.3	0.0	25.0	14.5	232	6.8	17.1	71.7
	Bioaccess conc.	0.1	0.0	0.1	2.8	55.0	0.0	3.30	68.4
	%Bioaccessible	4.7	0.0	0.3	19.2	23.7	0.0	19.3	93.3
USEPA	**TDI_{oral}**	0. 3	N/A	15	160	N/A	12	3.6	600

% Bioaccessible = $\frac{C_{Bioaccessibility} \times 100}{C_{total\ conc}}$, Where $C_{Bioaccessibility}$ – PTE bioaccessible concentration (mg/kg) in soil samples obtained by the application of simplified bioaccessibility extraction test and $C_{total\ conc}$ -PTE total concentration (mg/kg) in soil samples obtained using microwave digestion method**; Total conc-** total concentration of PTEs in soil**, Bioaccess conc –** bioaccessible concentration of PTEs in soil, **SGV –** soil guideline values of soil**,** $\mathbf{TDI_{oral}}$ **–** USEPA tolerable daily ingestion rate of PTEs in soil

4.6TOTAL CONCENTRATION OF PTEs IN VEGETABLES

Due to rapid urbanisation and lack of adequate space for agricultural activities, vegetables are often planted around dumpsites. Being an important constituent of daily human diet, it is very necessary to ensure the quality of vegetables as they can bioaccumulate quantities of PTEs such that they cause serious health problems both to animals and human beings after consumption(Khan *et al.*, 2015).As compared to fruit and grain crops, metals get easily accumulated in edible portions of vegetables and mainly in leafy vegetables (Garg *et al.*, 2014). The mean concentrations of PTEs in leaves and roots of the vegetables investigated in this study are presented in Tables 4.10 – 4.15. It was observed from values obtained that PTE uptake levelsdiffered considerably from one vegetable type to another.According to Xian *et al.* (2015), different vegetables may accumulate different heavy metals and the absorption ability varies in different biological species due to their diverse physiological character.

PTE concentrations were observed to be significantly higher in the root of vegetables grown in MFM soil than their leaves. For other soils however, no trends was found, it was also noticeable from the results that for some PTEs, the level of uptake was higher in the leaves than the roots while higher in the roots for others than in the leaves. For instance, PTE concentrations were found to be higher in the leaves of waterleaf, spinach, celosia and okro than the roots whereas, for pumpkin leaf, the concentrations of PTEs were found to be higher in the roots than in the leaves in most cases. These findings suggested that translocation of PTEs in different parts of the vegetables did not follow a particular trend of uptake.

However, since this study is based on human health risk assessment, only the edible parts of the vegetables (i.e. leaves) were considered for risk assessment study.

Table 4.10: Results of total concentration of PTEs in waterleaf (mg/kg)

Soil sample	Waterleaf	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	Leaves	< 0.2	0.42 ± 0.02	< 0.5	16.7 ±1.6	213 ± 26	2.3 ± 0.2	3.1 ± 0.2	628±13
	Root	0.42 ± 0.1	1.31 ± 0.1	4.2 ± 0.3	26.7 ± 2	187 ± 13	4.0 ± 0.7	9.1 ± 0.6	55.8 ±29
ORL	Leaves	0.21 ± 0.02	< 0.04	1.3 ± 0.1	12.4± 1.1	87.7 ±11	2.6 ± 0.2	3.1 ± 0.2	98.3 ±14
	Root	0.85 ± 0.1	0.12 ±0.02	1.7 ± 0.2	24.9 ± 2	143 ± 12	5.3 ± 0.5	2.6 ± 0.2	169±13
KATANG	Leaves	0.49±0.03	3.51 ± 0.3	4.5 ± 0.4	238 ± 60	188 ± 23	5.0 ± 0.1	50.2± 9.5	591±57
	Root	0.46±0.03	4.62 ± 0.2	1.7 ± 0.1	170 ± 5	98.3± 4.7	2.7 ± 0.2	21.5± 0.7	348±12
OWD	Leaves	2.24 ± 0.2	1.61 ± 0.1	10.1± 0.6	132 ± 14	528 ± 78	12.6± 1.7	29.6± 3.7	918±79
	Root	1.5 ± 0.1	0.33± 0.1	8.2 ± 0.3	43.1± 1.3	360 ± 15	6.2 ± 0.2	18.2 ± 1.2	270 ± 8
IBF	Leaves	0.31 ± 0.02	0.31 ± 0.01	2.1 ± 0.3	38.1± 4.7	396 ± 39	2.0 ± 0.2	8.6 ± 0.5	383 ± 32
	Root	0.24±0.02	0.52 ±0.01	< 0.5	28.5± 1.5	172 ± 10	1.9 ± 0.1	3.4 ± 0.1	317±17
FSS	Leaves	0.21 ± 0.02	2.3 ± 0.2	1.6 ± 0.2	40.5± 2.0	136 ± 15	1.4 ± 0.1	5.5 ± 0.5	574±74
	Root	0.25±0.03	1.42 ±0.1	1.5 ± 0.3	14.6± 0.1	40.8± 0.7	1.3± 0.01	4.6 ± 0.2	172± 2.2
CONT	Leaves	< 0.2	0.3 ± 0.01	< 0.5	10.4± 0.2	41.7 ± 1	0.8± 0.01	4.1 ± 0.4	187±12
	Root	< 0.2	0.4±0.01	0.91 ±0.01	7.2 ± 0.1	86.4± 1.2	0.9± 0.01	1.7 ± 0.1	78.2±2

Results are expressed as means of triplicate determinations± standard deviation, "<" denotes below detection limits

Table 4.11: Results of total concentration of PTEs in pumpkin(mg/kg)

Soil sample	Pumpkin	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	**Leaves**	0.3±0.01	0.6 ± 0.1	1.6± 0.1	15.2± 1.7	128 ± 9	2.5± 0.1	6.4 ± 0.4	108 ± 9
	Root	0.7±0.1	3.7 ± 0.5	5.0 ± 0.7	28.3± 3.6	14.1± 2	6.78 ± 1	8.8 ± 1.1	131 ± 15
ORL	**Leaves**	0.6± 0.01	0.1 ±0.01	12.4± 1	32.5± 4.2	180 ± 16	12.9 ± 1	8.8 ± 0.8	149 ± 6
	Root	2.2±0.03	3.2±0.1	5.3 ± 0.3	26.7± 2.1	13.1 ± 1	7.4± 0.5	7.0 ± 0.4	489 ± 16
KATANG	**Leaves**	0.4±0.001	1.2 ± 0.1	7.5 ± 0.6	118 ± 9	215 ± 61	7.1± 0.8	30.8 ± 2	346 ± 15
	Root	0.5±0.1	4.8 ± 0.2	7.1 ± 0.7	6970±210	5.9 ± 0.6	8.8± 0.8	68.4± 1.8	635 ± 48
OWD	**Leaves**	1.2±0.1	0.3± 0.01	7.5 ± 0.7	56.9 ± 6	244 ± 29	9.8± 0.7	18.8± 1.6	303 ± 34
	Root	0.82±0.05	1.0±0.17	5.3 ± 0.1	248 ± 14	20.7± 0.2	13.0±1.1	18.2± 1	616 ± 30
IBF	**Leaves**	0.6±0.001	1.3± 0.01	4.9 ± 0.1	37.8 ± 0.6	191 ± 3	5.4± 0.1	16.7± 0.2	148 ± 8
	Root	< 0.2	< 0.04	2.4 ± 0.3	23 ± 3	7.9 ± 0.8	2.1± 0.3	8.22 ± 1	132 ± 15
FSS	**Leaves**	< 0.2	0.5± 0.01	3.2 ± 0.3	35.9 ± 4	101 ± 2	2.2± 0.2	11.7± 1.2	204 ± 36
	Root	< 0.2	0.3±0.01	0.9 ± 0.8	29.8± 5	1.6 ± 0.6	2.3± 0.2	3.6 ± 0. 2	123 ± 34
CONT	**Leaves**	0.4 ± 0.03	0.6±0.01	2.8 ± 0.2	27.8 ± 3	402 ± 32	3.8± 0.3	9.91 ± 1	147 ± 40
	Root	< 0.2	0.3±0.02	1.3 ± 0.4	177 ± 2	1.6 ± 0.1	3.84 ± 1	2.6 ± 0.1	59.1 ± 10

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limits

Table 4.12: Results of total concentration of PTEs in spinach (mg/kg)

Soil sample	Spinach	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	**Leaves**	< 0.2	1.1 ± 0.01	1.4± 0.01	17.4± 0.9	135 ± 18	3.3 ± 0.1	5.1 ± 0.2	201 ± 60
	Root	0.06±0.01	0.5 ± 0.01	0.5± 0.01	9.4 ± 0.1	0.8 ± 0.01	1.0±0.01	0.94±0.03	166 ± 2
ORL	**Leaves**	0.3 ± 0.01	0.2 ± 0.001	8.1 ± 0.3	33.3± 2.3	284 ± 23	9.9 ± 0.4	108 ± 8.1	365 ± 59
	Root	0.9 ± 0.1	0.1 ± 0.001	1.7 ± 0.2	25.0± 1.8	143 ± 12	5.3 ± 0.5	2.6 ± 0.2	169 ± 13
KATANG	**Leaves**	< 0.2	8.4 ± 1.2	0.6 ± 0.1	100 ± 4	241 ± 53	4.8 ± 0.5	9.7 ± 1.2	937 ±95
	Root	0.23±0.01	4..2 ± 0.2	3.1 ± 0.2	96.8± 5	2.5 ± 0.1	3.2 ± 0.1	18.7 ± 0.6	340 ± 16
OWD	**Leaves**	< 0.2	1.4 ± 0.1	1.2 ± 0.1	69.4± 6.9	156 ± 11	4.4 ± 0.6	22.4 ± 2.3	313 ± 31
	Root	0.52±0.02	0.84 ± 0.04	3.5 ± 0.2	105 ± 4	8.1 ± 0.4	3.6 ± 0.1	23.9 ± 0.8	220 ± 8
IBF	**Leaves**	< 0.2	0.6 ± 0.01	2.7 ± 0.3	30.2 ± 4	86.7±11.6	3.3 ± 0.4	1.9 ± 0.1	105 ± 62
	Root	0.32±0.03	0.46 ± 0.05	2.3 ± 0.2	20.3± 2.1	3.7 ± 0.4	1.8 ± 0.2	1.9 ± 0.3	88.9 ±18
FSS	**Leaves**	< 0.2	1.0 ± 0.01	0.9 ± 0.1	23.7± 2.3	86.9±11.5	4.9 ± 0.1	5.1 ± 0.2	291 ± 35
	Root	< 0.2	1.0 ± 0.1	3.1 ± 1.2	18.8± 0.8	1.09±0.07	1.4 ± 0.3	2.7 ± 0.1	132 ± 6
CONT	**Leaves**	< 0.2	0.5 ± 0.01	< 0.5	17.3± 1.2	360 ± 51	1.5 ± 0.1	2.4 ± 0.1	103 ± 61
	Root	0.22±0.01	0.40 ± 0.04	1.2 ± 0.1	13.3± 0.4	2.6 ± 0.1	1.3±0.01	1.8 ± 0.1	51.8± 5

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limits

Table 4.13: Results of total concentration of PTEs in *celosia* (mg/kg)

Soil sample	Celosia	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	**Leaves**	< 0.2	1.3 ± 0.1	0.8 ± 0.1	18.2± 1.6	82.8 ± 10	2.3 ± 0.2	3.4 ± 0.5	157 ± 40
	Root	0.33±0.01	1.20±0.03	3.4±0.1	17.6± 0.3	6.4 ± 0.1	2.37±0.05	5.8 ± 0.2	117 ± 8
ORL	**Leaves**	0.5 ± 0.04	0.8 ± 0.1	11.3 ± 0.8	48.8 ± 4.2	400 ± 59	7.0 ± 0.9	19.5±2.2	382 ± 32
	Root	0.22±0.01	0.51±0.02	8.6 ± 0.5	22.7 ± 1.1	20.5± 1.2	5.7 ± 0.3	4.0 ± 0.2	172 ± 8
KATANG	**Leaves**	< 0.2	12.5 ± 0.7	2.6 ± 0.1	96.3±13.3	1680±227	6.3 ± 0.7	19.7±1.5	828±70
	Root	0.22±0.01	0.6 ± 0.01	2.0 ± 0.1	12.6 ± 0.3	4.1 ± 0.1	1.8 ± 0.1	2.9 ± 0.1	402 ± 13
FSS	**Leaves**	< 0.2	3.4 ± 0.2	1.4 ± 0.1	27.4 ± 2.1	38.6 ± 3.3	2.7 ± 0.2	8.2 ± 0.5	253 ± 32
	Root	0.17±0.03	0.9 ± 0.1	1.8 ± 0.2	20.9 ± 1.6	2.1 ± 0.2	2.4 ± 0.2	5.4 ± 0.5	155 ± 13
CONT	**Leaves**	< 0.2	1.9 ± 0.2	2.4 ± 0.2	21.9 ± 2.1	1120±112	3.7 ± 0.3	5.5 ± 0.4	151 ± 52
	Root	0.22±0.03	0.4 ± 0.01	1.21±0.07	13.3 ± 0.4	2.6 ± 0.1	1.28±0.04	1.8 ± 0.1	122 ± 5

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limits

Table 4.14: Results of total concentration of PTEs in okro (mg/kg)

Soil sample	Okro	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	**Leaves**	< 0.2	1.5± 0.1	1.5 ± 0.1	23.3± 1.7	192 ± 15	2.8 ± 0.3	3.0 ± 0.2	116 ± 87
	Root	< 0.2	0.62± 0.06	1.4 ± 0.1	12.7± 1.1	2.2 ± 0.2	2.5 ± 0.2	2.8 ± 0.2	51.2 ± 14
ORL	**Leaves**	< 0.2	0.9 ± 0.1	7.4 ± 0.6	33.5± 3.4	312 ± 41	3.9 ± 0.3	4.9 ± 0.5	270 ± 34
	Root	< 0.2	0.13± 0.01	0.94 ± 0.01	10.8± 0.1	3.11±0.04	1.88±0.02	1.78±0.03	74.8± 1.4
KATANG	**Leaves**	< 0.2	6.9± 0.3	5.2 ± 0.2	277 ± 11	221 ± 19	4.61 ± 0.3	40.7 ± 3.3	728 ± 56
	Root	< 0.2	3.84± 0.42	1.6 ± 0.2	69.9 ± 7	1.6 ± 0.2	2.73 ± 0.2	16.5 ± 1.7	178 ± 18
FSS	**Leaves**	< 0.2	2.61 ± 0.2	2.7 ± 0.2	45.8 ± 3	124 ± 8	2.52 ± 0.2	14.7 ± 1.1	296 ± 46
	Root	0.25±0.01	1.7± 0.1	5.1 ± 0.2	34.4± 1.5	4.3 ± 0.2	3.51 ± 0.1	15.6 ± 0.8	148 ± 7
CONT	**Leaves**	< 0.2	2.4± 0.2	1.5 ± 0.1	26.1 ± 3	727 ± 90	2.6 ± 0.2	3.61 ± 0.4	140±17
	Root	< 0.2	0.38± 0.02	0.77±0.14	6.8 ± 1	15.2 ± 0.4	1.3 ± 0.1	1.4 ± 0.2	48.2 ± 6

Results are expressed as the mean of triplicate determinations ± Standard deviation, "<" denotes below detection limits

Table 4.15: Results of total concentration of PTEs in Amaranthus (mg/kg)

Soil sample	Amaranthus	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	**Leaves**	0.4 ± 0.02	1.5 ± 0.1	3.4 ± 0.2	39.1 ± 2	418 ± 21	3.1 ± 0.2	11.7 ± 0.5	177 ± 31
	Root	< 0.2	0.53 ± 0.02	< 0.5	5.82 ± 0.2	0.44 ± 0.05	0.71 ± 0.04	0.44 ± 0.03	32.1 ± 7
FSS	**Leaves**	< 0.2	0.8 ± 0.1	1.8 ± 0.1	31.6 ± 0.8	66 ± 10	1.5 ± 0.1	6.3 ± 0.2	221 ± 17
	Root	< 0.2	0.64 ± 0.02	0.85 ± 0.04	15.2 ± 0.6	1.22 ± 0.06	1.26 ± 0.05	2.2 ± 0.1	84.8±2.7
CONT	**Leaves**	< 0.2	0.8 ± 0.1	1.8 ± 0.2	26.6 ± 3.0	358 ± 17	3.1 ± 0.3	5.6 ± 0.5	114 ± 82
	Root	0.4 ± 0.0	0.92 ± 0.02	2.35 ± 0.06	19.0 ± 0.5	4.25 ± 0.14	2.06 ± 0.05	5.8 ± 0.1	68.2 ± 4

Results are expressed as means of triplicate determinations ± standard deviation,"<" denotes below detection limit

The mean concentrations of PTEs in edible parts of the vegetables used in this study are presented in Figures 4.13 – 4.20. The values obtained ranged between<0.2 – 2.2 mg/kg for As, < 0.04 – 12.5 mg/kg for Cd, < 0.5 – 12.4 mg/kg for Cr, 10.4 – 277 mg/kg for Cu, 38.6 – 1680 mg/kg for Mn, 0.8 – 13.0 mg/kg for Ni, 2.0 – 108 mg/kg for Pb and 98.0 – 1040 mg/kg for Zn.

From the results in Figure 4.18, it was observed that the highest concentration of As (2.2 mg/ kg) was found in waterleaf grown in Owode soil, while pumpkin leaf similarly grown in Owode soil rank second highest with concentration of 1.2 mg/kg. These levels were found to be much higher than WHO acceptable safe limit for As in food, whereas the other vegetables grown on the seven soils (spinach, okro and celosia) had values less than detection limit (< 0.2 mg/kg). Arsenic is a very toxic element with no physiological function in the body considered to be the first in the list of priority pollutants that are carcinogenic in nature (ASTDR, 2007).

Cd and several Cd compounds are also known human carcinogens and can induce many types of cancer.Long period of exposure may lead to kidney failure and permanent lung damage (Kalaghor *et al*., 2014) The highest concentration of Cd was found in vegetables grown in Katangua soil, with*celosia*having a concentration of 12.5 mg/kg, spinach had a concentration of 8.4 mg/kg whileokro leaf had a concentration of 6.9 mg/kg. These levels were observed to be 10 to 15 times higher than WHO acceptable limit (Table 4.16). However, the lowest Cd concentration was found in waterleaf and pumpkin leaf.

For Cr uptake, it was observed that vegetables grown in Orile soils had the highest concentrations. Pumpkin leafhad the highest concentration (12.4 mg/kg), followed by celosia with 11.3 mg/kg, spinach with 8.1 mg/kg and okro with 7.4 mg/kg respectively. The levels of Cr in these vegetables were much higher than WHO acceptable limits (Table 4.16). Also, when compared with previous studies(Table 4.16) the levels found in this study were much higher most of results report but very

few exception , for instance, the level of spinach in this study was observed to be lower than the results obtained in a similar study by Gupta *et al*., (2012). The lowest concentration of Cr in the vegetables examined in this study was found in waterleaf.

Futhermore,vegetables grown in Orile soils had the highest concentration of Ni, with pumpkin leaf recording a concentration of 13.0 mg/kg, spinach with concentration of 9.92 mg/kg, celosia with concentration of 7.2 mg/kg, while the lowest concentrations were found in vegetables grown on Control and MFM soil. The results obtained for the level of Ni in the vegetables of this study were in agreement with previousstudies (Table 4.16).

Copper is an essential micronutrient which functions as a biocatalyst required for body pigmentation in addition to iron, maintain a healthy central nervous system(Doherty *et al.,* 2012). The highest concentration of Cu was found in vegetables grown in Katangua soil with Okro having a concentration of 277 mg/kg, waterleaf with concentration of 238 mg/kg, pumpkin with concentration of 118 mg/kg while the lowest Cu concentration was found in vegetable grown in Control and MFM soils.

Similarly, manganese and zinc are known micronutrients required by the body, however, their concentrations were found to be very high in all the vegetables studied. When compared to previous published works (Table 4.16), the levels found were much higher.

The highest concentration of Pb was found in spinach grown in Orile soil, while the lowest concentrations were found in MFM and control soil. The concentrations in the vegetables were higher than WHO acceptable limits. When compared with other similar publishedworks, the levels found in this study were in agreement. Furthermore, it was observed that uptake of PTEs by vegetables was greater from soils with elevated levels than soils with lower concentrations in most cases.

Generally from the findings in this study,the observed difference in the degree of accumulation of PTEs by individual vegetables species and even the same type of vegetables grown on different soils may be attributed to their different uptake efficiencies for PTE from soil solution, bioavailability of metals in the soils, physicochemical properties of the soil and plant response (El Hamiani *et al*., 2015). The values of total PTEs obtained from the results of this study when compared with previous studies on uptake of PTEs in vegetables from contaminated sites (as shown in Table 4.16)were found to be much higher than previousstudies reported especially for Mn, Pb and Zn. However,the results were observed to be very consistent with the reports of Kalaghor *et al*., (2014), Gupta *et al*., (2012), Tsafe *et al*., (2012) and Olayinka *et al*., (2011)in terms for trends in PTE uptake.

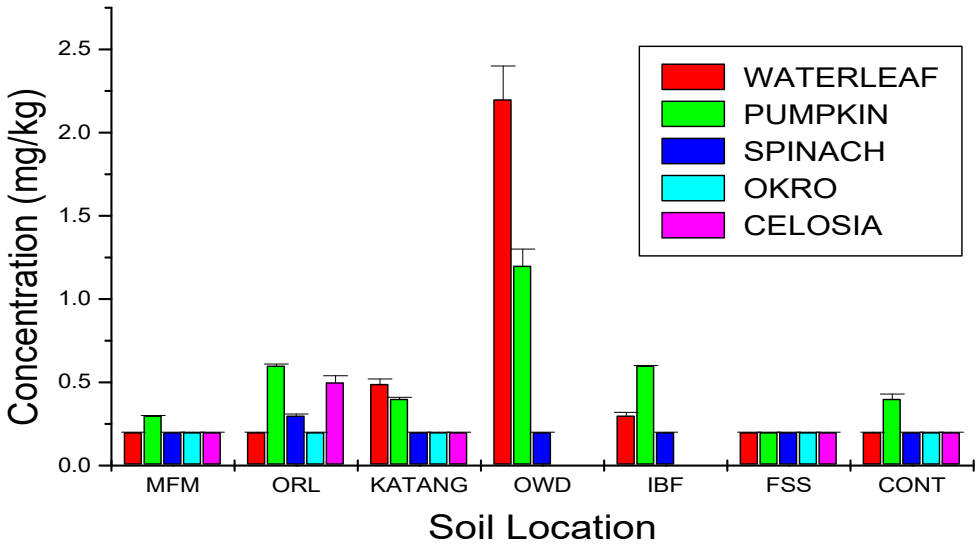


Figure 4.13: Mean concentration of arsenic in edible part of vegetables

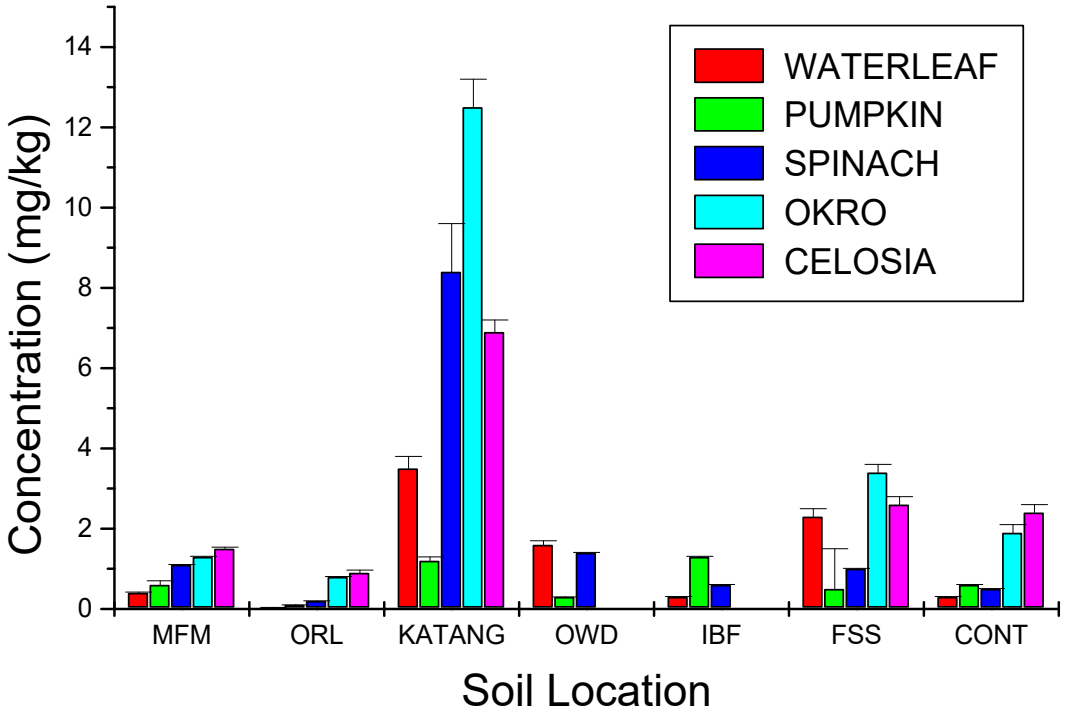


Figure 4.14: Mean concentration of cadmium in edible part of vegetables

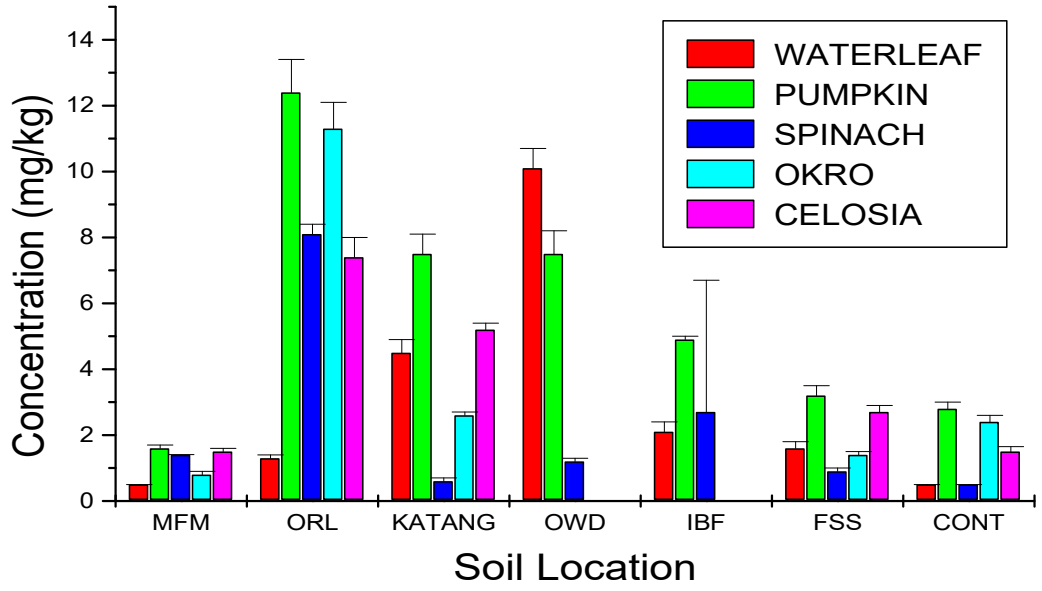


Figure 4.15: Mean concentration of chromium in edible part of vegetables

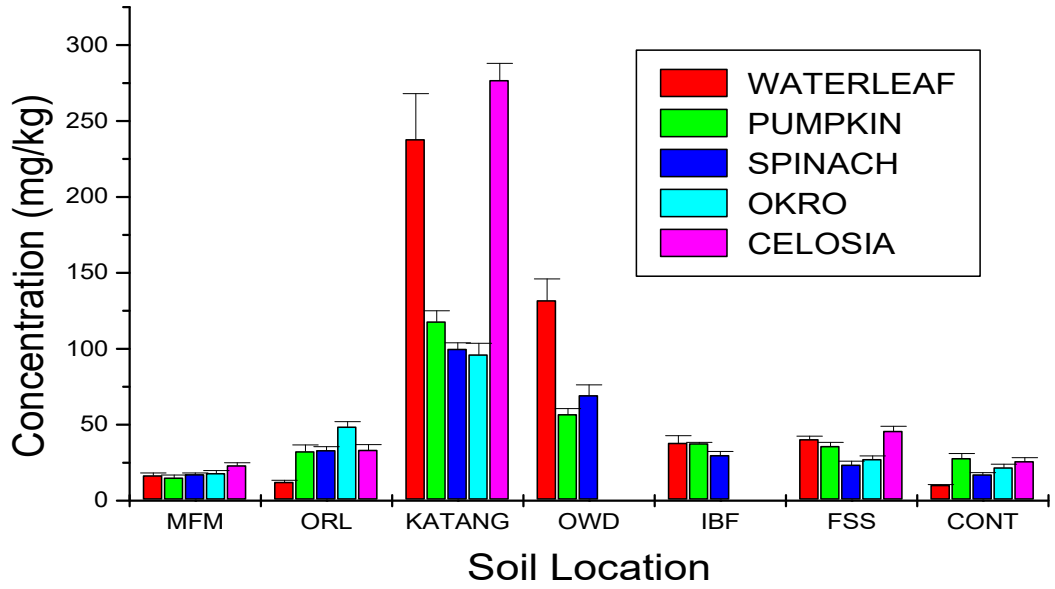


Figure 4.16: Mean concentration of copper in edible part of vegetables

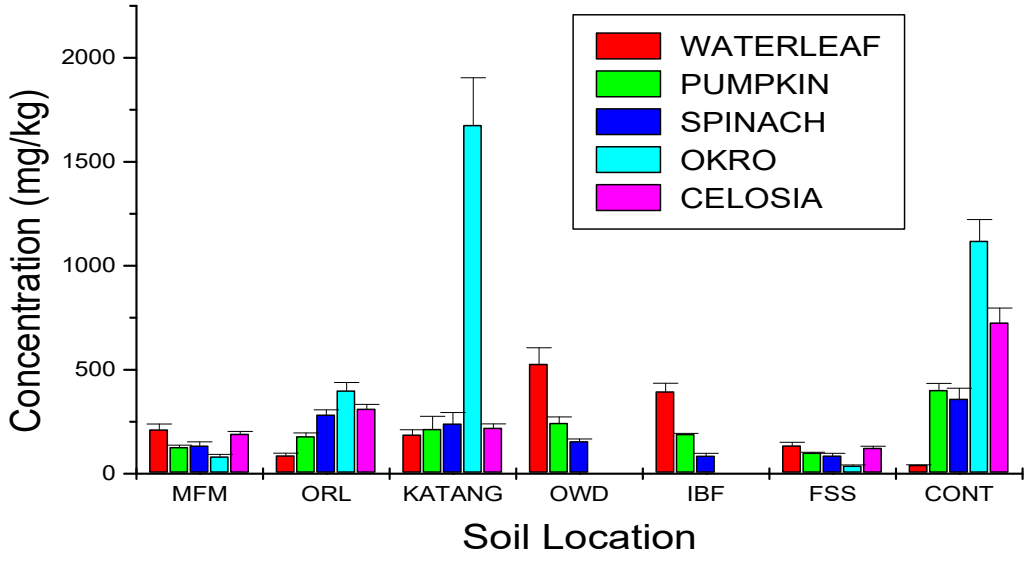


Figure 4.17: Mean concentration of manganese in edible part of vegetables

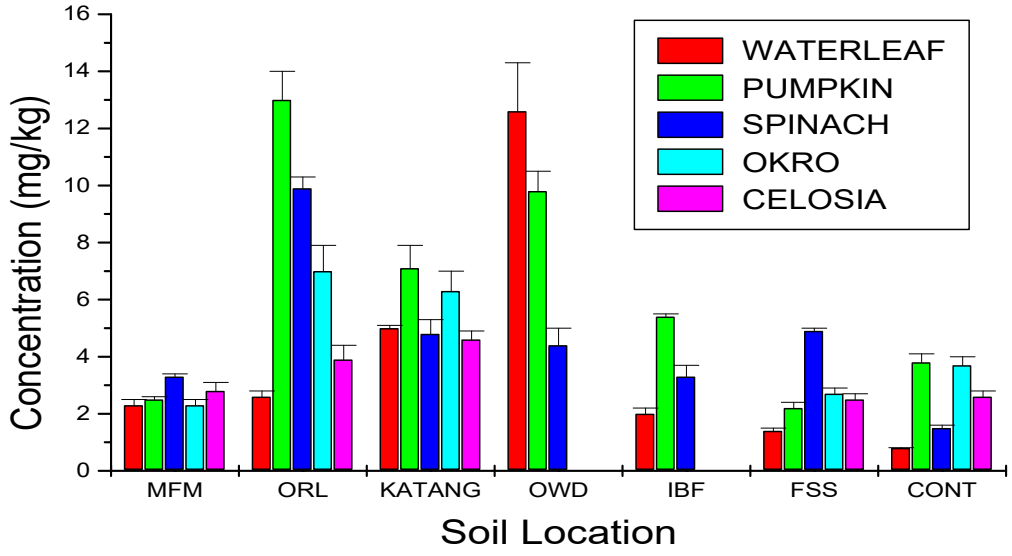


Figure 4.18:Mean concentration of nickel in edible part of vegetables

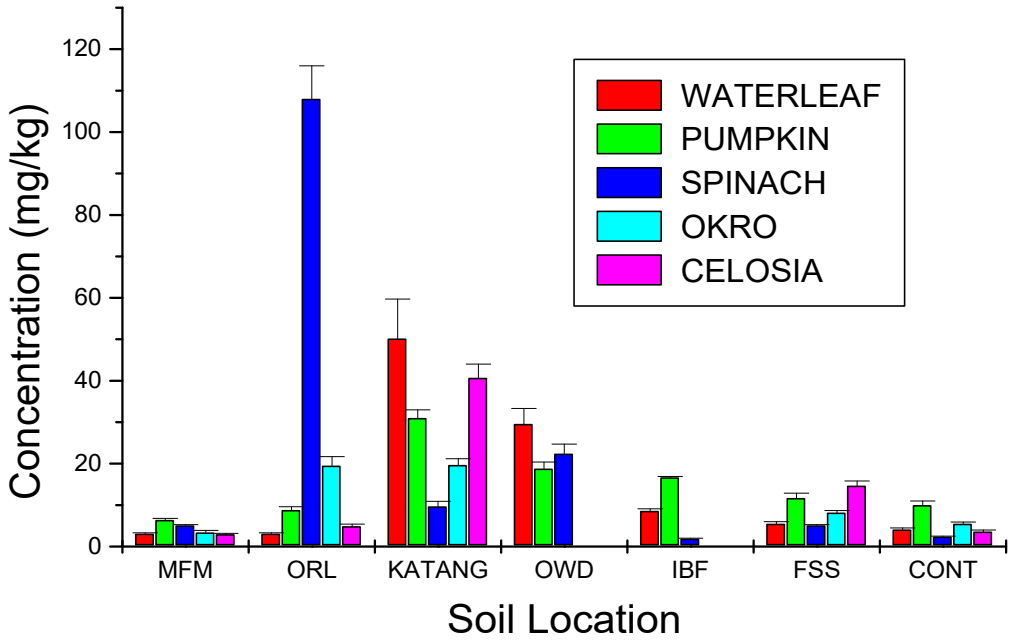


Figure 4.19: Mean concentration of lead in edible part of vegetables

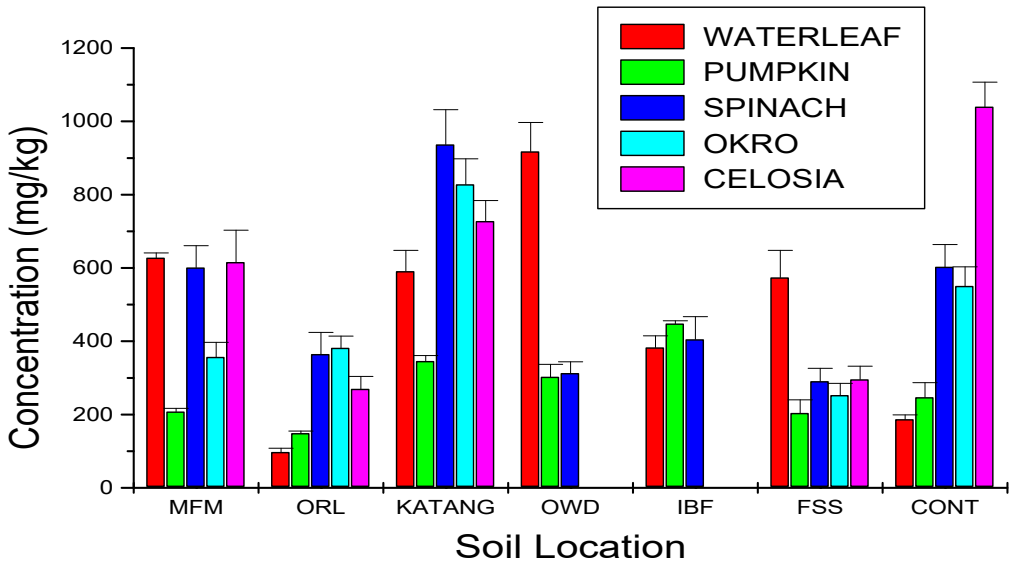


Figure 4.20:Mean concentration of zinc in edible part of vegetables

Table 4.16.Comparison of studies ontotal concentration of PTEs in some vegetables grown on contaminated sites (mg/kg)

Vegetables	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	References
Pumpkin	n/a	3.25	1.0	n/a	n/a	n/a	2.4	56.9	Orubite *et al*.,(2015)
Pumpkin	n/a	0.11	n/a	n/a	7.74	0.03	0.06	0.32	Chukwuemeka *et al*.,(2015)
Pumpkin	n/a	1.25	1.75	11	42.5	16.8	6.75	79.8	Kalaghor *et al*.,(2014)
Waterleaf	n/a	1.5	5.75	7.8	62.8	19.0	8.0	186.8	Kalaghor *et al*.,(2014)
Spinach	0.05	13	95.8	32.1	n/a	68.7	47.7	148	Gupta *et al*.,(2012)
Spinach	n/a	n/a	n/a	16.3	1.1	n/a	4.4	26.3	Vinod *et al.,(*2012)
Spinach	n/a	n/a	3.08	0.64	44.6	7.55	5.93	26.3	Tsafe *et al*.,(2012)
Spinach	0.36	0.21	n/a	0.28	n/a	0.02	0.07	0.03	Opaluwa *et al*.,(2012)
Celosia	n/a	4.1	79	n/a	n/a	n/a	26.6	103	Olayinka *et al.,* (2011)
Greenleaf	n/a	5.3	96	n/a	n/a	n/a	49.5	112	Olayinka *et al.,* (2011)
Spinach	n/a	2.1	n/a	11.0	n/a	7.0	18.0	n/a	Khan *et al*.,(2010)
Permissible Limit	n/a	0.2	2.3	40	500	n/a	5	60	FAO/WHO (2007)

n/a = not available;

4.7BIOACCUMULATION(TRANSFER) FACTOR

Soil to plant transfer of PTEs is a major pathway of human exposure to soil contamination. It provides a useful indication of relative metal availability from soils to plants(Chi *et al*., 2004).The transfer factor determined in this study was based on the total PTE concentration in the edible part of the vegetables (leaves) without taking into consideration the other part (root) of the vegetables studied.

The results of the transfer factor values of the PTEs for the vegetablesunder investigation are presented in Table 4.17- 4.18. The results obtained indicate that the transfer values of PTEs (As, Cd, Cu, Cr, Mn, Ni, Pb and Zn) for various vegetables varied greatly between plant species and soil locations and was found be to maximum for Cd, Cu, Mn and Zn. As and Cr were found to be have the lowest transfer factor but amongst the vegetables it was observed that both spinach and pumpkin leaves showed a higher transfer factor from soil to plants than other vegetables. Variations in transfer factor among different vegetables may be as a result of differences in the concentration of elements in the soil on which the vegetables were grown and dissimilarities in element uptake by different vegetables (Cui *et al.,* 2004).

The transfer factor of the PTEs summarised in Table 4.17 – 4.18, ranged from 0.01 to 3.1 for Cd, 0.001- 0.070 for Cr, 0.02-0.76 for Cu, 0.03-2.7 for Mn, 0.002- 0.36 for Ni, 0.002- 0.65 for Pb and 0.03- 2.89 for Zn. According to Sajjad *et al*. (2010), if the transfer coefficient of a metal is greater than 0.5, the plant has a greater chance of the metal contamination by anthropogenic activities. Arsenic, Cr, Cu, Ni and Pb had transfer factors less than 0.5, suggesting that the PTEs had very low uptake by the vegetables while Cd, Mn and Zn had transfer factors much greater than 0.5, an indication that great risk is associated with their uptake in the vegetables.The high transfer factors of Zn and Cd may be because these elements have been found to be very mobile and easily removed from soils (Sahuquillo *et al.*,

2003; Oyeyiola *et al.*, 2014; Ferri *et al.,* 2015) and are therefore easily taken up. However, it was noticeablefrom the results that transfer factors of some PTEs (e.g. Mn and Zn in Katangua, Orile and Owode soils; Pb in Katangua soil;) decreased when the vegetables were grown in soils with a higher contamination, but increased when grown on soils with less contamination as observed in the high transfer factor for vegetables grown particularly in MFM and Control soil.This confirms previous studies that indicated plant uptake is not governed solely by soil PTE levels (Ferri *et al.*, 2015).The general trend of transfer factor followed the order Cd > Zn > Mn > Cu > Pb > Cr > Ni>As

Table 4.17:Bioaccumulation (Transfer) factor of PTEs from soils toVegetables

	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
			Waterleaf					
MFM	0.007	0.514	0.007	0.394	0.69	0.176	0.078	2.906
ORL	0.008	0.011	0.001	0.021	0.027	0.002	0.019	0.035
KATANG	0.016	0.164	0.019	0.015	0.167	0.034	0.008	0.119
OWD	0.072	0.539	0.007	0.032	0.210	0.046	0.125	0.343
IBF	0.023	0.397	0.030	0.476	0.836	0.135	0.139	0.813
FSS	0.012	0.318	0.010	0.072	0.167	0.016	0.012	0.209
CONT	0.000	0.485	0.002	0.285	0.102	0.066	0.211	1.630
			Pumpkin leaf					
MFM	0.230	3.190	0.025	0.358	0.830	0.189	0.164	1.163
ORL	0.021	0.066	0.005	0.054	0.056	0.012	0.053	0.053
KATANG	0.012	0.056	0.032	0.008	0.191	0.049	0.005	0.070
OWD	0.038	0.102	0.005	0.014	0.097	0.036	0.079	0.113
IBF	0.049	1.497	0.070	0.472	0.404	0.360	0.270	0.949
FSS	0.008	0.064	0.020	0.064	0.124	0.025	0.026	0.074
CONT	0.032	2.960	0.044	0.760	0.983	0.319	0.529	1.670
			Spinach					
MFM	0.009	1.408	0.022	0.411	0.437	0.242	0.130	2.777
ORL	0.008	0.188	0.003	0.055	0.088	0.009	0.650	0.129
KATANG	0.000	0.396	0.003	0.007	0.214	0.033	0.002	0.190
OWD	0.000	0.479	0.001	0.017	0.062	0.016	0.094	0.117
IBF	0.000	0.712	0.039	0.378	0.183	0.221	0.031	0.858
FSS	0.000	0.138	0.006	0.042	0.107	0.055	0.011	0.106
CONT	0.002	0.699	0.008	0.473	0.880	0.127	0.125	2.244

Table 4.18: Bioaccumulation (Transfer) factor of PTEs from soils to Vegetables

	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
			Okro leaf					
MFM	0.003	1.566	0.012	0.429	0.270	0.170	0.087	1.654
ORL	0.019	0.702	0.005	0.081	0.124	0.007	0.117	0.135
KATANG	0.007	0.589	0.011	0.006	1.486	0.043	0.003	0.167
FSS	0.000	0.449	0.009	0.049	0.047	0.030	0.018	0.092
CONT	0.018	2.850	0.037	0.599	2.727	0.309	0.286	2.795
			Celosia					
MFM	0.000	1.893	0.023	0.551	0.621	0.213	0.077	2.851
ORL	0.000	0.816	0.003	0.055	0.096	0.004	0.029	0.095
KATANG	0.000	0.325	0.022	0.018	0.196	0.032	0.006	0.147
FSS	0.006	0.350	0.017	0.082	0.152	0.028	0.033	0.107
CONT	0.003	0.601	0.023	0.714	1.777	0.220	0.185	3.054

4.8 RISK ASSESSMENT OF PTEs FROM CONSUMPTION OF VEGETABLES

4.8.1 Estimation of daily intake rate

The plant bioaccumulation factordoes not represent the risk associated with consumption of PTE in vegetables; the toxicity to human actually depends upon how much of the contaminated vegetables are consumed (Anita *et al.,* 2010). Based on the concentrations of PTEmeasured in the edible parts of vegetables, the estimated daily intake rate values were calculated. The risk to human health arising from consumption of vegetables grown on contaminated soils were expressed using the daily intake rate of PTEs for each vegetable type as presented in Tables4.19 – 4.23. It was observed from the results that daily intake rate of PTE such as Cd, Pb and Zn in both adults and children were very high when compared with the Provisional tolerable daily intake (PTDI)limits established by USEPA while daily intake rate for As, Cr, Cu, and Ni were below the PTDI values.

It was also observed that values obtained for daily intake rate were much greater for children than for adults as shown in Tables 4.22 -4.26, suggesting that the former may be more vulnerable to PTE exposure. However, this finding was based on the assumption that children have a greater daily intake of vegetables relative to their body weight than adults; young children may therefore be highly exposed to environmental hazards than adults, also children exhibit a higher absorption of PTEs than adults due to their less developed digestive systems. The estimated daily intake rate of Cd, Pb and Zn for both adult and children through the consumption of vegetables exceeded the provision tolerable daily intake (PTDI) values recommended by USEPA but the intake of As, Cr, Mn and Ni was less than the PTDI values.

However, it was observed that the highest daily intake of potentially toxic elements such as Pb, Ni, Cu and Cr were from vegetables grown in KATANG Soil, which was the soil with the

highest pollution level of all the soils under study. Only Zn was observed to have very high daily intake rate for all the vegetables in the different soil samples.

Thus, the findings of this study regarding daily intake rate suggest potential health risk for both adults and children with respect to high amount of daily intake of Cd, Cu, Pb and Zn through ingestion of vegetable crops grown on contaminated soils.

Table 4.19: Estimated daily intake rate of PTEs from waterleaf

Soil		As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	Adults	0.001	0.002	0.002	0.082	1.050	0.012	0.015	3.090
	Children	0.002	0.003	0.003	0.118	1.510	0.017	0.022	4.460
ORL	Adults	0.001	0.000	0.006	0.061	0.437	0.013	0.015	0.480
	Children	0.002	0.000	0.009	0.088	0.629	0.019	0.022	0.698
KATANG	Adults	0.002	0.017	0.022	1.170	0.926	0.024	0.247	2.910
	Children	0.003	0.025	0.032	1.690	1.330	0.035	0.356	4.190
OWD	Adults	0.010	0.008	0.050	0.655	2.602	0.062	0.150	4.520
	Children	0.002	0.011	0.072	0.943	3.750	0.089	0.210	6.510
IBF	Adults	0.001	0.002	0.010	0.188	1.950	0.010	0.043	1.890
	Children	0.002	0.002	0.015	0.270	2.810	0.014	0.061	2.720
FSS	Adults	0.001	0.012	0.008	0.199	0.669	0.007	0.027	2.830
	Children	0.001	0.017	0.011	0.287	0.963	0.010	0.039	4.070
CONT	Adults	0.001	0.002	0.001	0.051	0.207	0.004	0.020	0.924
	Children	0.001	0.002	0.001	0.074	0.297	0.006	0.029	1.330
USEPA Limit	PTDI	0.002	0.001	0.120	0.500	12	0.210	0.004	1.000

"PTDI" denotes provisional tolerable daily intake rate; USEPA denotes United State environmental protection agency

Table 4.20: Estimated daily intake rate of PTEs from spinach

Soil		As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	Adults	0.001	0.006	0.007	0.086	0.665	0.016	0.025	2.96
	Children	0.001	0.008	0.010	0.123	0.958	0.023	0.036	4.261
ORL	Adults	0.001	0.001	0.040	0.164	1.401	0.049	0.537	1.801
	Children	0.002	0.002	0.058	0.237	2.017	0.070	0.773	2.593
KATANG	Adults	0.001	0.041	0.003	0.494	1.188	0.024	0.048	4.619
	Children	0.002	0.059	0.004	0.711	1.710	0.034	0.068	6.649
OWD	Adults	0.001	0.007	0.006	0.34	0.770	0.022	0.111	1.544
	Children	0.000	0.01	0.009	0.489	1.108	0.031	0.159	2.223
IBF	Adults	0.001	0.003	0.013	0.149	0.427	0.016	0.009	1.995
	Children	0.001	0.004	0.019	0.215	0.615	0.023	0.014	2.872
FSS	Adults	0.001	0.005	0.005	0.117	0.428	0.024	0.025	1.436
	Children	0.001	0.007	0.007	0.168	0.617	0.035	0.036	2.067
CONT	Adults	0.000	0.002	0.003	0.085	1.776	0.007	0.012	2.973
	Children	0.000	0.003	0.004	0.122	2.556	0.011	0.017	4.279
USEPA Limit	PTDI	0.002	0.001	0.120	0.500	12.00	0.210	0.004	1.000

"PTDI" denotes provisional tolerable daily intake rate; USEPA denotes United State environmental protection agency

Table 4.21: Estimated daily intake rate of PTEs from pumpkin leaf

Soil		As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	Adults	0.002	0.003	0.008	0.075	0.629	0.012	0.032	1.026
	Children	0.002	0.004	0.011	0.107	0.905	0.018	0.046	1.477
ORL	Adults	0.001	0.000	0.061	0.160	0.888	0.064	0.043	0.736
	Children	0.002	0.001	0.088	0.230	1.278	0.092	0.062	1.059
KATANG	Adults	0.002	0.006	0.037	0.583	1.062	0.035	0.153	1.707
	Children	0.003	0.008	0.053	0.839	1.529	0.050	0.220	2.457
OWD	Adults	0.001	0.001	0.037	0.281	1.204	0.048	0.093	1.494
	Children	0.002	0.002	0.053	0.405	1.733	0.069	0.134	2.151
IBF	Adults	0.001	0.006	0.024	0.186	0.942	0.027	0.083	2.207
	Children	0.002	0.009	0.035	0.268	1.356	0.038	0.119	3.177
FSS	Adults	0.001	0.002	0.016	0.176	0.499	0.011	0.058	1.005
	Children	0.001	0.003	0.022	0.253	0.718	0.016	0.083	1.446
CONT	Adults	0.002	0.003	0.014	0.137	1.983	0.019	0.050	1.218
	Children	0.003	0.004	0.020	0.197	2.854	0.027	0.072	1.753
USEPA Limit	PTDI	0.002	0.001	0.120	0.500	12.00	0.210	0.004	1.000

"PTDI" denotes provisional tolerable daily intake rate; USEPA denotes United State environmental protection agency

Table 4.22: Estimated daily intake rate of PTEs from okro leaf

Soil		As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	Adults	0.001	0.006	0.004	0.09	0.411	0.011	0.017	1.762
	Children	0.002	0.009	0.006	0.129	0.591	0.016	0.024	2.537
ORL	Adults	0.003	0.004	0.055	0.240	1.973	0.035	0.096	1.882
	Children	0.004	0.006	0.080	0.346	2.839	0.05	0.139	2.709
KATANG	Adults	0.001	0.061	0.013	0.475	8.265	0.031	0.097	4.079
	Children	0.002	0.088	0.018	0.683	11.898	0.045	0.140	5.872
FSS	Adults	0.001	0.016	0.007	0.135	0.190	0.013	0.041	1.248
	Children	0.001	0.023	0.010	0.194	0.274	0.019	0.058	1.796
CONT	Adults	0.001	0.009	0.012	0.108	5.499	0.018	0.027	2.718
	Children	0.002	0.013	0.017	0.155	7.916	0.026	0.039	3.913
USEPA Limit	PTDI	0.002	0.001	0.120	0.500	12.00	0.210	0.004	1.000

"PTDI" denotes provisional tolerable daily intake rate; USEPA denotes United State environmental protection agency

Table 4. 23: Estimated daily intake rate of PTEs from cockscomb

Soil		As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	Adults	0.001	0.008	0.007	0.115	0.945	0.014	0.015	3.038
	Children	0.002	0.011	0.010	0.165	1.361	0.020	0.021	4.374
ORL	Adults	0.001	0.005	0.036	0.165	1.536	0.019	0.024	1.329
	Children	0.002	0.007	0.052	0.237	2.211	0.027	0.035	1.913
KATANG	Adults	0.002	0.034	0.026	1.364	1.092	0.023	0.201	3.589
	Children	0.001	0.049	0.037	1.964	1.572	0.033	0.289	5.166
FSS	Adults	0.001	0.013	0.013	0.227	0.609	0.012	0.073	1.459
	Children	0.002	0.018	0.019	0.326	0.877	0.018	0.105	2.100
CONT	Adults	0.001	0.002	0.007	0.128	3.584	0.013	0.018	5.132
	Children	0.001	0.003	0.010	0.185	5.159	0.019	0.025	7.388
USEPA Limit	PTDI	0.002	0.001	0.120	0.500	12.00	0.210	0.004	1.000

"PTDI" denotes provisional tolerable daily intake rate; USEPA denotes United State environmental protection agency

4.8.2 Estimation of health risk index of PTEs

The health risk indices (HRIs) were calculated by dividing daily intake of PTEs by theirreference doses. HRI is usually adopted to assess the non-carcinogenic risks of hazard materials in foods. An HRI more than 1 is usually considered as not safe for human health. The results ofthis study are presented in Figures 4.21- 4.26. It was observed that values of HRI were greater than one for As, Cd, Cu, Pb and Zn in most of the vegetables studied, but less than one for only Cr and Ni. The HRI from consumption of vegetables was also observed to be higher high from all the PTEs in vegetables grown in KATANG soil than any other soil site with HRI values ranging from 0.9 – 62 while the lowest HRI was found in CONT and MFM soils, with values ranging from 0.0-1.8.

Values of HRI for individual elements were generally greatest for Cd, followed sequentially by Pb, Cu, Zn, Ni, As and Cr, although the values for Pb and Cu, occasionally exceeded those of Cd. The findings generally suggest that PTEs especially Cd, Cu and Pb contamination were high enough to cause potential human health risk from consumption of the vegetables studied while As, Cr, Ni and Zn with HRI less than one indicated no relative health risk associated with their ingestion.

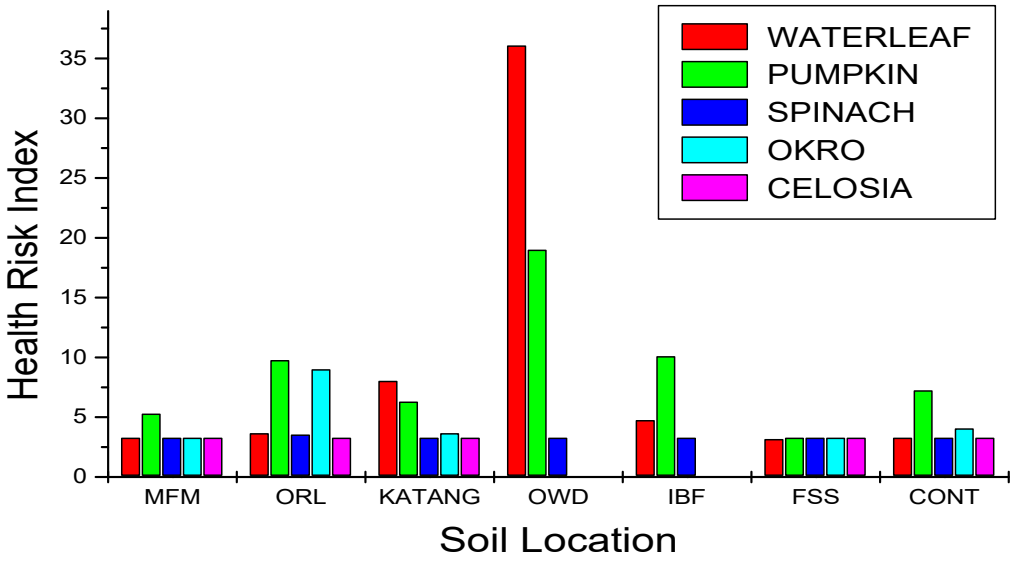


Figure 4.21: Health Risk Index of arsenic

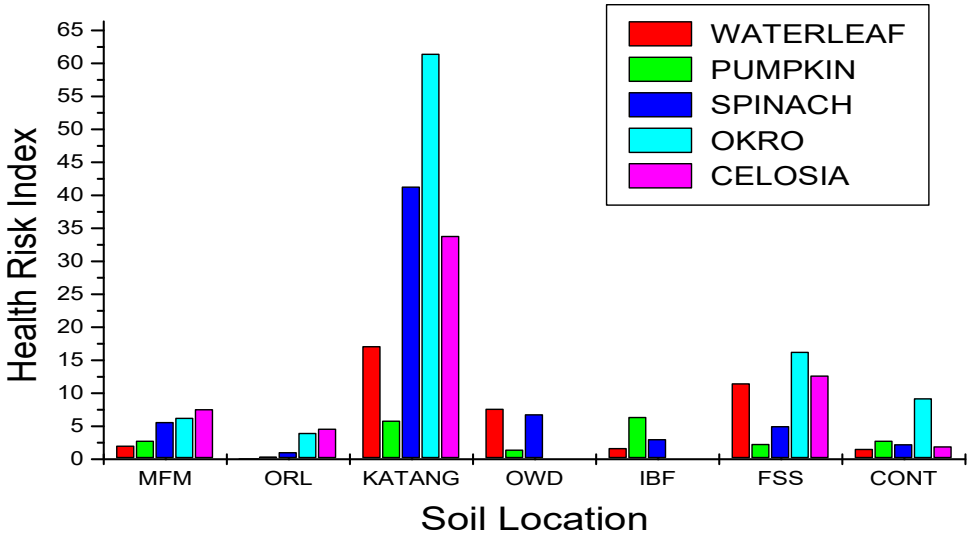


Figure 4.22: Health Risk Index of cadmium

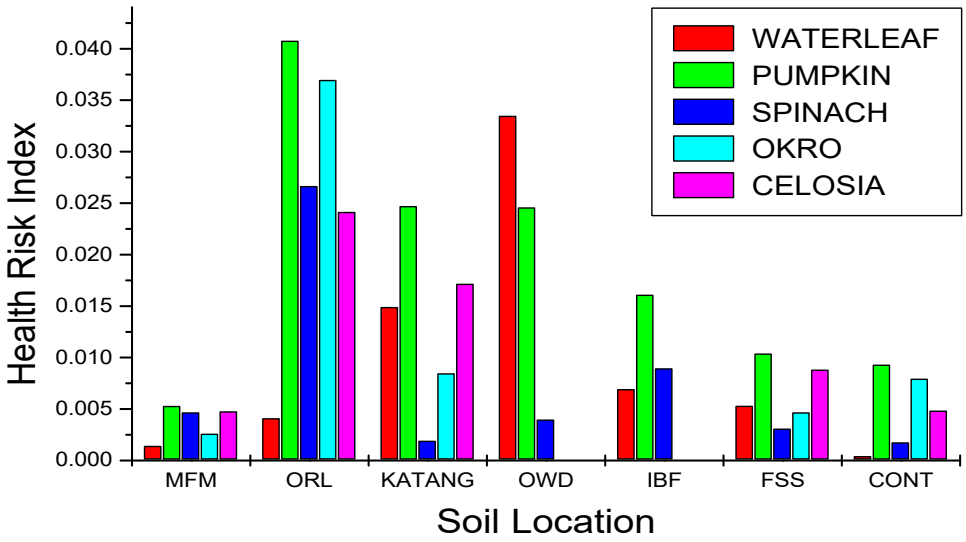


Figure 4.23: Health Risk Index of chromium

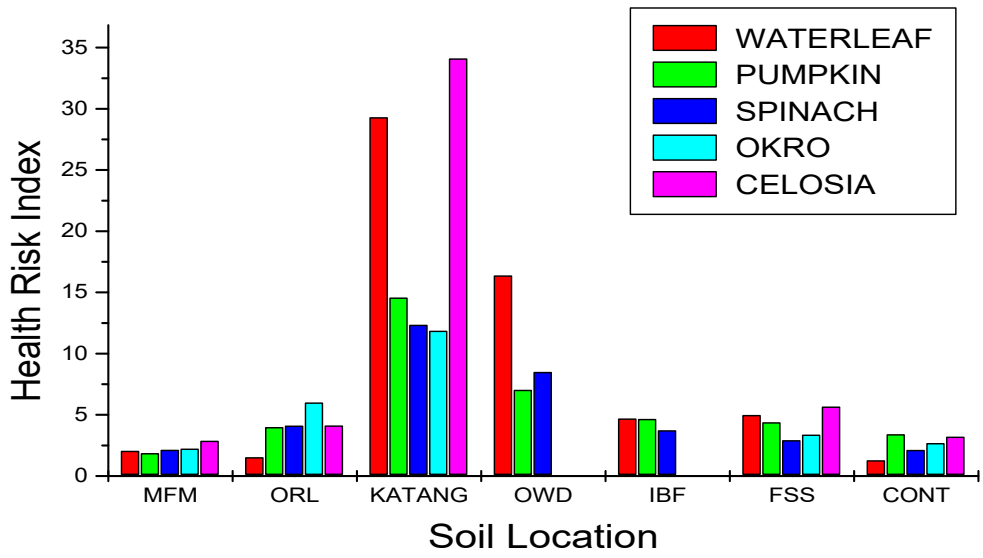


Figure 4.24: Health Risk Index of copper

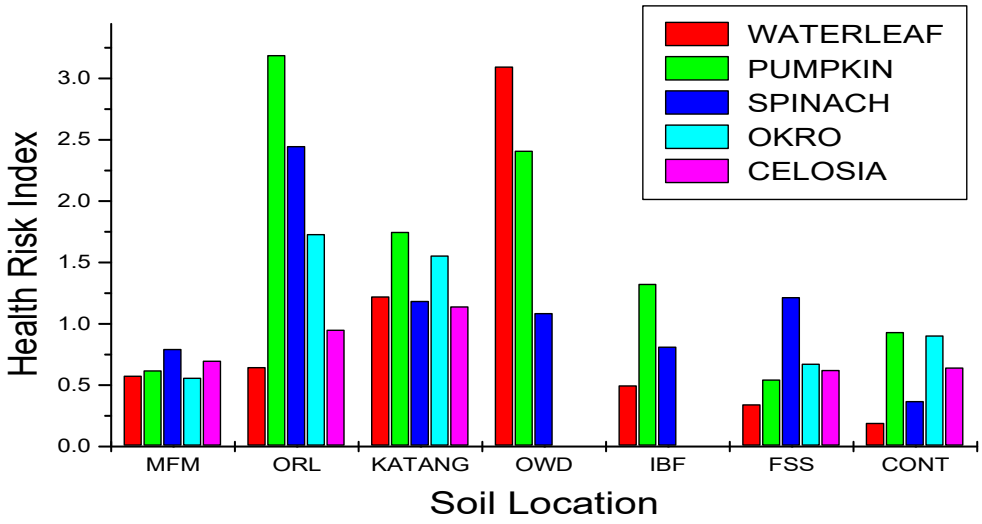


Figure 4.25: Health Risk Index of nickel

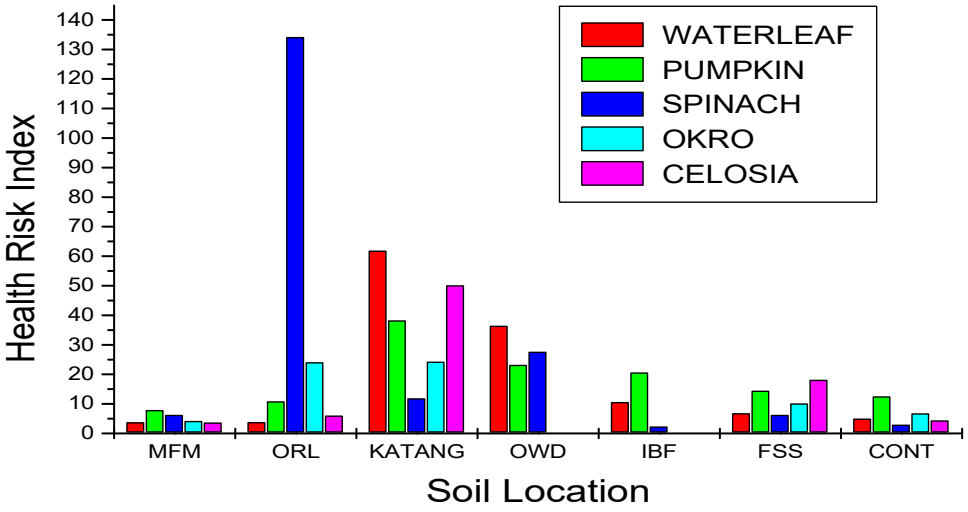


Figure 4.26: Health Risk Index of lead

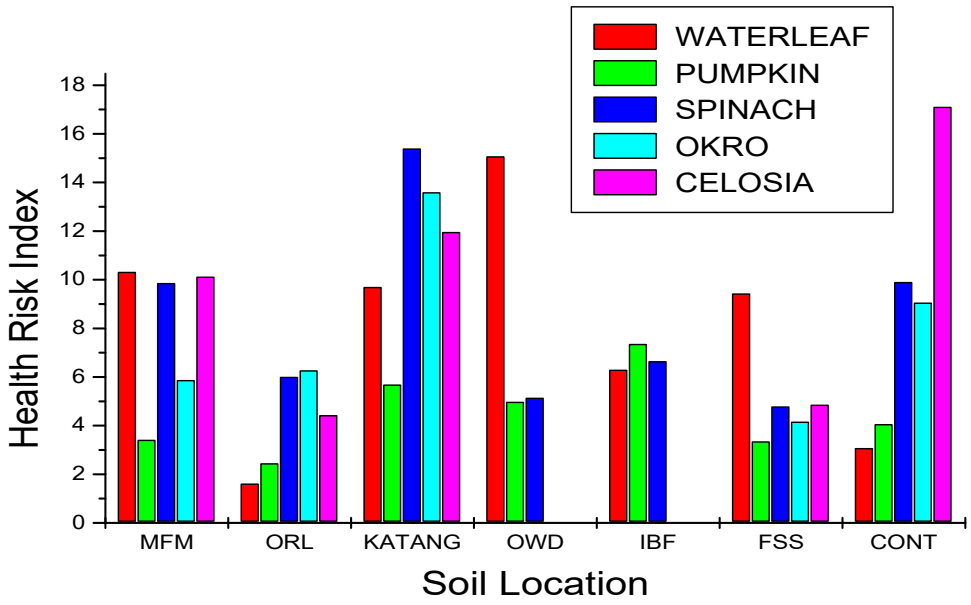


Figure 4.27: Health risk index of zinc

4.8.3Bioaccessible Studies of PTEs concentration in vegetables

4.8.3.1 PBET bioaccessible concentrations of PTEs

The results obtained for bioaccessibility studies for re-evaluation of health risk are presented in Tables4.24 – 4.32.

The physiologically based extraction test (PBET) results are presented in Tables4.24 – 4.27. Generally, the results obtained showed that the PTEs were more bioaccessible in the gastric phase (stage I) than in the intestinal phase (Stage 2). Itwas observed that only Cd, Cu and Zn were solubilized in the gastric phase with values obtained lower thanFAO/WHO recommended safe limits. The values varied in the range of0.1-2.4 mg/kg for Cd, 0.4-114.4 mg/kg for Cu, and 0.8-137 mg/ kg for Zn, while Cr and Pb had values below detection limits. However,in the intestinal phase,little or no bioaccessibility was observed for the PTEs, an indication that they were not really solubilised in the small intestine (where absorption into the blood stream actually occurs). According tothestudyof Ning *et al*., (2015), higher solubility of PTEs have been found in the acid environment (gastric phase) rather than in neutral or weak alkaline environment (intestinal phase), as they will be precipitated at higher pH. Similarly, Roussel *et al.*, (2010) reported that the higher bioaccessibility in the gastric phase was expected because the solubilisation of these PTEs is higher in the more acidic environment (gastric phase) than in the higher pH medium (intestinal phase) where re-adsorption and precipitation occurs.

Bioaccessibility of PTEs differed for the different vegetables, the solubility of Cd was observed to be highest in celosia with percent bioaccessibility between 16 to 109% in the gastric phase, followed by 26.2 to 50% in waterleaf and 41.4% in pumpkin leaf, while

spinach had only 17% solubility. For the intestinal phase, bioaccessibility (12%) was observed for celosia only.

Bioaccessible Cu content across the different vegetable samples was 11.6 to 86% for pumpkin leaf, 5.5 to 71.8% for spinach, 5.6 to 55% for celosia, and 1.3 to 50% for waterleaf. The results indicated that most of the Cu content accumulated in the plant was recovered in the residual phase where the metals are not available for absorption.

The bioaccessibility of Zn in the gastric phase was 6 to 28% for pumpkin leaf, 10 to 59.8% for waterleaf, 23 to 66% for celosia, and 27% for spinach, while 7.4 to 16.1% was solubilized in the intestinal phase. Most of the concentrations of Zn in the vegetables were found not to be available for absorption.

However, it has been noted that reporting bioaccessibility results only in terms of percent bioaccessible fraction conceals the exact concentration of the PTE in the sample extract (EPA, 2007).

Table 4.24: Results of bioaccessibility studies (PBET) of PTEs in waterleaf (mg/kg)

Soil	Phase	Cd	Cr	Cu	Pb	Zn
MFM	**Gastric**	< 0.5	< 3.5	0.45	< 7.5	45.5
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
ORL	**Gastric**	0.6	< 3.5	5.12	< 7.5	5.6
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
KATANG	**Gastric**	0.67	< 3.5	30.1	< 7.5	21
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
OWD	**Gastric**	1.31	< 3.5	22.4	< 7.5	6.4
	Intestinal	< 0.5	< 3.5	0.73	< 7.5	< 0.4
IBF	**Gastric**	< 0.5	< 3.5	<25.6	< 7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
FSS	**Gastric**	< 0.5	< 3.5	18.3	< 7.5	22.3
	Intestinal	< 0.5	< 3.5	1.5	< 7.5	< 0.4
CONT	**Gastric**	< 0.5	< 3.5	< 0.4	<7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4

"<" denotes detection limit

Table 4.25: Results of bioaccessibility studies (PBET)of PTEs in spinach(mg/kg)

Soil	Phase	Cd	Cr	Cu	Pb	Zn
MFM	**Gastric**	< 0.5	< 3.5	0.5	< 7.5	47.7
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
ORL	**Gastric**	< 0.5	< 3.5	1.4	< 7.5	4.8
	Intestinal	< 0.5	< 3.5	<0.4	< 7.5	< 0.4
KATANG	**Gastric**	0.64	< 3.5	22	< 7.5	60.4
	Intestinal	< 0.5	< 3.5	22.7	< 7.5	< 0.4
OWD	**Gastric**	< 0.5	< 3.5	< 0.4	< 7.5	6.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
IBF	**Gastric**	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
FSS	**Gastric**	< 0.5	< 3.5	7.4	< 7.5	28.1
	Intestinal	< 0.5	< 3.5	1.2	< 7.5	< 0.4
CONT	**Gastric**	< 0.5	< 3.5	< 0.4	<7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4

"<" denotes detection limit

Table 4.26: Results of bioaccessibility studies (PBET)of PTEs in pumpkin leaf(mg/kg)

Soil	Phase	Cd	Cr	Cu	Pb	Zn
MFM	**Gastric**	< 0.5	< 3.5	< 0.4	< 7.5	87.5
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
ORL	**Gastric**	< 0.5	< 3.5	9.3	< 7.5	32.9
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
KATANG	**Gastric**	1.1	< 3.5	17.1	< 7.5	12.7
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	8.5
OWD	**Gastric**	< 0.5	< 3.5	5.3	< 7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
IBF	**Gastric**	< 0.5	< 3.5	3.2	< 7.5	6.3
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	4.7
FSS	**Gastric**	< 0.5	< 3.5	0.7	< 7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	38.0
CONT	**Gastric**	< 0.5	< 3.5	< 0.4	<7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4

"<" denotes detection limit

Table 4.27: Results of bioaccessibility studies (PBET)of PTEs incelosia(mg/kg)

Soil	PTE	Cd	Cr	Cu	Pb	Zn
MFM	**Gastric**	< 0.5	< 3.5	3.35	< 7.5	136
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
ORL	**Gastric**	< 0.5	< 3.5	5.12	< 7.5	33
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
KATANG	**Gastric**	0.6	< 3.5	114	< 7.5	43
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
FSS	**Gastric**	1.5	< 3.5	3.77	< 7.5	23.2
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4
CONT	**Gastric**	< 0.5	< 3.5	1.41	<7.5	< 0.4
	Intestinal	< 0.5	< 3.5	< 0.4	< 7.5	< 0.4

"<" denotes detection limit

4.8.3.2SBET bioaccessibility studies of PTEs in vegetables

The results of simplified bioaccessibility extraction test (SBET)are presented in Tables 4.27-4.31. The results obtained showed that the bioaccessible concentration of most PTEs (As, Cr, Cu, Mn, Ni and Pb) studied with the exception of Zn were within the tolerable safe limit recommended by FAO/WHO for edible vegetables. The concentration of the bioaccessible PTEs range between < 0.2 – 0.43 mg/kg, 0.06 – 4.4 mg/kg, < 0.5 – 1.5 mg/kg , 1.5 – 81 mg/kg, 28 – 680 mg/kg, < 0.2 -2.5 mg/kg, 0.6 – 23.2 mg/kg and 5.7 -316 mg/kg for As, Cd, Cr, Cu, Mn, Ni, Pb and Zn respectively. It was observed that Zn levels were higher than the safe limits for almost all the vegetables studied, though, according to RAIS (2010), harmful effect of Zn is felt at ingestion level up to 250 mg, but fromthe results obtained in this study, Zn level in most of the vegetables was bioaccessible only at concentrations less than 250 mg with only very few exception from some vegetables grown on Katangua soil.

It was also observed from the results obtained that the PTEs behaved differently in gastric fluid for most vegetables studied,the results showed that Cd, Cu and Pb were more bioaccessible from waterleaf (Table 4.31) and spinach (Table 4.32) than any other vegetables in all soils. The trend followed the order; spinach > waterleaf > pumpkin > okro > celosia. Zn bioaccessibility on the otherhand varied significantly amongst vegetable types, no particular trend was observed.However, the bioaccessible concentrations of As, Cr and Niwere found to be very low, with most values obtained less than detection limits, with exception in vegetables grown in Katangua soils which had values higher than the detection limits most particularly for Cr and Ni found in okro and waterleaf. Significant positive correlations ($P < 0.05$) were observed between total and bioaccessible concentrations of Cd, Cu, Pb, and Zn while no significant correlation ($P > 0.05$) was observed for As, Cr, Mn, Ni.

Generally, the results obtained for bioaccessible concentration of most PTEs (As, Cr, Cu, Mn, Ni and Pb,) studied using PBET and SBET methods were found to be within the tolerable safe limit recommended by FAO/WHO for edible vegetables, which suggested no potential health risk associated when these vegetables are consumed by humans.It was noticeable however, that bioaccessible concentrations of PTEsobtained using PBET were much higher in the gastric phase than the intestinal phase which may be because the solubilisation of these PTEs is higher in the more acidic environment (gastric phase) than in the higher pH medium (intestinal phase) where re-adsorption and precipitation occurs (Rousse*et al.,* 2010). However, a comparison of PBET and SBET gastric phase bioaccessible concentrations revealed that SBET gave much higher gastric bioaccessible concentrations.

Table 4.28: Results of bioaccessibility studies (SBET)of PTEs inwaterleaf (mg/kg)

Soil	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	< 0.20	0.24±0.1	< 0.50	6.0±1.0	148±23	1.1±0.01	0.6±0.4	106±40
ORL	< 0.20	0.06±0.01	< 0.50	10.0±3.0	131±8	1.1±0.4	0.7±0.1	28.3±6.5
KATANG	0.43±0.02	1.44±0.10	0.92±0.07	81.0±6.0	171±3	2.0±0.2	15±1	316±60
OWD	< 0.20	0.7±0.01	< 0.50	20.0±1.0	66.4±0.2	< 0.2	1.1±0.01	174±53
IBF	0.23±0.01	0.06± 0.02	< 0.50	22.4±0.5	320±9	< 0.2	3.6±0.1	70±5
FSS	0.24±0.01	0.25±0.03	< 0.50	24.1±1.4	116±5	< 0.2	1.7±0.2	137±10
CONT	< 0.20	0.14±0.04	< 0.50	6.5 1.3	293±27	< 0.2	0.6 ± 0.2	144±52
FAO/WHO	N/A	0.20	2.30	40	500	80	5	60

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limit,

N/A denotes "Not available", FAO/WHO (2011).

Table 4.29: Results of bioaccessibility studies (SBET)of PTEs in spinach (mg/kg)

Soil	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	< 0.2	0.2±0.1	< 0.5	1.6±0.5	95±12	< 0.2	0.5± 0.2	104± 4
ORL	< 0.2	0.1±0.01	0.72±0.1	5.0±0.3	422±32	0.9 ± 0.2	23.2±0.4	132± 9
KATANG	< 0.2	4.4±0.1	< 0.5	40.6±1.7	28±2	0.51±0.08	3.3± 0.1	315± 5
OWD	< 0.2	0.26±0.01	< 0.5	23.2±1.1	68±3	< 0.2	10.0±0.1	89± 6
IBF	< 0.2	0.26±0.01	0.6±0.0	11.1±0.2	91±3	1.0± 0.1	1.8± 0.2	290± 11
FSS	< 0.2	0.34±0.21	0.6±0.1	12.8±4.4	117±15	2.5± 1.1	3.0± 1.1	203± 9
CONT	< 0.2	0.15±0.02	< 0.5	2.4±0.9	61±2	< 0.2	2.1 ± 0.2	110± 5
FAO/WHO	N/A	0.2	2.3	40	500	N/A	5	60

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limit,

N/A denotes "Not available", FAO/WHO (2011)

Table 4.30: Results of bioaccessibility studies (SBET)of PTEs in pumpkin leaf (mg/kg)

Soil	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	< 0.2	0.13±0.001	< 0.5	2.1 ± 0.4	89.8± 6.3	<0.2	1.3 ± 0.1	56.4± 4.5
ORL	< 0.2	0.07±0.01	< 0.5	5.3 ± 2.7	71.2± 3.8	< 0.2	1.8 ± 0.6	7.2 ± 0.6
KATANG	< 0.2	0.5 ± 0.01	< 0.5	29.0± 2.3	118± 2	< 0.2	7.3 ± 0.4	59 ± 9
OWD	< 0.2	0.16±0.01	< 0.5	8.0 ± 0.6	101± 3.9	0.5 ± 0.1	2.03±0.06	27 ± 4
IBF	< 0.2	0.13±0.001	< 0.5	1.5 ± 0.6	33.5± 1.7	<0.2	1.37±0.04	30 ± 6
FSS	< 0.2	0.12±0.04	< 0.5	5.9 ± 0.8	105± 8.8	<0.2	3.7± 0.6	5.7 ± 0.7
CONT	< 0.2	0.14±0.03	< 0.5	2.1 ± 0.2	38.8± 3.3	<0.2	1.7 ± 0.3	20 ± 5
FAO/WHO	N/A	0.2	2.3	40	500	N/A	5	60

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limit,

N/A denotes "Not available", FAO/WHO (2011)

Table 4.31: Results of bioaccessibility studies (SBET) ofPTEs from consumption of okro leaf (mg/kg)

Soil	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	< 0.2	0.10±0.01	< 0.5	3.8 ± 0.2	62.9 ± 0.3	<0.2	0.3 ± 0.1	132± 7
ORL	< 0.2	0.22±0.02	< 0.5	8.9 ± 2.3	155 ± 7	<0.2	2.8 ± 0.4	94.3±6.6
KATANG	< 0.2	3.1± 0.1	< 0.5	27.0 ± 1.4	680 ± 13	0.44±0.03	2.46±0.04	310± 6
FSS	< 0.2	0.2 ± 0.2	< 0.5	4.7 ± 0.7	30.3 ± 5.7	<0.2	0.5 ± 0.1	14.8±1.0
CONT	< 0.2	0.1 ± 0.0	< 0.5	2.4 ± 0.8	448 ± 7	<0.2	0.7 ± 0.1	150± 6
FAO/WHO	N/A	0.2	2.3	40	500	N/A	5	60

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limit,

N/A denotes "Not available", FAO/WHO (2011)

Table 4.32: Results of bioaccessibility studies (SBET) ofPTEs from consumption of celosia (mg/kg)

Soil	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
MFM	< 0.2	0.13±0.02	< 0.5	4.6 ± 0.2	130 ± 1	<0.2	2.5±0.5	82 ± 25
ORL	< 0.2	0.4 ± 0.01	1.55±0.04	4.41±0.04	77.6± 1.2	<0.2	1.37±0.02	39 ± 6
KATANG	< 0.2	0.12±0.001	< 0.5	5.5 ± 1.1	170 ± 1	<0.2	1.75±0.01	57 ± 8
FSS	< 0.2	0.2 ± 0.1	0.55±0.06	6.3 ± 0.1	72 ± 9	<0.2	3.8±0.1	40 ± 10
CONT	< 0.2	0.1 ± 0.2	< 0.5	3.7 ± 0.8	75 ± 18	<0.2	2.0 ± 0.6	126 ± 36
FAO/WHO	N/A	0.2	2.3	40	500	N/A	5	60

Results are expressed as means of triplicate determinations ± standard deviation, "<" denotes below detection limit,

N/A denotes "Not available", FAO/WHO (2011)

CHAPTER FIVE

CONCLUSION

5.1SUMMARY OF FINDINGS

Concentrations of PTEs have successfully been determined in five commonly cultivated vegetables in urban soils from Lagos state, which were from locations impacted by the nearby presence of dumpsitessituated near residential and commercial areas, in order to assess potential public health risk. Vegetables commonly consumed by humans were planted on these soils to determine the level of uptake of the potentially toxic elements from the soils to vegetables, and the potential human health risk associated with the consumption of these vegetables was evaluated using estimation approaches (daily intake rate and health risk index) proposed by USEPA. Human health risk was further assessed by investigating the oral bioaccessibility of these PTEs using in-vitro bioaccessibility models via the application of physiologically based extraction test and simplified bioaccessibility extraction test.

The concentration of PTEs in soils samples investigated varied for the different soil samples, while some soils were observed to be heavily contaminated with PTEs (KATANG, OWD, ORL and FSS), others had mild contamination(MFM, IBF). Generally, levels of PTEs werefound to be below soil guideline values for mildly contaminated soils (MFM, IBF) but above for the heavily contaminated soils (ORL, KATANG, OWD and FSS).However, the concentrations of PTEsfound in vegetables studied differed considerably from one plants specie to the other with uptake of PTEs greater from vegetables grown on soils with elevated levels than soils with lower levels. Relatively high concentrations of the PTEs were observed in the vegetables with the most of the values obtained higher than the recommended tolerable safe limits established by FAO/WHO. The highest level of As, Cr and Ni were found in pumpkin leaves, Mn and Cd in celosia leaves, Pb in spinach, however Zn was found to be

high in all the vegetables. The trend of transfer factor from soil to vegetables followed the order Cd >Zn > Mn > Cu > Pb> Ni> Cr>As.

Evaluation of human health risk bythe estimation of dietary daily intake rate and health risk index of vegetables suggested Cd, Cu, Ni, Pb, and Zn contamination in all the most of the vegetables studied. It was observed from the results that daily intake rate of PTEs such as Cd, Pb and Zn in both adults and children were very high when compared with the Provisional tolerable daily intake (PTDI) limits established by USEPA while daily intake rate for, Cr, Cu, and Ni were below the PTDI values.The estimation of non-carcinogenic risks using health risk index revealed values of HRI were greater than one for As, Cd, Cu and Pb in most vegetables studied, but less than one for Cr and Ni. The major non-carcinogenic risk contributors were Cd, Cu and Pb while the other PTEs (Cr, Ni, Zn) do not pose any major potential risk through consumption of these vegetables.Re-evaluation of risk associated with consumption of these vegetables using *in- vitro*bioaccessibility modelssuggested that PTEs taken up by the vegetables were available for absorption (solubilized) in the gastric extraction phase rather than in the intestinal phase. The level of PTEs absorbed in the gastric phase were found to be relatively low.Generally, the results obtained for bioaccessible concentration of most PTEs (As, Cr, Cu, Mn, Ni and Pb,) studied using Physiological based extraction test (PBET) and simplified bioaccessibility extraction test (SBET) were found to be within the tolerable intake level recommended by FAO/WHO for edible vegetables, which suggested no potential health risk associated these vegetables when consumed by humans but bioaccumulation of these PTEs could take place over time in the human system through continuous consumption of the contaminated vegetables, possibly resulting in deleterious health effects in humans.

Futhermore, the oral bioaccessibility study has also been able to illustrate that some levels of potentially toxic elements could actually be solubilized in the human gut when taken in through consumption of soils or contaminated vegetables, though in low concentrations, while some portion of these PTEs which were not solubilised are retained in the gastrointestinal tract and eventually ejected out of the body system.

5.2 CONTRIBUTION TO KNOWLEDGE

The research has been able to add the following contributions to the body of existing knowledge:

1. This research is one of the first studies on in-depth human risk assessment of PTEs and the first study on human risk assessment of arsenic in urban soils and tropical vegetablesconsumed in Lagos, Nigeriavia the estimation of dietary exposure rate and non-carcinogenic risk (health risk index).

2. The use of physiologically based extraction test (PBET)for the evaluation of the oral bioaccessibility and human health risk assessment of PTEs in vegetables from Sub Saharan Africa, (specifically Nigeria) was carried. As at the time of this work to the best of my knowledge, none exist in literature.

3. The determination of bioaccessible concentrations of PTEs in tropical vegetables and urban (Lagos) soils through oral ingestion using simplified bioaccessibility extraction test (SBET) has also been studied for the first time in Nigeriato the best of the knowledge of the researcher.

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7.0 APPENDICES

Appendix I: **Soil guideline values (SGVs) of PTEs from different countries**

Countries	Potentially Toxic Elements (mg/kg)							
	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Australia	100		100	1000	1500	600	300	7000
Canada	12	1.4	64	63	NA	50	70	200
England	32		Cr(III) 3000 Cr(VI) 4.33	2330	NA	130	450	3750
Germany	50		400	NA	NA	140	400	NA
Netherland	55		380	190	NA	210	530	720
South Africa	48	32	Cr(III) 9600 Cr (VI) 13	2300	1500	1200	230	1900

Appendix II: Optimal operating conditions and dynamic working range of FAAS

Element	Wavelength (nm)	Lamp current (mA)	Linear working range (mg/L)	Absorbance	LOD_{inst} (mg/kg)	LOD_{pro} (mg/kg)
Cd	228.8	7.5	0.1 – 0.5	0.04 – 0.10	0.02	0.5
Cr	357.9	10	2 - 25	0.05 – 0.22	0.16	3.5
Cu	324.8	4	2 -10	0.11 – 0.50	0.024	0.5
Pb	283.3	10	5 -100	0.05 – 0.60	0.25	12.7
Zn	213.9	5	0.1 – 0.4	0.05 – 0.40	0.02	0.4

Appendix III.Operating conditions of ICP-MS

Parameter	**Operating Mode**
RF Power	1550 W
Carrier gas flow	1.05 L / min
Nebulizer pump	0.1 rps
Quadrupole bias	-15 V
Omega Lens	8.4 V
Reaction cell	ON
Sampling period	0.31 s
Internal standard	^{115}In

PUBLICATIONS

1. ODUJEBE, F.O., OYEYIOLA, A.O., DAVIDSON, C.M., OLAYINKA, K.O.& ALO B.I. (2017). Bioaccumulation and Human health risk assessment of potentially toxic elements in Vegetables grown in the vicinity of dumpsites in a rapidly urbanising tropical megacity. *Journal of Hazardous materials* Ms. Ref. No: HAZMAT-D-17-01649.

2. ODUJEBE, F.O., OYEYIOLA, A.O. & OLAYINKA, K.O. (2016). Use of Physiologically based extraction test for the assessment of bioaccessibility of toxic metals in vegetables grown on contaminated soils. *Journal of Health and Pollution*, **6** (10):74-83