Environmental risk assessment of inorganic and organic pollutants in raw and cooked food in African countries

by

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Declaration

This master thesis is my own work and has not been submitted for a degree in any other University.

……..

Yannick Belo Nuapia

Abstract

Exposure to inorganic and organic pollutants residues causes negative effects to human health. They can cause damage to the living organism as well as to the environment. Humans take up these pollutants through skin absorption, respiration and ingestion of contaminated food and also the drinking water as a potential contaminants exposure route for inorganic and organic pollutants to humans. Studies have shown that inorganic and organic pollutants can be retained in food crops at higher than the permissible levels, thus posing some health risks to consumers.

This study sought to assess the potential risk posed by inorganic and organic pollutants to human health as a result of consuming raw food and processed food

cooked in traditional utensils. The food samples and the traditional utensils were collected in Kinshasa and Johannesburg markets. In total ninety- two food samples were assessed. The food crops included: cabbage, cassava, beans, beef meat and fish (tilapia). Ten trace elements, namely: Al, As, Cd, Cr, Cu, Hg, Mn, Pb, Se and Zn were analysed in raw food samples using ICP-OES, ICP-MS and mercury analyser. These metals were also analysed in food cooked with the traditional utensils. The health risk indices calculated were: Daily Intake of Metal (DIM) and Hazard Index (HI). The results obtained reveal that the raw food collected in Johannesburg markets contained a significant ($p < 0.05$) higher concentration in all elements than the food samples collected in Kinshasa markets. Hg was detected only in fish samples. It was observed that cooking utensils can leach some significant quantities of trace elements into food during processing, hence resulting increase the concentration of these metals in processed foods. The DIM exceeded the oral reference dose (Rfd) and the HI were > 1 in all the food cooked. The results of metal found in this current investigation were above the standard permissible limit set by FAO/WHO.

On the other hand, the levels of 17 organochlorine pesticides were assessed in uncooked and cooked beans, cabbage, cassava, fish and beef meat collected in Kinshasa and Johannesburg markets. It was noticed from the results recorder that

the mean concentration of organochlorine pesticides in uncooked food collected in Johannesburg market were significantly high ($p < 0.05$) than the uncooked food from Kinshasa markets. DDE recorded its highest mean level (253.58±4.78 µg kg^{-1}) in beef meat samples collected in Johannesburg markets. The results from the current study revealed that after cooking the amounts of organochlorines were significantly reduce than the uncooked food. Residue amounts of organochlorine pesticides found in this study are considerably lower than the recommended amount of organochlorines set by WHO/FAO (300 µg kg^{-1}).

Dedication

This thesis is dedicated to the almighty God, my creator and provider. To him alone be all the praise and adoration. I also dedicated my research to lovely wife Flora Tshisalu Kena and my children, Zoe Kena Belo and Immacule Mbuyi Iyodja.

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Abbreviations

ADI: Acceptable Daily Intake

ATSDR: Agency for Toxic Substances and Disease Registry

BHC: Benzene Hexachloride

CCD: Coupled Charge Detection

CRM: Certified Reference Material

CVAAS: Cold Vapour Atomic Absorption Spectrometry

DDD: Dichlorodiphenyldichloroethane

DDE: Dichlorodiphenylethane

DDT: Dichlorodiphenyltrichloroethane

Dw: ww dry weight; wet weight

EDI: Estimated Daily Intake

EICD: Electrolytic Conductivity Detector

EPA: Environmental Protection Agency

FAO: Food and Agriculture Organisation

FEPA: Federal Environmental Protection Agency

FI: Flow Injection

FIA: Flow Injection Analysis

FIMS: Flow Injection Mercury System

GC Gas: Chromatograph

GC-ECD: Gas Chromatograph with Electron Capture Detector

HCB: Hexachloro Benzene

HCH: Hexachlorocyclo Hexane

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy

JHB: Johannesburg

Kin: Kinshasa

LD50: lethal dose 50

LOD: Limit of Detection

MRL: Minimal risk level

ND: No detect

PTFE/TFM: Polytetrafluoroethylene/tfm (teflon)

PTFE: Polytetrafluoroethylene (or Teflon ®)

RDA: Recommended dietary allowance

RNI: Reference nutrient intake

RSD: Relative Standard Deviation

SUL: safe upper level

U.S EPA: United States Environmental Protection Agency

UL: tolerable upper intake level

UNEP: United Nations Environment Programme

WHO: World Health Organization

Chapter 1: Introduction

1.1 Background

It is of general knowledge that the accumulation of pollutants in the human body through food consumption is of concern for public health. Food contamination might occur during its production, processing, transportation and handling (Fries, 1995; Tricker and Preussmann, 1990; D'Mello 2003; Cabrera et al., 2003). This contamination can be classified into two categories: long term contamination of low concentration chemicals which is a result of a gradual diffusion of contaminants in the food and short-term contamination at higher concentration of chemicals which usually results from accidental release or contamination from the source (Rashmi et al., 2013).

The level of pollutants in human body is determined from human samples such as hair, blood, urine, adipose tissue and breast milk but can also be measured indirectly by determining the amount of the pollutant in the consumed food in relation to the daily intake of food (FAO/WHO, 1999).

Among the existing pollutants, heavy metals are of interest due to their persistence and tendency to bioaccumulate in organisms (Pitot and Dragan, 1996). Food contains appreciable quantities of inorganic elements such as Na, K, Fe, Ca, B, Mg, Se, Cu and Zn. These elements have a biological function in major and trace quantities for cellular processes. Hg, Pb, Cd and As have a toxic effect and cause harmful effects on health. These metals can be lethal at extremely low concentration depending on the species. The negative effects of these metals are due to the fact that the metals can accumulate in human body. Bioaccumulation process of metals occurs in all existing organisms as a result of exposure to metals in environment and food (Abou-Arab et al., 1999). The organo forms of mercury tend to pose the most harmful effects on health. For example, methyl-mercury is a neuron toxic. Pb also causes injury to the brain cells and delay intellectual development in children. These metals can also damage the kidneys, immune and reproductive systems and the brain (Scheummer, 1987).

Cd is a toxic to the kidney. Exposure to inorganic arsenic can damage some cells and cause a cancer. In view of varied toxic effect and the fact that these metals can accumulate in the body, it is important to assess the levels of the metals in foodstuffs in order to protect human health (WHO, 2008).

The amounts of Hg, Pb and Cd in foodstuffs have been set by FAO and WHO. FAO/WHO set maximum levels (MLs) for the metals in large range of foodstuffs such as meat, milk, fish, cereals, vegetables, fruit and fruit juices, and also sets a maximum level for Hg in fish and fish products (FAO/WHO,2000).

Other trace nutrients like Cu, Zn, Cr and Mn can damage human health if taken in high concentration (Onianwa, 2001).

On another hand, although organochlorine pesticides have constantly proved their importance by increasing total agricultural productivity, and plant illnesses. Many studies have demonstrated that persistence organic pollutants such as organochlorine pesticide (Patandin et al, 1999) as well as polychlorinated biphenyls (PCBs), and poly aromatic hydrocarbons (PAHs) have negative effects in human's health (Levi et al., 2001; Azaroff et al., 1999; Gomes et al., 1999). The organochlorine pesticides are more accumulated in the human fat. They tend to stay until the fat is break down for energy. The pesticides can pass through the mother placenta to the unborn child. They lead to many harmful effects such as abnormal development of the immune system, birth defects and fetal death (Davis et al., 1993). This is why pesticides are considered as one of the main environmental and human health concerns in the world (Schafer et al., 2002; Albo et al., 1979).

Moreover, Dichlo-diphenyl-trichloroethan (DDT) is an organochlorine insecticide which is very persistent in the environment. Its half-life is estimated for 15 years. Its metabolites products in the soil environment are dichloro-diphenyl-dichloro-ethane (DDD) and dichloro-diphenyl-dichloro-ethylene (DDE). They are also extremely persistent and have comparable physical and chemical properties (Dorner and Plagemann, 2002). In 1945, DDT was used for the first time in Mexico for malaria control. In 1994, DDT was banned and substituted by pyrethroids (Webster et al., 2000). WHO and FAO have reported high levels of

DDT compounds in vegetables, fish and meat eaten by many people in Africa (WHO, 2000). DDT can cause immunosuppression, reproductive effects, shortened duration of lactation, neurological and behavioral effects, and genotoxicity (Svensson et al., 1994; Vine et al., 2001; Rylander et al., 1996; Longnecker et al., 2001; Gladen and Rogan, 1995; Miersma et al., 2003; Dorner and Plagemann, 2002; Rabello et al., 1975; Yanez et al., 2004). Vin et al. (2001) have reported that DDE can modulate immune responses in exposed children.

Due to problems related with organic and inorganic pollutants, many nations and international organizations such as UNEP, WHO and EU have recognized those pollutants as a major risk for health and particularly for children's health (WHO Food Safety, 1999). They have progressively issued various guidelines or recommendations aimed at limiting or forbidding the use those pollutants. For example, the addition of tetramethyl lead in gasoline in many countries has been banned resulting to the decrease of lead in the environment (Landrigan, 1989).

1.2 Problem statement and motivation

Polluted food has been known to cause many diseases to humans. It is still one of the most common causes of health problems in the world. In all countries, polluted food pose serious health problems, ranging from minor illness to fatal diseases (D'Mello, 2003). Unfortunately, diseases originating from polluted food are not well defined totally (WHO Food Safety, 1999). Estimations of these problems are complicated due to the fact that a very few diseases can be clearly related to food. In some cases, diseases from to the polluted food often go undetected or underreported. It is estimated that 1,8 million peoples die due to illnesses caused by polluted food (WHO Food Safety, undated).

Kinshasa and Johannesburg are two big cities in Africa. Kinshasa is a political capital of DRC and Johannesburg is an economic capital of Republic of South Africa. These cities are characterized by a high population density, intensive motor car traffic, especially during peak hours and a number of industrial activities. In both cities, many people sell food on the street with millions of consumers relying on this source of provision. At some places these "street

markets" are close to public roads where the traffic is very high. Therefore, foods sold in those areas are not only exposed to chemicals used to grow the vegetables but also to pollutants from vehicle emissions. The situation is very much similar to many other countries in Africa.

To add, in Kinshasa, many women cook food in the traditional metal pots made of metals such as Al, Pb, Cr and Mn. Dabonne et al (2010) demonstrated that the metals can be transferred into the food during cooking. Tuakuila et al (2010) found important amount of Pb in children blood from some areas in Kinshasa and reported that the source of Pb contamination was believed to be the traditional metal pots used for cooking.

In 2001, the Stockholm Convention on Persistent Organic Pollutants (POPs) was signed to decrease and eradicate the release of POPs into the environment. Controlled use of insecticides to reduce malaria in some parts of South Africa and DRC is allowed. These areas are Kwazulu Natal and Limpopo provinces. Over their daily activities in and around the house, people could be exposed to DDT even under controlled usage. Chen et al. (2006) found the uptake of DDT by the root of rice, a step in food chain accumulation. Vazquez et al (2000) has shown the accumulation of DDT in number of chicken, beef and fish.

Monitoring of pollutants levels in food becomes a necessity so as to compare the results to the recommended maximum limits as given by WHO/FAO. Such studies help to prevent a potential catastrophic situation that might occur from ignorant consumption of polluted food (WHO/FAO, 1999). A continued monitoring of pollutants levels in food in Africa countries is of importance for governments in order to implement and/or improve policies regarding food production and processing.

Chapter 2: Literature review

2.1 Introduction

Food is the major source of nutrient supply for human body. Apart from basic substances of nutrition as proteins, carbohydrates and lipids, human body requires fibers, vitamins, enzymes and range of essential chemicals. Macro elements such as Ca, K, Mg, Na and micro-elements such as Cu, Fe, Mn, Mo, Se, Va and Zn are needed for vital functioning of human body. Specific or natural environmental conditions with interconnection of anthropogenic influences can affect chemicals content in food. For example, chemical content in soil, air, dust such as pesticides, heavy metals can influence composition of vegetation that is consumed by animals or is used as human food. It can result in the specific chemical transfer into food chain which can affect human health, longevity and life quality in general (Combs, 2005; Fraga, 2005; Nabrzyski, 2007).

Food contamination with toxic pollutants such as, As, Cd, Cr, Hg, Pb, pesticides and other may occur resulting in reduced quality of daily nutrition and adverse health effects. Therefore, the assessment of the content and concentration of microelement, macro-elements and other pollutants such pesticides in connection with estimation of environmental and anthropogenic impacts are the issues of a high importance. Quantitative data of pollutants concentration in food is an indispensable tool for risk assessment analysis that can be associated with occasional intakes of pollutants in too high levels and can lead to the toxic adverse effects in human body (Goldhaber, 2003).This chapter presents a review of the literature relating to:

- Characteristics of inorganic elements in food and human health;
- Organochlorine pollutant and their impact in human health;
- Risk assessment.

2.2 Characteristics of inorganic elements in food and human health

Basic elements such as C, H, N and O form up to 97% of the matter of living systems. Content of these elements in the tissues and organs of an average human body weight is estimated as follows: O (61%) > C (23%) > H (10%) > N (2.6%). Other elements can be subdivided in macro and micro-elements. Ca, Cl, K, Mn, Na, P, S and Si are macro-elements; each of them accounts about 0.03-1.4% of human body weight. Trace element concentration in human body is variable from 0.1 mg kg^{-1} to 100 mg kg^{-1}. Trace element group involves such elements as Ag, Al, As, Au, B, Ba, Br, Cd, Co, Cr, Cu, F, Fe, I, Mn, Mo, Ni, Pb, Ru, Se, Sr, Va and Zn, but the list can vary due to the traits of the lifestyle, diet and habitation environment of an individual (Aras and Ataman, 2006; Baltes, 1998; Fraga, 2005; Garrett, 2005; Goldhaber, 2003).

Nutrition, including food, beverages and drinking water, is the main source of elements for the living organism. Therefore, the composition of chemical elements in diet can be assessed as transition matter for the uptake of nutritionally as well as potentially toxic elements. In this section importance of inorganic element, inorganic element pathways routes in food from the environment, contamination sources and natural occurrence of inorganic elements in food, bioavailability of inorganic elements from food to human organism and toxicity of inorganic elements in respect to human health are discussed.

From all known chemical elements about 50 elements can be found in living organisms, including plants, animals and humans. However the physiological relevance for humans has been revealed only for 23 elements (Combs, 2005; Fraga, 2005). Elements are essential if they are vitally important and irreplaceable for normal physiology of the organism (Aras and ataman, 2006; Goldhaber, 2003). Importance to the element is attributed if the consumption of element at levels above the safe adequate intake induces alterations in physiological functions of organs and tissues (Aras and Ataman, 2006). The group of essential chemicals involves not only chemical elements, but also amino acids, fatty acids and vitamins compounds that cannot be synthesized by the organism itself but are unambiguously needed for the biological functions (Combs, 2005). However, the

term essentiality is a relative designation due to the specific needs of biological systems for various elements in different amounts. Some elements considered as essential for certain species can be non-essential or even toxic for another species and some elements can be essential in certain amount while in higher concentration they become toxic. For example, Va and Co are essential elements for blue-green algae, one within the function of photosynthesis and another is involved in the process of nitrogen fixation. At the same time function of Co in higher plants is unknown and, if attributed to humans, biological function of vanadium is not clearly understood (Kabata-Pendias, 2011).

Bowman et al. (2003) listed elements such as Co, Cu, Fe, K, Li, Mg, Mn, Mo, S and Zn as essential for all living organisms. The role of some elements such as Al, As, B, and Va in human health is still under scientific investigation and, therefore, the term possibly essential elements can be attributed. Elements can be toxic, potentially toxic or non-toxic elements, nevertheless, it depends on the element concentration (ATSDR, S.A.; Szefer and Grembecka, 2007; WHO, 1996).

According to Kabata-Pendias and Mukherjee (2007), elements that are found in detectable levels in human body can be listed as follows:

- Essential elements such as B, Br, Ca, Cl, Co, Cr, Cu, F, Fe, I, Li, Mn, Mg, Mo, P, K, S, Se, Si, Na, Va and Zn;
- Possibly essential elements such as Ba, Ge, Ni, Rd, Sn, Sr and Ti;
- Non-essential elements such as Ag, Au, Cs, Hf, In, Ir, Sb, Ta, Te, U, Y, Zr, including rare earth elements;
- Non-essential and highly toxic inorganic elements such as Be, Bi Cd, Hg, Pb and Th.

General contribution of elements in human physiology is following:

- Ca, F, Mn and P are involved in structures of bones and membranes;
- Cl, K and Na supports the balance of water and electrolytes;
- Cu, Mg, Mo, Se and Zn participate in metabolic catalysis;
- Fe is involved in binding of O;
- I and Cr are associated with hormone formation and activity (Aras and Ataman, 2006; combs, 2005).

Nutritionally important elements can be subdivided in macro-elements or macro-nutrients such as Ca, Mg, Na and micro-elements or micro-nutrients such as Co, Cr, Cu, Mo, Ni, Se, depending on the required daily consumption amounts. Daily requirement of trace elements usually is less than 100 mg day^{-1}, while macro-nutrients for human body are necessary in larger quantities (Abrahams, 2002; Fraga, 2005). Requirements usually are calculated taking into account the studies of regional dietary surveys. Several terms are used to set and describe required element concentration for humans. For example, Reference Nutrient Intake (RNI), Recommended daily allowance (RDA), Upper Intake Level (UIL), Estimated Average Requirement (EAR) (Duffus et al., 2007; FSA, 2006; Iom, S.A.; Schrauzer, 2002; White and Broadley, 2005). Requirements of essential inorganic elements for human derived from USA and the UK documents are summarized in appendix I.

2.2.1 Transfer route of inorganic element pollutants in food

Food is the main sources that supply human body with nutrients including micro and macro-elements; slight amounts of elements can be inhaled from air. Nowadays industrial food production has been developed rapidly and is steadily restricted by laws and regulations that set down limitations for composition of food. However, food products derived from plant or animal origin and produced by small farms or individual households still are exposed to the influence of regional or site-specific environmental conditions. Although element circulation in ecosystems is a process without cease in between the main constituents of biosphere, soil, water and air; vegetation is the primary recipient of elements from environment, mainly taking up trace elements and macro elements by roots and foliage (Kabata-pendias, 2011; Kabata-pendias and Mukherjee, 2007; Voutsa et al., 1996).

Element transfer into the food chain continues from plants to animals, finally reaching food of plant source and food of animal source and human nutrition (Figure 2.1). If compared with animals, plants can be assessed as the major components of ecosystem involved in element transfer from environment to food due to their capacity to take up, store chemical elements, synthesize or

resynthesize chemical compounds containing certain elements available in soil and water. As soil is the main environmental provider of elements, the research of element transfer into food and mineral nutrition can be linked as well to geological issues (Bowman et al., 2003; Combs, 2005). Plants can take up and accumulate from the environment where they grow not only essential elements needed for plant nutrition but also toxic elements, thus successive element transfer into the food chain can appear and may cause potential risks for both, plant and human or animal health (Voutsa et al., 1996).

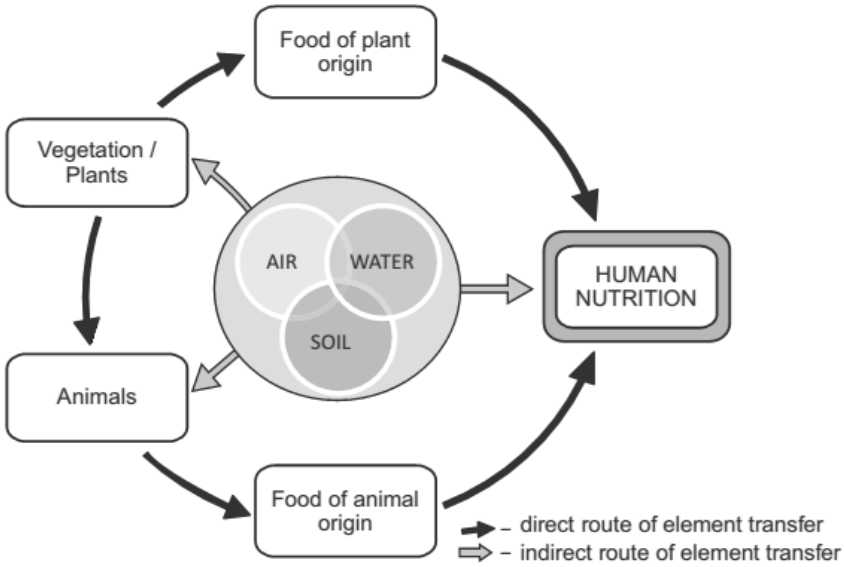

Figure 2.1: Schematic overview of element transfer from environment to human nutrition (Combs, 2005)

Element transfer from environment to food chain via atmospheric deposition is dependent on the size of particulate matter; also chemical composition of particles is important.

Particulate matter containing toxic substances can deposit on the surface of soil, water and plants. As smaller particles are, as easier they can be absorbed by plants and inhaled by animals and humans bringing subsequent effects on nutrition and human health (Allen et al., 2001).

Some authors mention also direct route of element transfer to human nutrition from soil. Small soil particles can be digested with consumption of unwashed

vegetables and fruits thus increasing the possibility also of airborne particle deposition and admission into food chain (Abrahams, 2002; Combs, 2005; Kabata-Pendias and Mukherjee, 2007).

2.2.2 Contamination sources and natural occurrence of inorganic element pollutants in food

Consumer safety is the main reason why food contamination is a question of high importance. Soil contamination and possible subsequent site specific and vegetation pollution with potentially toxic elements can occur due to the natural environmental processes and anthropogenic activities. Respectively, volcanic releases, atmospheric depositions, mining and use of chemicals in agricultural practices can be mentioned as well recognized examples. Geochemical anomalies, variable site-specific content of elements in soil, agrochemical applications, and pollution influence element content and concentration in plants consumed by humans as foodstuffs resulting in dietary intake of pollutants (Hao et al., 2009; Kabata-Pendias, 2004; Kabata-Pendias and Mukherjee, 2007). Background element content in environment is not constant but influenced by natural processes in the environment and affected also by human impact or anthropogenic factors which mainly bring moderate diffuse inputs of chemicals into the environment, therefore, concentration of elements can vary in time (Diez et al., 2009; Kabata-Pendias and Mukherjee, 2007). Contamination with pollutants may be caused via environmental pollution, applied agricultural practice in food plant cultivation and animal breeding, food manufacturing, processing, packaging and storage that is discussed in this section.

2.2.3 Environmental pollution as a source of toxic inorganic elements in food

The main environmental pollution sources of trace inorganic pollutants are industry, mining and smelting, brick, pipe, glass manufacture, cement, ceramic, chemical plants, power generation, combustion of fossil fuels and municipal wastes, nuclear reactions, agricultural activities, transport and urban pollution. It has been estimated that the most hazardous inorganic elements for the biosphere

are Ag, As, Au, Cd, Cr, Cu, Hg, Pb, Sb, Th and Zn. While among the least hazardous elements, Ga, La, Nb, Sr, Ta and Zr are ranked (Kabata et al, 2007).

However, pollution induced by industry, traffic or other anthropogenic activities recently is significant source of elements that revert back to ecosystems. Elements with natural origin as well as elements from anthropogenic sources are circulating in the biosphere and are involved in biogeochemical cycles apart from the site of their origin (Voutsa et al., 1996).

It is proven by experiments that dust outflows from industrial or urban areas influence the concentration of chemical elements on foliage surface significantly (Allen et al., 2001). Dust can contain high amounts of Al, Ba, Fe, Mn and Pb, and the accumulation of elements is dependent on the intensity of traffic and the air quality in urban areas (Simon et al., 2011). On the contrary, investigating roots of plants grown in an industrial area in Nigeria, it was detected that most elemental concentration detected in vegetables was not above the admissible levels (Tsafe et al., 2012). Just some elements, such as Cr, Mn, Pb and Zn appeared in high levels in vegetable leaves that were also verified by analysis of particulate matter (Tsafe et al., 2012). There were wide distinctions between the elemental composition of vegetables, soil and air. Root vegetables were found to accumulate soil Cd much more efficiently than other microelements (Voutsa et al., 1996).

Such elements as Sb, As, Be, Co, Hg, Cu, Cr, Mo, Mn, Se, Ni, and Zn principally enter the atmosphere from fossil fuel combustion processes but largest worldwide emitter of As, Cd, Cu, Ni and Zn is metallurgical industry. Gasoline and diesel that are the most popular fuels used by motor vehicles make regular emissions of Cd, Cu, Fe, Ni, Pb and Zn into air in various amounts (Allen et al., 2001).

Quantitative analysis of particulate matter can be used not only for the assessment of air pollution but also for estimation of the risks that can arise from possible element contamination for humans, animals and plants. There are several trace elements such as Cd, Hg and Pb that are toxic metals for living organisms even in very low concentration therefore particulate matter containing traces of these elements can be hazardous within environmental systems. It has been discovered that ultra-small particles (<100 nm) has significantly higher value of toxicity than

particles of larger size, and toxicological effects extends if particles are smaller (Meresova et al., 2008).

Food contamination is important issue within the assessment of food safety. Several pollutants entering food chain via environmental pollution are found in food, for example, organochlorine pesticides, potentially toxic metals are among the most concerning compounds (Khan et al., 2008; Koplik et al., 2004;Sharma et al., 2008; Villalobos et al., 2009). Environmental pollution impact on the concentration of elements in food has been studied in industrial and urban areas where locally grown or breed food has obvious contact with potential contaminants. Release of metallic elements and metalloids from outflows of manufactures and mining areas as well as transport exhausts, are well known sources of environmental pollutants (Villalobos et al., 2009). According to environmental research, food study may be used as instrument for the possible environmental impact assessment (Wunderlich et al., 2011).

The pollutants can easily move in the food chain. The animals or humans can be exposed to potential effects of contamination. For example, ingesting food such as cabbage, spinach and cassava grown along the roadsides or in urban areas (Eggen et al., 2011; Samsoe-Petersen et al., 2002; Wunderlich et al., 2011). Several cases has shown food crop contamination with such elements as Cd, Pb, Zn from leaching wastewaters as well from use of wastewater in irrigation of agricultural fields. High levels of Cd has been found in rice in Japan and in vegetables in UK, Hg in tissues of domestic animals in Brazil, as in vegetables in Urals, in Russia that have been grown near industrially active areas (Reilly, 2007).

Nowadays the problem of possible human health hazards by consumption of contaminated food crops has been recognized not only for large scale agricultural production but also concerning small scale production and community gardens (Alexander et al., 2006; Wunderlich et al., 2011).

2.2.4 Agricultural impact on the concentration of elements in food

Agro ecosystem is the environment used by human activities through the agricultural practice. Widely applied soil fertilizers usually contain impurity of

trace elements and can affect concentration of elements in soil in a great extent. Continuous use of these substances can lead to significant variations of soil physical, chemical and biological character that can be irreversible or reversible over a long time period. It is common that agriculture intensifies the weathering of soil that can result in the changes of the quantitative mobile forms of the elements through biogeochemical processes. This is assessed positively due to the increase of bioavailability of essential elements for crops; however, accumulation of potentially toxic elements also can occur (Kapoba, 2005).

Elements that are basic trace elements in agricultural and crop sciences are B, Cu, Co, I, Mn and Zn (Kapoba, 2005). In agricultural areas as well as in areas used for agriculture in the past, natural soil composition can be transformed by frequent applications of fertilizers and other agrochemicals. Use of mineral fertilizers may lead to higher concentration of Strontium in the soils, but application of organic fertilizers conveys significant amounts of As, Cu, Ni and Zn into the soils. Phosphorous fertilizers are the ones that substantially can affect soil element concentration by input of such elements as As, Cd, Cr, F, Ni, Pb, Sr and Zn (Kapoba, 2005).

Apart from the food chain contamination that can occur as consequences of excessive transfer of metallic elements or metalloids and pesticides from soils to edible plants, environmental effects such as extinction of vegetation cover induced by plant intoxication can also occur (Kabata-Pendias, 2004).

Transfer of inorganic and organic pollutant from use of agrochemicals, fertilizers and sewage sludge in agriculture can be detected. For example, fungicides containing copper applied in grape plantations can lead to wine contamination, use of some antifungal seed dressings can result in food contamination by organic mercury, but lead arsenate used as insecticide in orchards can contaminate fruits and their products with as in high levels. Sewage sludge can be applied as soil fertilizer, but in several cases they contain high concentration of Cd, Cu, Fe, Pb, Zn that can be transferred from soil to plants and food (Reilly, 2007). Application of fertilizers on agricultural soils is one of the main sources of nutrient element input in soils with subsequent transfer into biota. Long-term use of fertilizers

should not be encouraged due to prospective soil contamination with such element as heavy metal and pesticides (Jiao et al., 2012).

2.2.5 Impact of food processing on inorganic element concentration in food

Impact of food processing on food quality and composition has been established concerning several nutritional constituents as vitamins, antioxidants, proteins or in favor of organoleptic values such as taste, colour or smell (Awuah et al., 2007; Dewettinck et al., 2008; Odriozola-Serrano et al., 2013; Pingret et al., 2013). However impact of processing on the elemental content and concentration in food has not been studied fully mainly due to the fact that assessment of food processing is a complicated issue affected by many factors. Food is not homogeneous and therefore has to be brought under different processing conditions that are specific for various product groups, starting from cutting and milling of vegetables and cereals up to cooking and fermentation of meat and milk products.

Harvesting of food crops and processing of food of animal or plant origin can induce food contamination through contact with equipment or substances used. For example, fish and meat products can become exposed to painted or varnished surfaces. Processing of food can be done in various ways. For example, fermentation, sterilization, drying, chemical preservation depending on the hygiene and processing requirements. The most common material of food contact surfaces is stainless or aluminum steel ; however, other contacting surface materials can come into contact with food, such as acryl-based coatings on the walls, polyester or epoxy-based powder coating products on metal substrates, epoxy or polyurethane coatings on the floors, polytetrafluoroethylene (PTFE) coated standard conveyor systems (Forrest, 2009). Transfer of metallic elements and metalloids from processing equipment during food and beverages canning (lead), from using cooking utensils (e.g. Cu, Pb) and ceramic ware (mainly lead) as well from plastics and other wrapping materials (Cd, Co, Cr, Hg, Pb) has been detected (Reilly, 2007). Even use of certain detergents in cleaning of food processing equipment can lead to leaching of As, Cd, Hg and Pb from utensils made from stainless steel or aluminum.

Mineral content is inherent in natural foodstuffs. Apart being a substantial part of many enzymes, trace and macro-element presence play important role in food processing, for example, in fermentation producing alcoholic beverages, fermented dairy products and meat aging. Processing as well can decrease element concentration in food and therefore food fortification with certain elements such as Ca, Fe, Mg, and Se is practiced frequently. Compounds used as dietary supplements or food additives with antimicrobial, sequestrant, antioxidant, flavour enhancing and leavening, texturizing, and buffering properties contain metallic cations that can change natural element balance in food (Karley and White, 2009; Nabrzyski, 2007).

Also domestic food processing can influence element concentration in food. For example, milling of grains can reduce concentration of Ca, Fe, P and Zn. But fermentation of grain product increases bioavailability of Ca, Fe, P and Zn. Cooking and storage over 6 months of fortified blended food reduces trace elements concentration (Callanan, 2000). Although historical facts confirm possibility of extremely high food pollution by Pb in the past from ceramic kitchen ware with Pb enamel, dishes from Zn containing high impurity of Pb, also nowadays there still certain risks remain of possible food contamination with potentially toxic elements (Baltes, 1998).

2.2.6 Food contamination from storage and packaging materials

The metals primarily used in food packing are Al and Sn. Al is used to brand foil, cans, for lamination of paper and plastics. Mg or Mn is regularly added to Al to increase its properties. Al is extremely resistant to most forms of corrosion. It's normal covering of Al_2O_3 provides very real barrier to the effects of air, odours, temperature, light, humidity, chemical attack and microorganisms. Another metal widely used in food packaging materials is tinplate is produced from low-carbon steel coated with thin layers of Sn from both sides. Depending on industrial process, there are hot-dipped tinplate and electrolytic tinplate. Sn offers to steel some corrosion resistance, but for more protection tinplate containers are often lacquered to offer inert barrier between the food and metal. Also tin-free steel

recognized as electrolytic Cr or chrome oxide coated steel is used in food packaging materials (Forrest, 2009; Rial-Otero et al., 2009).

Food storage and packaging materials can influence not only food organoleptic and nutritional values but also may lead to food contamination if inappropriate materials are used and may lead to long term adverse health effects. For example, Al and Sn are widely used in cans for food storage as metals of low impact on human health. However, recent research studies suggest correlations between ingested Al and Alzheimer's disease development (Rial-Otero et al., 2009). It is detected that despite high Al resistance it can leach from the material of cans, cookware or other kitchen utensils to food (Joshi et al., 2003).

Packing materials and systems for food storage contain numerous substances which can migrate to food during processing and storage. However, the major concern regarding safety of food-contact materials is that the effects of prolonged exposure in humans are still poorly known (Rial-Otero et al., 2009). Information of food packaging materials and possible leaching of element is summarized in appendix II.

2.2.7 Food contamination from traditional utensils used in Africa

The cooking method requires the usage of utensils which are also liable for the presence of inorganic elements in food. Placed at the finale of the chain of food preparation, packing and utensils contaminate food via the leaching of materials used in their design.

In Democratic Republic of Congo, as in most Africa countries, much of the Africa population and almost all in the poor areas still used traditional cooking utensils. Most of the artisanal utensils are manufacturing by using a number of raw materials such as car and motor bike engine parts, waste aluminum and computer component.

The traditional utensil is not expensive and durable. In Kinshasa, the housewives like to cook rice, beans, meat, chicken and cassava in this utensil. The traditional utensil is the common utensil used to cook street food, where several people come to eat during launch time.

The study done on transfer of inorganic element in food from traditional utensil confirms that the utensils are a main source of food contamination by inorganic pollutants in developing countries (Dabonne et al, 2010). Uriah et al, (2014) compared the presence of inorganic pollutants in raw food and process food cooked in the traditional utensil, and found significant amount of inorganic pollutants such as Al, Cd, Co, Cr, Cu, Fe, Pb, Sb, Se, Ni and Zn. The traditional utensils constitute a potential source of contamination of food from inorganic elements.

2.2.8 Bioavailability of inorganic elements

Bioavailability can be defined as the levels of a nutrient that is potentially presented for absorption from a matrix (e.g., from soil for crops or from food for humans) and when absorbed, utilisable for metabolic processes in the organism (Welch and Graham, 2005). Total content of elements is the value that can characterize certain matrix like soil, plant or processed food, in general. Detailed information about the bioavailability of elements can be achieved if it is known in what chemical compounds they are involved. The investigation of element bioavailability is complicated interdisciplinary issue dependent on chemical, environmental, nutritional, physiological and epidemiological impacts that in many cases is still at the level of suggestions and assumptions even if it has been investigated for decades. Trace and macro-elements reaching human body can be absorbed by three pathways:

- Gastrointestinal tract element absorption from food, water, food
- Supplements, drugs, soil, particular matter, atmospheric aerosols;
- Respiratory system element absorption from particular matter,
- Atmospheric aerosols, gases;
- Skin element absorption from soil, water, atmospheric aerosols, gases, chemical substances (Kabata-Pendias and Mukherjee, 2007).

Inorganic elements pass through several of organs before entering the blood stream. It can enter the organism by ingestion, inhalation or dermal absorption. Inhaled particles pass through the respiratory system, ingested particles pass

through the gastrointestinal tract and dermally absorbed elements enter directly into the blood stream. The consumption of an inorganic element also commands how much the body will absorb, for example only 6% of ingested Cd is absorbed in contrast to 50% of inhaled Cd. It accumulates in adrenal pericarp possibly binding with proteins. Elimination half-life period for Cd is very long 10 to 30 years. Contamination by high doses of Cd can result in chronic intoxication (Baltes, 1998; Bowman et al., 2003). Absorption of ingested metallic mercury is very minimal (0.01%). Organic mercury is absorbed from gastrointestinal tract up to 90% of uptaken dose. Absorption of inorganic mercury is medium, 7-15% (Liguts, 2001). Human body can absorb 5-10% of uptake amount of Pb which is tended to accumulate in bone tissues and viscera. Accumulation of Pb for humans is very dangerous due to the possible release of element in human body (Baltes, 1998).

Low element concentration in environment is not the only one reason for element deficiency in human nutrition. Important issue is the chemical form of element. For example, nowadays the Zn deficiency has been recognized in developing countries where local food contains Zn of low bioavailability. Other element, such as Fe, bioavailability can be affected by presence of phytate that is a plant substance and also by antagonist elements such as Co, Cu, Zn and Mn. Bioavailability of Zn also can be reduced by antagonism with phytates, Ca, Cu, Fe, Ni and P (Abrahams, 2002). Bioavailability of elements is dependent on element chemical form, the composition of diet and health situation of the individuals. The data of concentration of micro and macro-elements in food can give important information about dietary habits of special group, health situation of individuals and origin of elements. It is important to determine the daily dietary intake of microelements, their concentration and sources.

Fe is part of haemoglobin, Ca, P and other elements constitute a significant part of mass of teeth and bones, Na, K, P, S, Cl constituents of fluids inside and outside body cells. Other function regulation of biological activities Ca for normal blood clotting, Mg stimulates activity of many enzymes etc. (Aras and Ataman, 2006).

Determination of total content of elements in a matrix is a tool to assess the overall understanding about the element amounts but deeper investigation is needed for the assessment of bioavailability of elements. The investigation of bioavailability is a complex set of mutual factors.

The research of element bioavailability has been performed on main food crops such as wheat, rice, cassava, maize and beans (Welch and Graham, 2005). Heavy metals and their toxicity as well as a general overview on organochlorine pesticides and their impact in the human health. It is too complicated to do complex investigation of the bioavailability of various elements at the same time; therefore, the basic target is to understand the overall principles and to create example models. More deep studies of element bioavailability from food crops have been studied concerning Ca and Fe on an in vitro (Viadel et al., 2006). This model has been adopted also for determination of Zn bioavailability from food crops using such protein as metallothionein (Welch and Graham, 2005).

Zn absorption is lower for vegetarians than non-vegetarian diet. Therefore, it have been suggested that the Zn requirement for vegetarians is around 2 times bigger than for those consuming a non-vegetarian food. Poor bioavailability is one of the reasons of dietary deficiency in humans. It can be attributed to such element as Zn and can be connected with phytate presence in food of plant origin such as unrefined cereals and legumes. It is also connected with Mg and potassium salts. Phytate tends to make strong chelated with metal ions, particularly Zn, but also Fe and Ca and as a result insoluble complex compounds are derived that cannot be absorbed through gastro-intestinal tract of humans due to absence of intestinal phytate enzymes. High concentration of Ca can lead to even more insoluble complex formation such as calcium-zinc-phytate complex. Where Zn deficiency has been observed, also Fe deficiency is common, because these metals have similar distribution in nutritional sources and their bioavailability can be affected similarly (Abebe et al., 2007).

Several inorganic elements such as Ca, Cu, Fe, K, Mg, Mn, Na and Zn function in cationic forms that allow them to be involved in chelation processes in contact with proteins or small organic molecules. Besides insoluble chelates (with phytic

acid or oxalic acid) that cannot be assimilated in human organism, there are several chelates that are of high importance in metabolism. Here can be mentioned hemoglobin and myoglobin where Fe is chelated. Other chelates, such as amino acids, EDTA promote transportation, absorption and storage of mineral ions (Combs, 2005).

Macro-elements and trace elements bioavailability depends on physiological and exogenous factors. Physiological factors of element bioavailability comprise:

- Age-related regressions in the efficiency of enteric absorption of Cu and Zn;
- Premature postnatal deficiency of regulation of absorption of Cr, Fe, Zn;
- Adaptive rises in the absorptive efficiencies of Cu, Cr, Fe, Mn and Zn by receptor up regulation through stages of deficiency;
- Requirement on other nutrients for the bio-functions of Se and I in thyroid hormone metabolism, and Cu and Fe in catecholamine metabolism;
- Anabolic effects on tissue sequestration of Zn and Se;
- Catabolic effects on Cr, Se and Zn losses (ATSDR, S.A.; Combs, 2005).

The ability of living organism to absorb and use same micro- and macro element from food depends on several factors:

- The form of the element in food, e.g., Fe from meat (haem Fe) can be more simply absorbed than Fe contained in plant foods;
- Other components of the nutrition, which either enhance or inhibit element absorption, e.g., the absorption of Fe from plant foods is enhanced by eating food with high concentration of vitamin clike oranges or tomatoes, but inhibited by compounds such as tannin (present in tea) and phytate (present in cereals).

2.2.9 Toxicity of inorganic elements

The international Union and Applied Chemistry (IUPAC) estimate that a substance can be toxic if it has ability to cause negative effects to a living organism. Toxicity of a substance depends to the amount of substance administered, the type of injury causes, the route in which the chemical is administered and distributed in time (single or repeated doses), the necessary time

to produce the injury , the nature of the organism(s) affected and other relevant conditions (Duffus et al., 2007).

Toxic substances can cause cumulative and non-cumulative effects. Cumulative effects are adverse effects that occur after repeated exposure by a toxic substance, while non-cumulative effects may be caused by rapidly absorbable and rapidly eliminated toxic substances (Duffus et al., 2007). Cumulative effects usually are associated with accumulation of a substance in tissues or organs of living organisms or in elements of ecosystems.

Adverse effects can be initiated by excess or deficiency of elements in human body, including essential elements, but the exposure of toxic elements to human organism can be caused by very small concentration and may result in serious adverse health problems (Abrahams, 2002). Special attention should be focused on elements and substances with revealed or suspected Carcinogenity and neurotoxicity as the adverse effects caused by them are cumulative, more destructive and irreversible.

Chronic toxicity continuous and reiterative exposure of non-lethal doses of a substance that causes cumulative damage for organism. However, in the assessment of food contamination it is more correct to deal with Minimal Risk Levels (MRL) derived by the agency for toxic substances and disease registry in the USA. MRL is an estimate of the daily a living organism exposure to a toxic. Minimal risk levels are derived from acute-duration (1-14 days), intermediate-duration (>14-364 days) and chronic-duration (≥365 days) oral exposure studies (ATSDR, S.A.). Oral MRLS as well as estimated oral intake levels for several elements are summarized in appendix IV.

2.3 Organochlorine pesticides

Organochlorine pesticides are any chemical or mixture of chemical used to destroy, prevent or control any pest and undesirable plants or animals producing destruction or interfering with the production, processing, storage, transport or selling of food, agricultural commodities, wood and wood products or animal foodstuffs (FAO, 1983). Pesticides are categorized based on functional groups in

their molecular structures such as inorganic, organo nitrogen, organo halogen and organo sulphur compounds. They can be also classified on their particular biological activities or target species such as fungicides, herbicides, acaricides, and insecticides (Sverdrup et al., 2002; Adeyemi et al., 2008).

Pesticides contribute to increased quantity and variety of the diet, crop yields and help to limit the extent of some illnesses (Nakata et al., 2002). Pesticides are harmful to human health. The antagonistic health effects of pesticides are grave. Organochlorine pesticides can cause destruction of the nervous cells, dysfunction of the immune, lung damage, decrease the capacity of reproductive organs, and endocrine systems, cancer and birth defects (Ayejuyo et al., 2008).

Organochlorine pesticides compounds are synthetic organic insecticides that contain C, H, Cl and sometimes O. This group of synthetic chemicals was introduced for the first time in 1930s and was used extensively in agriculture immediately after the Second World War (Darko et al., 2007). Organochlorine pesticides have been widely used through the earth since the middle of the 20^{th} century and had been used globally mostly in public health activities in an attempt to combat vector borne diseases in agriculture and animal production (Darko et al., 2007). Consequently, pollution of the environment by organochlorine pesticides is found in many places due to the fact that these compounds are highly lipophilic and do not yet degrade in the environment hence stay in the environment and food web long after being applied .They can also be transported by air and water such that trace amounts are all over the world (Doong et al., 2002).

2.3.1 Dichloro-diphenyl-trichloro-ethane group

Dichloro-diphenyl-trichloro-ethane (DDT) is one of the most recognized synthetic compounds which were extensively used to control insects in agriculture and disease vectors. In this group the most important compound is DDT and its metabolites p, p'-DDE and p, p'-DDD. These metabolites can be observed or recovered in soil or foodstuff as residue several years after use. Exposure to these chemicals can be direct. For example occupational exposure or can be indirect

through consumption of foods containing residues of these chemicals (Essumang et al., 2009).

Accumulation along food chain has been investigated and noted that such accumulation can be mortal to humans. DDT was detected in various food commodities such as animal products, poultry, fish, human fat and milk (Forget et al., 2001). DDT has been associated with premature births as well as affecting neurobehavioral functions (Guan et al., 2009).

The concern about DDT persistence and negative health effects has had a significant impact on agriculture and vector control. Since DDT is known to induce microsomal enzymes, this may quantitatively alter the response to various drugs and toxic compounds as well as to naturally occurring substances in the body leading to alteration of homeostasis of biochemical processes. In the air DDT is broken down slowly top, p'-DDE and p,p'- DDD by microorganisms (ATSDR, 2000).

2.3.2 Cyclodienes group

This group includes aldrin used as ant killer in construction work, dieldrin, isodrin, heptachlor and endosulfan which are highly insecticidal. Endosulfan is of environmental importance because of its apparent persistence and toxicity to many non-target organisms like fish (Helberg et al. 2005).

2.3.3 Hexachlorocyclohexane Group

Hexachlorocyclohexane (HCH) exists in isomers and most common isomers include α-HCH, ß-HCH, γ-HCH and δ-HCH had been the most used for the control of insect pest on agriculture field. Lindane (γ-HCH) is commonly used as dust in seed treatment by farmers. Although γ-HCH is persistent and hard to biodegrade, some microorganisms had been isolated which can be able to degrade one or more HCH isomers under aerobic conditions (Martinez et al. 1997).

2.3.4 Harmful effects of organochlorine pesticides

The presence of organochlorine residues in human tissue fatty have been associated with development of breast cancer due to anti-androgenic and estrogenic properties and their effects on sexual activity (Lopez et al. 1996). After metabolism of the residues in the living organism, they are accumulated in the fatty tissues from where they enter in blood circulation (Ahlborg et al. 1995; Hunter et al. 1997).There is uncertainty about the negative effects that these residues may have after a lengthy human exposure at low doses (Cerrkvenik et al., 2000; Nag, 2008).The compounds had been reported to cause several effects such as teratogenic, immunologic, reproductive, carcinogenic and neurologic problems in organisms (Kodba , 2007; Soler and Yolanda , 2007).

2.4 Risk assessment

Risk assessment is a procedure leaded to assess the menace to a given target organism or system. Risk assessment of pollutants leads to procedures and methods that apply to evaluate the negative effects caused by the pollutants. Risk assessment can be done to assess past, current and future exposure to any pollutants found in the environment. They may be qualitative or quantitative in nature.

Risk assessment is normally done in an approach that is improbable to undervalue the current menace. Anyway, substance risk assessments depend on scientific consideration of chemical activities, exposure, amount and toxicity. In overall terms, risk depends on the following factors: the quantity of a pollutant existing in an environmental medium, food and/ a product; the number of a person has with the pollutant in the medium; and the harmfulness of the pollutants (IPCS, 2004).

The information to describe these three factors is the foundation of most chemical risk assessments. The data are not always available, many risk assessments involve that evaluations or judgements be made concerning some data inputs or categorizations. Therefore, risk assessment results have linked uncertainties, which should be categorised as much as possible (IPCS, 2004). In this study, the

following variables or indices were used to assess the level at which the consumers are exposed to heavy metals risk through food consumption: Daily Intake of Metal (DIM) and Hazard Index (HI) of the pollutants.

2.4.1 Daily Intake of Metal (DIM)

The daily intake of metals (DIM) is the evaluation of the level of the pollutants contained in food eaten per day measured in mg kg^{-1} per body weight. It depends on the metal level in yields and the quantity of consumption of the respective food crop. DIM is calculated by the multiplication of the amount of the pollutants measured in the food sample by the estimated amount of the food eaten daily, divided by the average body weight of an individual. This index helps to determine the amount of the heavy metal consumed in a certain food material per day.

2.4.2 Hazard Index (HI)

The Hazard Index (HI) is a measure of the potential risk of negative health effects from a mixture of chemical constituents in various food samples. When exposure implicates more than one chemical, the amount of the individual hazard quotients for each chemical is used as a measure of the potential for harm. This sum is called the hazard index (Caylak, 2012)

2.4.3 Review of metals risk assessment

The amount of metals in edible vegetables and daily intake in Sarandaj, Iran has been reported by Maleki and Zarasvand (2009). They have found that the dietary intakes of Cd, Cu,Cr and Pb through consumption of Allium ampeloprasum, Ocimum basilicum), Petroselinum crispum), Lepidium sativum) and Artemisia dracunculus were estimated to 0.07, 2.50, 1.72 and 2.96 mg day^{-1}, respectively.

They have concluded that the vegetables grown in Sarandaj region of Iran are more polluted and can posed hazard for human consumption (Malek and Zarasvand, 2009). The heavy metals risk assessment of vegetables carried out by Harmanescu et al. (2011) in Banat city of Romania to determine the amount of

metals (Fe, Mn, Zn, Cu, Ni, Cd and Pb) found in parsley, carrot, onion, lettuce, cucumber and green beans. They compared plants in polluted mining sites with those grown in the reference area. They determined their potential negative effects via calculation of the daily metal intake (DIM) and Hazard index (HI). They have reported that the HI values calculated were under the permissible limit of HI < 1 for all vegetables grown in clear area. In mining area the HI were above 1.

Abbas et al. (2000) reported that the vegetables grown in Sindh province have a higher level of Cd, As and Mg than the permissible limit set by FAO and WHO. Mapanda et al., 2007 assessed the contamination of vegetables by metals in two city of Harare (Zimbabwe). The results from their research shown that the concentration of metal in the vegetables were above the permissible limit set by FAO/WHO and HI were above 1. They concluded from results that the vegetables were not safe from metal toxicity and suggested the need for an improved food quality assurance system to ensure compliance with existing standards on heavy metal concentrations

Nkansah and Opoku-Amoako (2010) investigated in Kumasi (Ghana), the amount of Pb, Zn, Ni, Cu, Fe and Hg in fifteen spices sold at the local markets in Kumasi. They have revealed that most of the spices have an acceptable level of metal according to the standard limit set by FAO/WHO (2000).

In Nigeria, Batagarawa and Lawal, (2010) studied the levels of some metals in moss plant harvested from the streets of Dutsinma town of Katsina State of Nigeria. They have observed that these plants content a higher amount of metal compared to the permissible limit set by FAO/WHO. They reported that the absorption of these metals was related to their concentrations in the atmosphere as a result of industrial and automobile emissions. They attributed the high concentrations to the increase in motor vehicles and some industries sited in the town over the recent time.

Chapter 3: Objectives of the study

3.1 General objective

Artisanal aluminum utensil has become one of the most used in Africa, due to its affordability and durability. The widespread use in developing countries has made it imperative to raise the investigation about the possible metal leach process during its use and the level of metal leached into food. Relevant data and accurate on metal leach in foodstuffs cooked with traditional utensil are important for accurate assessment of potential adverse human health effects and the environment. Studies on the interaction between metal from the artisanal aluminum utensil and different solutions have been investigated for a long time in terms of corrosion of the utensil. (Dabonne et al., 2010; Anthony et al., 2013).

In other hand, persistent organic pollutants like pesticides are still used to protect the crop against the insect. Some studies reported that the organochlorine pesticides used in Africa are the lowest overall for all continents because of poverty, instability, unreliable rains, and soils. These factors kept small owner agriculture from developing in many regions. There are some areas of intensive organochlorine pesticides used, such as Sudan, Egypt, Nigeria, Ivory Coast and South Africa. Many organochlorine pesticides have been banned or whose use has been severely restricted in Europe and North America but in Africa, these organochlorine pesticides are still marketed and used.

DDT, the pesticide most used in the world. In 1994, DDT was forbidden but in September 2006, the WHO commended extensive use of indoor residual spraying of insecticides. For example DDT was used to combat mosquitoes and other disease vectors. In many parts of African countries DDT is used and it accumulates in crops, contaminates water and pollutes the atmosphere (FAO/WHO, 2000). People are poisoned when they eat the vegetables that contain the residues of persistent organic pollutant. The persistent organic pollutant has the potential to cause serious environmental and health problems in African population.

The main objective of this study was to determine the presence of metals and organic pollutants (organochlorines) in African food (cooked and raw food) and to assess its health risk in Africa's population.

3.2 Specific objectives

- To determine metals and organochlorine pesticides content in foodstuffs collected in Kinshasa (Democratic Republic of Congo) and Johannesburg (South Africa).
- To compare levels of metals and organochlorine pesticides in foodstuffs in both countries.
- To determine the impact of traditional cooking utensils on trace element in food process
- To estimate organochlorine and metals dietary intakes by citizen in both countries and compare them with the WHO guidelines so as to asses potential health risk.

3.3 Research questions

The following questions were addressed in order to achieve above objectives:

- What are the concentrations of pollutants in the food samples and is there a reason to consider those levels as a potential risk for human health?
- Is it true that all vegetables sold in Africa market contain the pesticide residues?

Chapter 4: Materials and Methods

In this section the details of metals and organochlorine pesticides analysis are described.

4.1 Study area

The current study was carried out in Kinshasa and Johannesburg cities. Kinshasa is the political capital of Democratic Republic of Congo. The city has an approximate population of 9.5 million and is the third largest city in Africa. It covers an area of 583 sq. km^2. Kinshasa lies slightly to the south of the Equator. It is located on the south bank of the Congo River. Just a cross the river and directly opposite to it is located Brazzaville, capital of Republic of Congo. Kinshasa has a tropical wet dry type of climate with a long and wet rainy season (Tuakuila et al., 2012).

The second city of study was Johannesburg. It located in the Gauteng province and it is the largest city in South Africa. The population is estimated to at 7,860,781 (7, 2% of South African population). Johannesburg is economic capital of South Africa. It is a principal point for commerce, business, industry and retail. Johannesburg markets are provides by vegetables, fish, meat and other foodstuffs from many areas around the city.

4.2 Sample collection

As mention in study area, the food samples were collected in both cities Kinshasa and Johannesburg. In Kinshasa the food samples were collected in the time period of 25 Nov – 25 December 2013 and in Johannesburg, the food samples were collected on 17 Jan – 17 Feb 2014, in each city 46 food samples were collected. Food for sampling was selected as follows:

- The vegetable products : cabbage (n = 10), cassava (n = 10), beans (n = 10);
- Animal products : Beef meat (n = 10) ;
- Fish products : Tilapia (n = 6);

- Three traditional utensils from Kinshasa and other three from Johannesburg.

In Kinshasa, the food samples were collected in three biggest urban market of Kinshasa such as central market (Zando), Gambela and Matete market. All these markets are public and very busy. The foods are sold on the floor without any hygienic condition and in some areas near the high traffic road. The vegetables from Kinshasa were collected in the individual farms. The farms were located near the high ways where the traffic is very high and with a lot of dust around.

The beef meat from Kinshasa was bought in the markets and the source was not clarified and the fish was from to Congo River. All the food samples were kept in closed polypropylene plastic box in refrigerator (-4°C) at the University of Kinshasa before being courier to South Africa.

In Johannesburg, the food samples were obtained at Johannesburg town market from individual vendors. Concerning the vegetables (cabbage, beans and cassava) and the beef meat bought in Johannesburg market, the sources were not clarified. The tilapia fish bought in Johannesburg market was from Mpumalanga. All the food samples were kept in closed polypropylene plastic boxes in refrigerator (-4°C) at the University of Witwatersrand.

The raw food collected in Kinshasa were cooked with the traditional pots purchase in Kinshasa and the raw food collected in Johannesburg market were processed with the traditional pots collected in Johannesburg. The raw foods were cooked as follow as:

Beans were chosen because of its long cooking time (contact time), but also high temperature. To cook the beans, 500 g were put in both pot filled with water and the cooking took 4 hours on traditional pots in the oven. Cooked beans were dried in the freeze dryer for 3 days. Finally the samples were ground in a mortar in order to be reduced in small pieces (powder).

Fish (tilapia) samples were cooked according to the traditional recipes in DR Congo and Johannesburg. The fish was chosen because it is one the most food eaten. To cook the fish, 500 g were put in both pot filled with water and 4ml of acetic acid. The cooking took 30 minutes on traditional pots in the oven. Cooked

fish were dried in freeze dryer for 3 days. The samples were put in mortar in order to be reduced in small pieces.

Beef meats were boiling in the traditional pots collected in Kinshasa and Johannesburg. The boil these meat, 100 g were put in the pots filled with water. The cooking took 45 minutes on the traditional pots in the oven. Cooked meats were dried in the freeze dryer for 3 days. The samples were reduced in power in the mortar.

Cabbage and cassava were cooked conferring the way it cooked in South Africa and Johannesburg. To cook these vegetables, 100 g of cassava and 100 g of cabbage were put in the pots filled with water. The cooking took 40 minutes on the traditional pots in oven. Cooked vegetables were dried in the freeze dryer for 3 days.

4.3 Reagents

All used chemicals were of analytical grade or higher, provided for analysis of elements in trace levels. Concentrated analytically pure nitric acid (65% w/v, supra pur grade) and hydrogen peroxide (30% w/v, supra pur grade) were obtained from Merck (South Africa). Hydrochloric acid; acetic acid; sodium chlorate, magnesium sulfate, acetonitrile pur, hexane pur and toluene pur were obtained from Sigma – Aldrich. For the ICP-OES and ICP - MS, stock solutions were also supplied by De Bruyne (South Africa) at a concentration of 10 mg L^{-1}. All instrument standard solutions were prepared on the day of use.
Certified reference material samples such as cabbage (BCR-679R), fish (BB422 – Fish ERM) and beef meat (BCR -185R beef meat) were obtained from Sigma – Aldrich (Germany).

4.4 Cleaning protocol

In trace analysis, contamination is a major source of error in environmental analysis. Clean up may reduce or minimize of any contamination in the sample. The best way to control the degree of contamination at any step of sample treatment is to use a blank. It is important to work under a clean environment for

successful analysis at very low concentration levels. A rigorous cleaning procedure for all vessels was used in sampling and sample preparation. The following cleaning procedure was used (Rosamond, 2014):

- Running tap water was used to initially rinse all vessels (plastics and Glassware) so as to remove all solid and liquid dirt.
- All vessels were washed with soap after which they were rinsed thoroughly with running tap water and then de-ionized water with an electrical resistivity of 18.2 MΩ cm (Millipore, USA). For organic analysis, the vessels were rinsed with appropriate organic solvent.
- All vessels were then soaked in a 10% (v/v) HNO_3 analytical grade (Merck) solution for 3 days after which they were thoroughly rinsed with deionized water.

4.5 Moisture determination

All samples were freeze dried at -40°C for a period of 3 days using the Labconco freeze drier (Vacutec, South Africa). After drying, the dry mass and % moisture content of the food sample was determined using the following equation:

$$\%\ \textbf{Moisture content} = \frac{w_2 - w_3}{w_2 - w_1}$$

Where;

W_1 = weight of container (g),

W_2 = initial weight of container with food sample,

W_3 = final weight of container with sample

The food samples were then crushed and homogenized using a mortar and pestle with the aid of liquid nitrogen.

4.6 Metal analysis

In this sector the details of metal analysis are described.

4.6.1 Microwave digestion of food sample for metals analysis

The food samples were digested in microwave digestion system as follows:

A mass of 0.25 ± 0.005 g of homogenized algae samples were weighed using an analytical balance (Precisa 180A, Switzerland) and placed into acid washed digestion tubes (PTFE-TFM liners) onto which 16 ml HNO_3 and 4 ml H_2O_2 were added before being placed into the Multiwave 3000 microwave digester (Anton Paar, Switzerland) shown in (Figure 4.1).

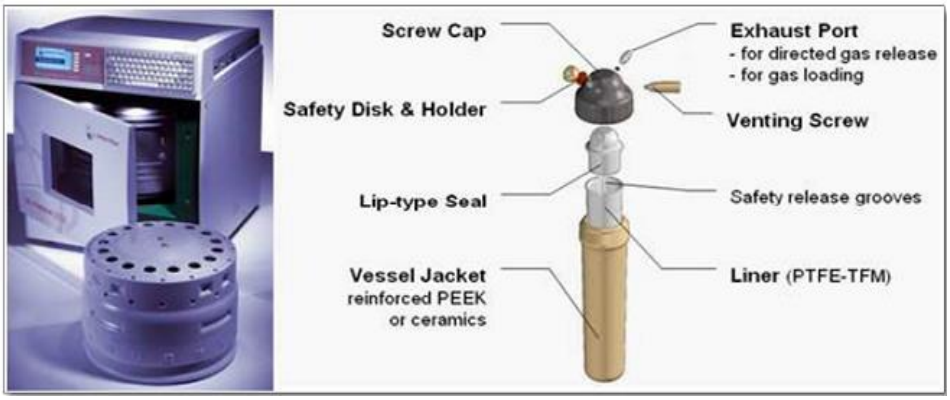

Figure 4.1 The Multiwave 3000 MAE system and the vessel design

Table 4.1 shows the microwave program for the sample digestion process. The digested samples were transferred into 50 ml acid washed volumetric flasks and the volumes were completed with deionised water after which they were kept at 4°C until further analysis.

Table 4.1: Microwave program for sample extraction

Phase	Power (W)	Ramp (min)	Hold (min)	Fan
1	600	10:00	10:00	1
2	0	05:00	05:00	3

Sample weight: 0.25g; Reagent: HNO_3 (16 ml); H_2O_2 (4 ml)

4.6.2 Inductively coupled plasma-optical emission spectroscopy (ICP - OES) measurement

Total metal concentrations of food samples were obtained by using an Inductively Coupled-Plasma-Optical Emission Spectroscopy (ICP-OES) instrument (Spectro,

Kleve, Germany) (Figure 4.2) with coupled charge detection (CCD) system. The instrumental conditions (Table 4.2) were optimized to obtain sufficient sensitivity and precision and the concentration of each element was determined at various wavelengths.

Wavelengths that represent a good compromise between maximum sensitivity and the ability to encompass a large range of concentrations are selected. The stock solutions supplied at a concentration of 10 mg l^{-1} were used to make working standards of 0.05 to 1 mg l^{-1}.

Table 4.2: Optimized parameters of the ICP-OES

Parameters	Value
Coolant flow	14 ml min^{-1}
Plasma power	1400 W
Auxiliary flow	1 ml min^{-1}
Nebulizer flow	1 ml min^{-1}
Type of nebulizer	Cross-flow
Injector tube diameter	0.889 mm

Figure 4.2 Inductively coupled plasma – optical emission spectroscopy (ICP-OES).

4.6.3 Inductively coupled plasma – mass spectrometry (ICP –MS)

An ICP-MS (Agilent technology, 7700 series ICP - MS) instrument, shown in Figure 4.3 was used for trace elements determination. Acid-washed torch and spray chamber were used in order to minimize contamination. The nebulizer was also rinsed with deionized water and silico-steell tubings (Restek) were used to reduce mercury adsorption in the sample introduction system.

ICP-MS parameters such as ion optics voltages, mass scan, time scan, pump speed, argon flow were optimized for a better resolution and analyte-background intensity ratio. A standard solution were prepared on a day of analysis from a stock solution of 10 mg l^{-1} (De Bruyn Spectroscopic Solutions, SA) for the instrument calibration and analyses were performed on triplicates.

Figure 4.3 Inductively coupled plasma – mass spectrometry (ICP- MS)

4.6.4 Determination of total mercury concentration in food samples

The total mercury concentrations in food samples were determined using the Hydride generation Flow Injection Mercury System FIMS 400, (Perkin Elmer, USA) which uses a Cold Vapour Atomic Absorption Spectrometry (CVAAS) detection system. Quantification was with respect to reagent-matched mercury standard prepared by serial dilution of 10 mg l^{-1}. Tin chloride ($SnCl_2$) 1.1% (wt.)

in 3% (v/v) hydrochloric acid (HCl) was used as a reductant and 3% (v/v) HCl as a carrier for the Hydride generation Flow Injection Mercury System.

4.6.5 Quality assurance of analytical methods for metals

To ensure reliability of the results, appropriate quality assurance procedures were carried out. Distilled deionised water was used throughout the study. Glassware was correctly cleaned, and the reagents were of analytical grade. Blank solutions were used to correct the instrument readings. The reference materials (SRM) obtained from sigma-Aldrich, including cabbage (BCR-679R), fish (BB422 – Fish ERM) and beef meat (BCR -185R beef meat) was used for validation of the analytical procedure.

4.6.6 Method Limit of Detection

The Limit of Detection (LOD) is the lowest analyte concentration which can be detected by the machine used (Armbruster et al., 2008). This was calculated using the following formula (Hutter, 2011)

LOD = 3 x standards deviation of a blank sample

4.6.7 Precision

Seven measurements were performed in each replicate sample so as to assess the repeatability and reproducibility of the analysis**.**

4.6.8 Accuracy

The accuracy of the analytical technique used was evaluated by relating the results obtained from CRMs analyses to certified values.

4.7 Organochlorine pesticides analysis

In this sector the details of analytical methodology of organochlorine pesticides are described.

4.7.1 QuEChERS Extraction method

Ten grams of food sample was homogenized using a mortar and stored in plastic bottle and kept in the freezer until analysis. The QuEChERS extraction method was done using the procedure reported by Ramalhosa et al., (2009). Ten grams of food sample was weighed in 50 ml Teflon tube and spiked sample with 2 µl (1 ng g^{-1}), 10 µl (5 ng g^{-1}) and 20 µl (10 ng g^{-1}) standards of seventeen organochlorine pesticides. The spiked samples were allowed to stand for 30 min and 10 ml of acetonitrile was added and shaken strongly for 1 minute. This was followed by salting-out step with 1.5 g sodium chlorine and 3 g of anhydrous magnesium sulphate into the tube and the mixture was shaken vigorously for 1 min and then placed in the centrifuge. After centrifuge, 6.5 ml of organic supernatant was transferred into the polypropylene centrifuge tube to clean-up with 1.65 g anhydrous magnesium sulphate and 27.5 g primary second amine (PSA). The solution was centrifuged for 5 minutes and filtered using a 0.45µm PTFE and injected in the GC –ECD for analysis.

QuEChERS extraction method was applied for food samples. Five grams of food samples were placed in a 50 ml of Teflon tube, 10 ml of acetonitrile added and samples shaken energetically for 1 minute. A mixture of 3 g of $MgSO_4$ and 1.5 g of NaCl was added and shaken vigorously for 1 minute. Then the mixture was centrifuged for 5 minutes at 40 rpm. After centrifuge, 6.7 ml of organic supernatant was collected in to 15 ml Teflon tube. 100 mg of $MgSO_4$ and 150 mg of PSA were added to the samples. Samples were shaken energetically for 1 minute and then placed in a centrifuge for 5 minutes at 40 rpm. After centrifuge, samples are filtered using a 0.45µm. The samples were analysed using GC-ECD.

4.7.2 Quality assurance of analytical method for organochlorine pesticides determination

Quality assurance of applied analytical methods was achieved by preparation of blank samples and standard solutions. The limit of detection (LOD) was determined based on the signal – to – noise method. The relative standard deviation (RSD) of the injection concentration is less than 20% (Armbruster and Pry, 2008). The accuracy of the extraction method is determined by its

reproducibility and its repeatability. The spiked concentrations were 1 ng g^{-1}, 5 ng g^{-1} and 10 ng g^{-1} of the pesticide Organochlorine standard were used for recovery calculation.

4.7.3 Gas chromatography – ECD procedure

A Gas chromatography, Agilent technologies 7890 A system equipped with ECD detector was used (Figure 4.4). Nitrogen (99.99%) was used as carrier gas flowing at 1.2 ml min^{-1}. The oven temperature was programmed from 60°C (1minute) to 180°C at a rate of 30°C min^{-1}, 180°C (3 minutes) to 300°C at a rate of 3°C min^{-1}. The temperature of the injector operating in split less mode (volume injected 1 ml) was held a 300°C and electron-capture detector temperature was 350°C.

Figure 4.4 Gas chromatography – Electron Captor Detector

4.8 Toxicological Evaluation

In this sector the details of risk assessment analysis are described.

4.8.1 Daily intake of metal (DIM)

For preliminary risk assessment of consumer exposure to inorganic element and organochlorine pollutants. The calculations of DIM are described as below:

DIM (mg/kg body weight/day) = $\frac{\boldsymbol{portion\ in\ Kg\ consumed\ per\ day\ X\ residue\ level}}{\boldsymbol{body\ weight}}$

Daily intake of metal of food was determined by conducting a survey to adult people (males and females) having a mean body weight of 55 kg. The daily intake of metals of particular foods was determined.

4.8.2 Health risk index (HRI)

To assess the human health risk of toxic elements, it is necessary to calculate the level of human exposure to that toxic by tracing the route of exposure of pollutant to human body. There are many exposures routes for toxic elements that depend upon a contaminated media. Many population use the food enriched with higher concentration of toxic elements which enters the human body leading to health risk (Bennion et al., 2005).

The health risk index for toxic elements by consumption of contaminated food was calculated by following equation:

HRI = $\frac{DIM}{R_{fd}}$

DIM = Daily intake of metal

R_{fd} = Reference oral dose

If HRI is bigger than 1, indicated that there is a potential risk associated with that toxic elements and if HRI is less than one that there is not a potential risk associated with that toxic elements.

4.9 Data analysis

Description of data was performed using Minitab 16 statistical software. The student's t-test was used to compare the results. The results obtained in this study were compared with FAO/WHO and other studies done in other countries in the world.

Chapter 5: Results and Discussion

5.1 Verification of analytical methods by analysis of metals in certified reference samples

Certified reference materials: BCR-185R -beef meat, BCR-679R-Cabbage, BB422 – Fish ERM were used for verification of applied analytical methods. Beef meat reference material has been designed for measurements of seven elements only. The analysis of cabbage reference material supported determination of accuracy and precision of measurements for ten elements. Obtained data revealed methodological distinctions and performance in respect to accuracy of measurements. The highest recovery was detected for Zn (99.44±1.83%) in fish reference material and the lowest was found for Manganese (81.20±4.09%) in cabbage reference material (Table 5.1). For the analysis of trace elements at mg kg^{-1} levels, accuracy and recovery of 70-120% considered acceptable (Kelly et al., 2013). The high recovery in various certified reference materials and low standard deviation indicate the accuracy of the method. Analysis of beef meat and fish reference materials revealed similar values of accuracy and precision of the method (Table 5.2 and 5.3).

Table 5.1 Accuracy of applied analytical methods detected by the analysis of certified reference material BCR-679R-Cabbage

Element	Certified value mg kg^{-1}	Recoveries (%)
Al	30	97.00±3.45
As	14.8	81.89±4.23
Cd	1.66	87.35±1.93
Cu	2.89	92.73±2.61
Mn	13.30	81.20±4.09
Cr	27	90.04±2.51
Se	11.80	87.29±1.89
Zn	79.70	96.50±2.71
Hg	6.30	96.35±3.19
Pb	20.60	96.46±2.03

Table 5.2 Accuracy of applied analytical methods detected by the analysis of certified reference material BB422 – Fish ERM

Element	Certified value mg kg^{-1}	Recoveries (%)
As	12.70	87.17±2.13
Cd	0.075	96.00±1.56
Cu	1.67	95.81±3.01
Hg	0.631	98.73±2.39
Mn	0.368	93.75±2.03
Se	1.33	97.74±2.51
Zn	16.09	99.44±1.83

Table 5.3 Accuracy of applied analytical methods (detected by the analysis of certified reference material BCR -185R-beef meat

Element	Certified value mg kg^{-1}	Recoveries (%)
As	0.033	93.94±1.64
Cd	0.544	99.26±4.72
Cu	277	98.56±2.82
Mn	11.07	90.70±1.38
Pb	0.172	90.70±3.45
Se	1.68	94.64±2.61
Zn	138.6	96.52±2.79

5.2. Concentration of metals in studied raw food samples from Kinshasa

5.2.1 Concentration of metals in raw vegetable foods

Samples of beans Phaseolus vulgaris (n_s= 10), cabbage Brassica rapa (n_s = 10) and cassava Mainihot esculenta (n_s= 10) were collected in three Kinshasa public markets. Nine metals such as Al, Zn, Cu, Cd, Pb, Mn, As, Se and Cr were quantified. Mean moisture content of samples was determined to be 94.4% for bean, 91.2% for cassava and 86.3% for cabbage. The sequence of analyzed microelements in vegetables samples from Kinshasa was detected as follows: Zn > Al > Cu > Mn > Pb > Cr > As > Cd > Se (based on mean values) (Figure 5.1).

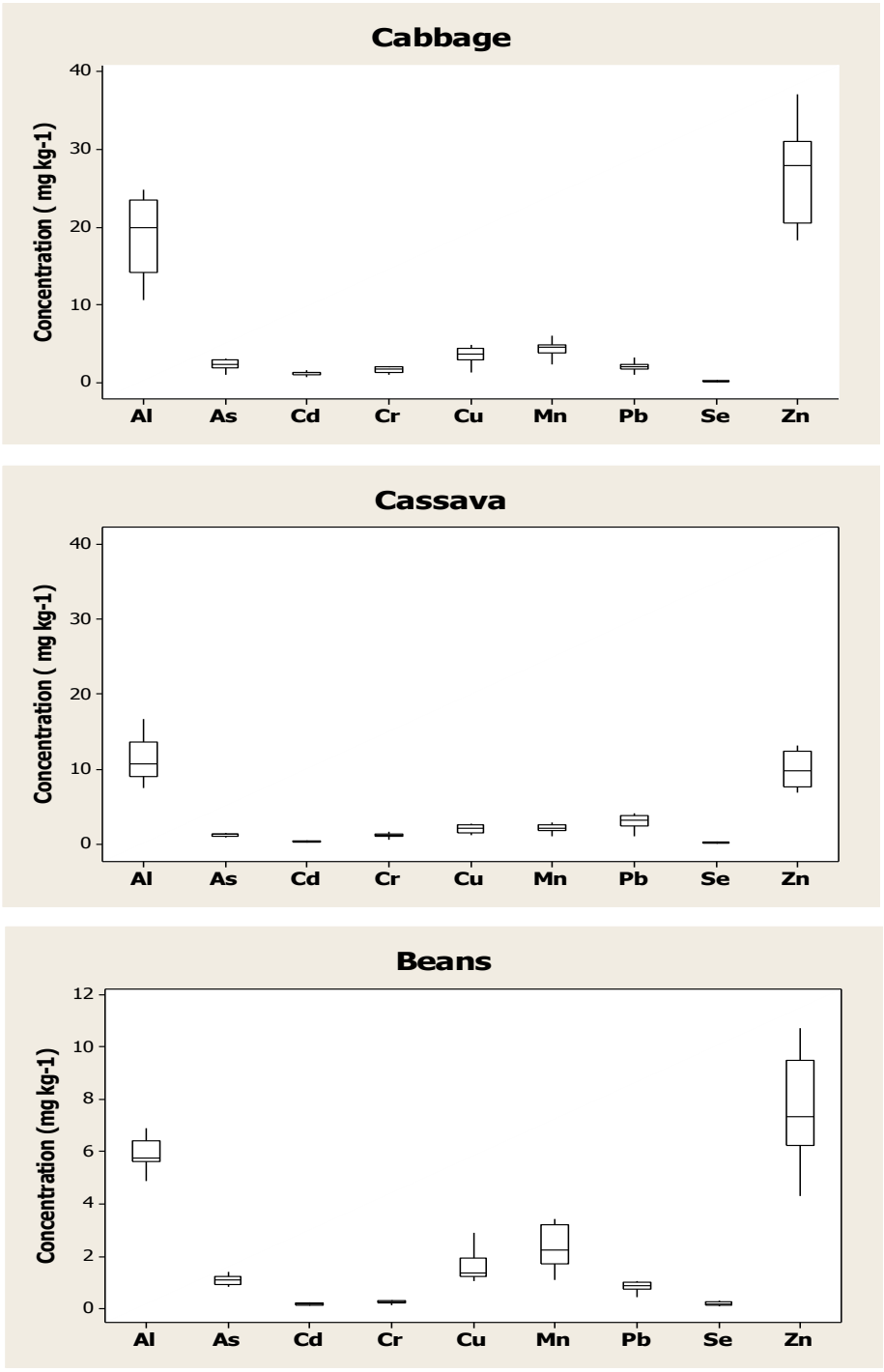

Figure 5.1 Concentration in mg kg^{-1} dry weight of metals detected in samples of vegetables form Kinshasa markets

In all the vegetable food samples, Zn was the element detected in the highest concentration. The widest range of Zn concentration was discovered for cabbage 18.25 to 36.98 mg kg^{-1} with a mean of 26.78 mg kg^{-1}. Cassava and beans

contained Zn content 6.91 to 13.23 mg kg^{-1} (mean 9.86 mg kg^{-1}), 4.28 to 10.71 mg kg^{-1} (mean 7.66 mg kg^{-1}), respectively. The level of Zn detected in the present study was similar to that reported by Jarzyńska and Falandysz (2007) in Cabbage and beans from Poland. Zn is bio-essential element for good health but very high ingestions can cause health complications (Zheng et al., 2007)**.** The recommended concentration of Zn is between 8 and 11 mg kg^{-1} (WHO/FAO, 2000).

The adequate concentration of these trace elements by Kabata – Pendias (2000) is around 10 mg kg^{-1} for Zn. 80% of examined food samples in this study were below and 20% exceeded the permissible limit for Zn set by the FAO/WHO (2000).

Al concentration ranged from 5.5 mg kg^{-1} to 24.78 mg kg^{-1}. The highest level of Al was detected in cabbages and the lowest was found in beans. Concentration detected in the present investigation was similar to that reported by Jarzyńska and Falandysz (2007) for Cabbage and beans.

The concentration of Al found in cassava samples were comparable to that reported by Tuakuila et al. (2012) in cassava and beans collected in Kinshasa market. Also data from study of vegetables in Nigeria were low than the results reported in the current study (Radwan and Salama, 2006). According to FAO/WHO (2000), the permissible limits for Al content was set for 12 to 71 mg kg^{-1} in foodstuffs. The examined vegetables samples in this study were within the permissible limit.

The highest Al content in cabbage can be due to the highest Al content in the soil where the cabbages were grown and in fact that cabbages have a capacity to accumulate the metals. Several authors have reported that the concentration of Al in the soil is an important factor to explain the contribution of total Al concentration in plant tissues (Dekker, 2001; Pruvot et al., 2006).

Cu concentration in vegetables grown in Kinshasa varied in range of mean values 1.62–3.52 mg kg^{-1}. Cu is essential element and the cofactor to many enzymes. Cu plays an important role in absorption, transport and utilisation of Fe in body. It use to prevention from the Fe deficiency (Chen et al., 2005). The concentrations of Cu detected in the present study were higher than those reported in Egypt by Abou-

Arab (2004) from Egypt markets. According to FAO/WHO (2000), the levels of Cu found in the current study were higher than the permissible limit set to 1.00 to 1.59 mg kg^{-1} in food.

Mn was detected in all vegetable food samples. Cabbage contained the highest values of Mn (2.31 to 4.98 mg kg^{-1} with mean of 3.74 mg kg^{-1}). The amount of Mn detected in the current study is within the standard limit for Mn (0.73 to 10.90 mg kg^{-1}). Mn plays an important role in the regulation of cell metabolism, receptor binding and signal transduction pathways, and an estimated safe adequate daily dietary intake of Mn for adolescents and adults ranges from 0.7 to 10.9 mg kg^{-1} (FAO/WHO, 2000).

As is an environmental toxicant detected in all studied food samples. The widest range of As concentration was discovered for cabbages, 1.01–3.09 mg kg^{-1} with the mean of 2.33 mg kg^{-1}. The amount of As in beans and cassava were lowest than the level detected in cabbages. In this study, the results were comparatively higher than those found by Mohammed (2006) in vegetables sold in Egypt market. They were similar to that found by Tuakuila et al. (2012) in vegetables sold in Kinshasa. The mean level of arsenic in cabbage was similar to the study of cabbage in Zimbabwe with a mean of 2.59 mg kg^{-1} in cabbage (Mapanda et al., 2007). According to a permissible limit establish by FAO/WHO (2000), the level of arsenic found in this study were higher than the permissible limit of arsenic in food.

Pb is one of the toxic elements that may cause significant adverse effects for human health (Hashmi et al., 2007; Kumar et al., 2007). Lowest concentration of Pb was detected in beans, 0.46 to 0.78 mg kg^{-1}). Cassava samples contained the highest concentration range 1.12 – 3.18 mg kg^{-1} with the of mean 2.20 mg kg^{-1} than any mean values for analysed vegetables. According to FAO/WHO (2000), which established limits for Pb level of 0.1- 0.2 mg kg^{-1} in food, 15% of examined food samples in this study were in the permissible limit and 85% exceeded the permissible limit. The means value of Pb found in the vegetables from Kinshasa were similar with that reported by Ahmed and Mohammed (2005) in food from Egypt market (1.21 to 3.12 mg kg^{-1}). But the results were higher than those

reported by Gonzalez-Weller et al. (2007) in food from Spain markets (6.94± 4.63 µg kg^{-1}) and Hounkpatin et al., (2012) in food from Tanzania markets (15.43±1.22 µg kg^{-1}).

Food is the major source of Cr intake by human. Cr as reviewed by Krejpcio (2009) is a bio-essential element for living organism. Cr plays many biologically active in the human body. Cr (III) is a cofactor of insulin metabolism. It is involved in glucose metabolism, lipid and protein metabolism. Cr VI has been shown to be potent occupational carcinogens (Nikfar et al., 2011).

In the current study, Cr was detected in all food samples with varying concentration ranging from 0.11 to 2.06 mg kg^{-1}. The highest Cr levels were detected in cabbage and the lowest was found in beans. The daily intake of Cr was set to 0.1µg – 25 µg per day (Krejpcio, 2009). Regarding to WHO/FAO (2000) permissible limit, the level of Cr in beans samples and some cassava samples was in the permissible limit for Cr in vegetables food but the level of Cr in cabbage samples (0.056 – 0.943 mg kg^{-1}) was high than the permissible limit for Cr.

Cd is a non-essential toxic metal has no biological role. Human toxicity occurs through food chain magnification. Foods are the principal source of Cd exposure for the non-smoking general population (Oniawa et al., 2000). In the present investigation, although Cd was found in all examined food samples. Wide range of Cd concentration was observed for all analyzed vegetables samples from 0.11 mg kg^{-1}in beans to 1.53 mg kg^{-1}in cabbage.

According to the FAO limits (0.30 mg kg^{-1}), the level of Cd in cabbage samples are above the FAO permissible limits.

Our mean Cd levels were higher than that reported by Mohamed (2006) in food from Egypt markets and Iwegbue et al. (2010) in street food (Nigeria). Other studies revealed different ranges of Cd concentration in vegetables. For example, vegetables grown in Nigeria contained 0.12 to 1.029 mg kg^{-1}, 02-0.318 mg kg^{-1} and 0.015-0.26 mg kg^{-1} of Cd in cabbage, beans and cassava, respectively (Radwan and Salama, 2006); but results from previous study in Kinshasa showed the following mean values of Cd concentration: in cabbage 0.12-1.28 mg kg^{-1}, in beans (Tuakuila et al., 2012). Other study in Tanzania revealed that cabbage can

contain Cd up to 3.39 mg kg^{-1} (Soceanu et al., 2007). Thus, the impacts of the basic soil composition, soil contamination level in each study site as well as agricultural practices (for example, composition and application of fertilizers) can have major impact on the metal accumulation.

Although Se was detected in all vegetable food samples ranging from 0.09 mg kg^{-1} to 0.28 mg kg^{-1}. The highest level was detected in cabbage and the lowest was found in cassava. The level of selenium found in this current study was corresponding to that reported by Jarzyńska and Falandysz (2007) in Poland. The dietary daily intake of Se for humans is 57 μg. The dietary daily intake can be insufficient for the expression of selenoprotein-P, which is considered to play a particular role in scavenging peroxy-nitrite and is required for the transport of Se and for certain brain functions (Iyaka, 2007). In the present study the level of selenium were below the permit limits fixed by FAO in food for Se.

5.2.2 Concentration of metals in raw beef meat food

Samples of beef meat (n_s= 10) were purchased from randomly selected individual vendors in Kinshasa. The metals were quantified in the following sequence: Zn > Al > Cu > As > Cr > Mn > Se > Cd > Pb (Figure 5.4). Concentration of Zn (mg kg^{-1}) in the examined meat ranged from 2.98 – 6.38 mg kg^{-1}. Zn concentration detected in present study is lower than reported by Iwegbue et al. (2008) in Food Street sold in Nigeria. The levels of Zn in the meat samples were below the permissible limit of 50 mg kg^{-1} set by the FAO/WHO (2000).

Al is common through water, plants, air, and food chain (Oniawa et al., 2000). Al was detected in all examined beef meat samples. The levels of Al in the beef meat samples were ranging from 3.57 to 4.19 mg kg^{-1} (mean value of 3.88 mg kg^{-1}). Al level in beef meat samples from the current study were lower than that reported by Abou-Arab (2001) in beef meat sold in Egypt market. The presence of Al in beef meat samples may be due to the high Al content in plants that are eaten by the animals due to the high soil content of this element. In the present study, Cu was detected in all the beef meat samples in the range of 0.46 to 0.94 mg kg^{-1} with the mean value of 0.69 mg kg^{-1}. The concentration of Cu recorded in this study is

lower than those reported by Salam et al. (2005) in Egypt and Iyaka (2007) in Nigeria.

Arsenic was detected in all the meat samples in the range of 0.35 - 0.86 mg kg^{-1} (mean of 0.62 mg kg^{-1}). The levels of As from the current investigation are considered lower compared to those reported by Gonzalez-Weller et al. (2008) in Spain and Iwegbue et al. (2007) in Nigeria that As level in food. According to the FAO/WHO limits (0.1mg kg^{-1} for beef meat), the obtained As values of all meat are above the permissible limits set by FAO/WHO (2000). Cr was detected in all meat samples in the present study with varying concentrations ranging from 0.45 to 0.78 mg kg^{-1}(with mean of 0.58 mg kg^{-1}). Wide range of Mn, Cd, Se and Pb concentration were observed for all analysed meat samples, 0.239 – 0.386 mg kg^{-1} (mean 0.338 mg kg^{-1}), 0.319 – 0.352 mg kg^{-1} (mean 0.337 mg kg^{-1}), 0.289 – 0.323 mg kg^{-1} (mean 0.310 mg kg^{-1}), 0.276 – 0.371 mg kg^{-1} (mean 0.371 mg kg^{-1}) and 0.270 – 0.376 mg kg^{-1}, respectively (Figure 5.2).

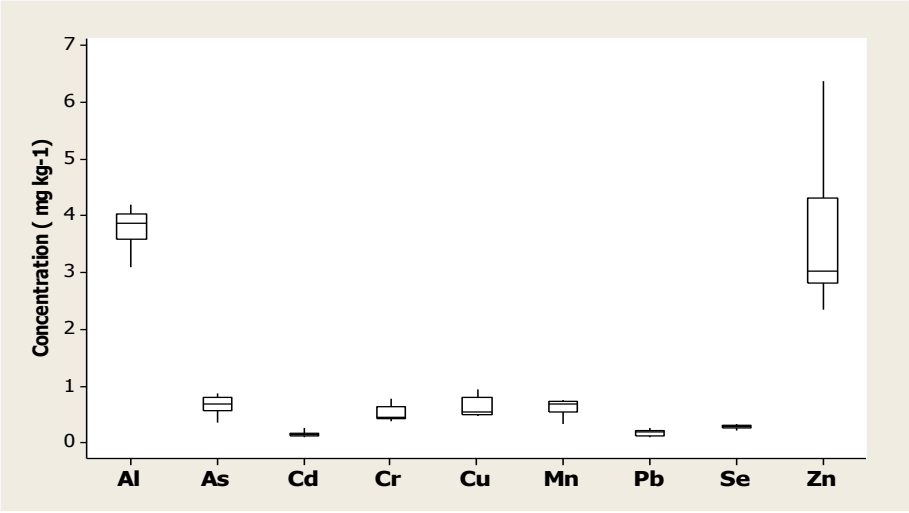

Figure 5.2 Concentration in mg kg^{-1} dry weight of metals detected in beef meat samples from Kinshasa markets

In the current study, beef meat contained the lowest amount of Mn, Cu, Cd, Se and Pb compared to the other studies in other Africa countries (Radwan and Salama, 2006; Stalikas et al., 2007).

5.2.3 Concentration of metals in raw fish

Fish sample (n_s= 3) were collected from individual vendors in Kinshasa. Ten metals were quantified in the following sequence: Zn > Cu >As > Al > Cd > Mn >Pb > Cr > Hg > Se. Mercury was found only in fish samples. The levels of Zn in fish samples were detected in the range of 12.76 – 15.17 mg kg^{-1} with the mean of 14.17 mg kg^{-1}. The level of Zn in the present study was extremely high as compared to the concentrations of other micronutrients that were considered in this study (Figure 5.3). In the current investigation the level of Zn in the fish samples were below the FAO/WHO permissible limits of 30 mg kg^{-1} of Zn for safe human consumption (FAO/WHO, 2000).

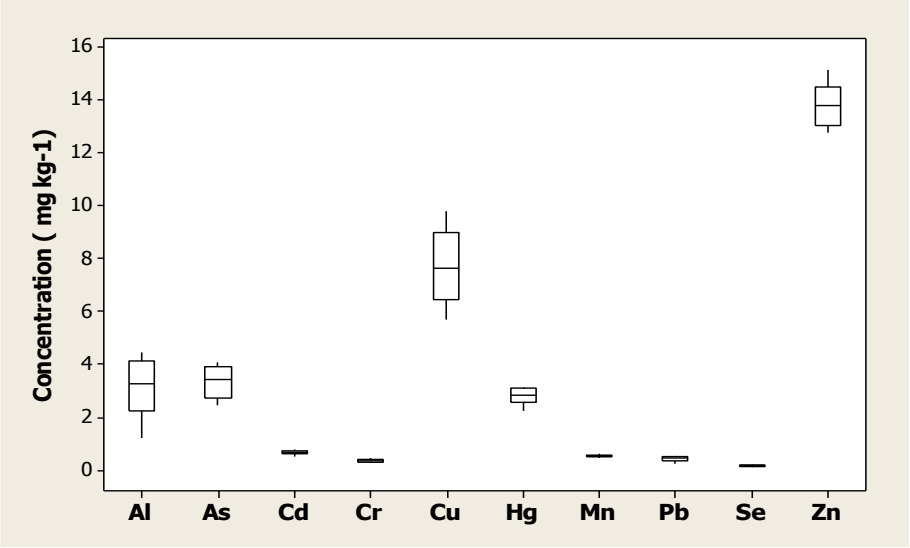

Figure 5.3 Concentration in mg kg^{-1} dry weight of metals detected in fish samples from Kinshasa markets

Cu was detected in the range of 5.17 to 9.78 mg kg^{-1} with mean of 6.53 mg kg^{-1}. FAO/WHO (2001) established limits for Cu in fish is 30.0 mg kg^{-1} for human health risk concerns. The levels of Cu in these samples were far below the permissible limit set by FAO/WHO.

As is a naturally occurring metal, found widely in the environment. As levels detected in the fish samples was in the range of 2.45–3.89 mg kg^{-1} with mean of 3.48 mg kg^{-1}. As concentration in the current study was lower than those reported by Abdel-Baki et al. (2011) in fish collected in Saudi Arabia (6.78 to 11.34 mg kg^{-}

[1] for As in fish). Relatively higher As concentrations have been reported for fish from Nigeria and Tanzania, were compared with our results (Oniawa et al., 2000; Stalikas et al., 2007). The results from this study were below the permissible limit set by FAO/WHO for arsenic in fish of 1mg kg^{-1} (FAO/WHO, 2000).

Al was found in the range of 1.23 to 4.45 mg kg^{-1}. Comparing to the permissible limit set by WHO/FAO, the Al content detected in the present study is lower than permissible limit of 30 mg kg^{-1} for Al established set by WHO/FAO (2000).

Hg is toxic metal elements. It is more bio-accumulated in the aquatic organisms. Methyl-mercury is the most toxic form of mercury. Consumption of fish contents high levels of mercury can cause severe health problems. The toxic effects of Hg can be associated with emaciation, decreasing in coordination, losing appetite and mortality (Duruibe et al., 2007). The average level of Hg found in present was 2.91 mg kg^{-1} in fish muscle which was higher than permissible levels of FAO/WHO (2000) of 0.5 mg kg^{-1}.

In the present study, Cd in fish samples ranged from 0.520 to 0.75 mg kg^{-1} (mean of 0.65 mg kg^{-1}). This level exceeded the maximum permissible limits set by FAO/WHO (2000) of 0.05 mg kg^{-1} for Cd. The levels of Cd observed in the current study are similar to those reported by Oguzie (2003) who detected Cd levels of 0.42 mg kg^{-1} in fish from Ikpoba River in Nigeria. But the levels of Cd in the current study were higher than the ones reported by Hounkpatin et al. (2012) of 0.23 mg kg^{-1} for Cd in Lacusrian village in Benin.

In the current study, Pb levels were detected in the entire fish samples and concentration ranged from 0.21 mg kg^{-1} - 0.45 mg kg^{-1} (mean value of 0.390 mg kg^{-1}). According to FAO/WHO, the levels of Pb found in the present investigation were below the permissible limits of 0.5 mg kg^{-1} for Pb. Levels of Pb observed in the present study were lower than those reported by Oguzie (2003) who found the Pb levels of 0.78 - 0.96 mg kg^{-1}.

5.3 Concentration of metals in studied food samples from Johannesburg market

5.3.1 Concentration of elements in raw vegetable foods

Samples of beans (n_s= 10), cabbage (n_s = 10) and cassava (n_s= 10) were collected in Johannesburg market.). Mean moisture content of samples was determined to be 98.71% for bean, 87.90% for cassava and 85.13% for cabbage. In total nine metals were quantified in analysed of vegetables from Johannesburg market. The highest concentration was found for Zn, Al, Mn, Cu, Cr, Pb, and As, 10.89 to 90.25 mg kg^{-1}, 7.89 to 60.67 mg kg^{-1}, 3.26 to 20.98 mg kg^{-1}, 1.34 to 20.09 mg kg^{-1}, 2.38 to 10.67 mg kg^{-1}, 0.62 to 6.42 mg kg^{-1} and 0.98 to 4.89, respectively (Figure 5.4).

Cd and Se in vegetable samples were detected in lower concentration. While Se in certain amounts is essential elements, Cd and Pb are elements of concern, especially if detected in food produced for infants and children (Ljung et al., 2011). The level of metals found in the vegetables collected in Johannesburg market exceeded the maximum permissible limits stipulated by Food and Agricultural Organization and World Health Organization (2000) for metals in vegetables foods.

The result of the analysis showed that significant differences existed in the elemental levels between the food samples analysed. This might be due to the ability of plants to accumulate metals or the geological status of the area where the food plants were cultivated.

In the results of this analysis, Zn concentrations were generally higher in the food samples than other metals. The summary of the analysis indicates that Hg was not detected in any of the food crops. The presence of the metals found in the crops was in the order Zn > Al > Mn >Cu > Cr > Pb > As > Cd > Se.

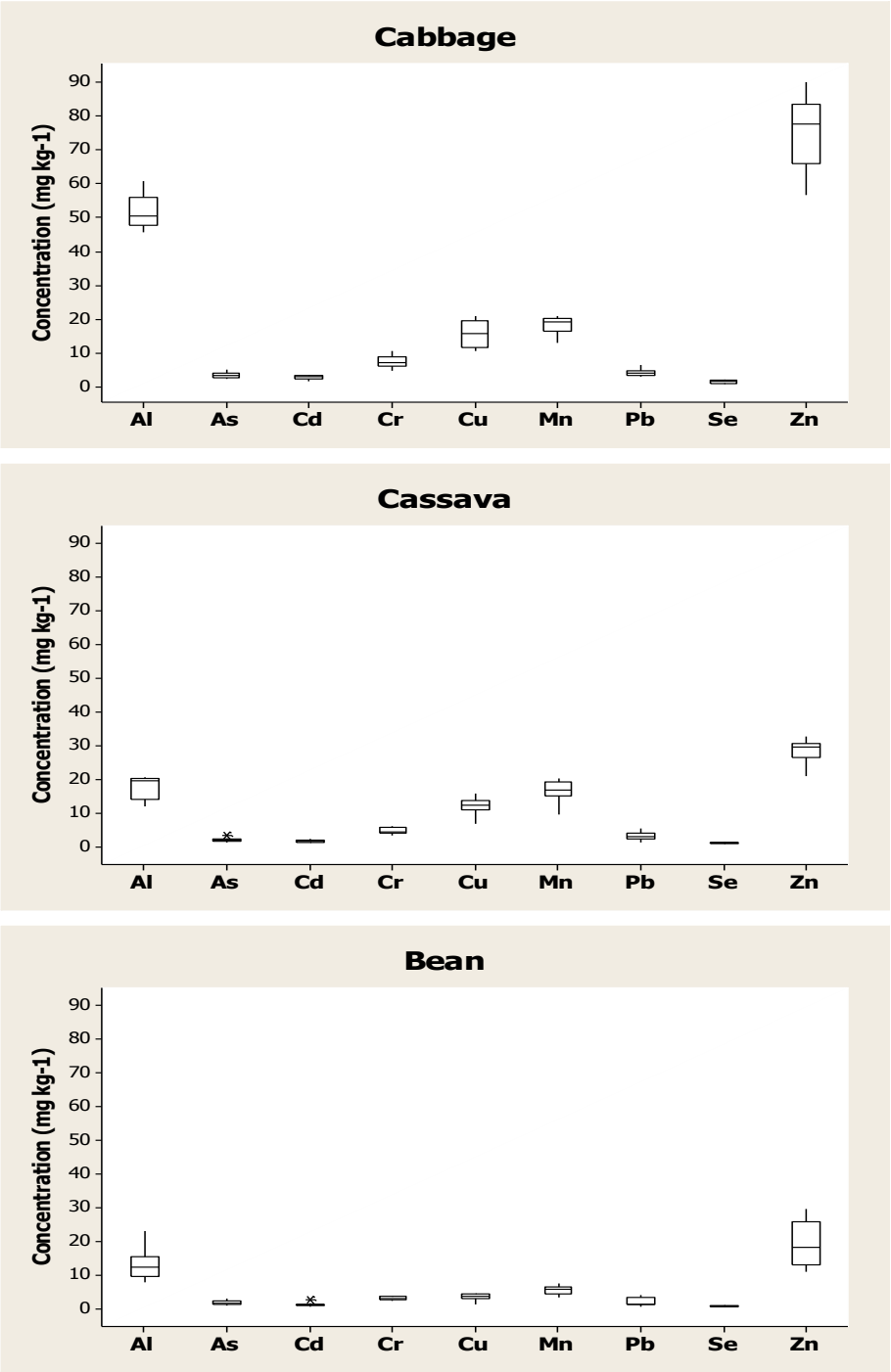

Figure 5.4 Concentration in mg kg^{-1} dry weight of metals detected in samples of vegetables form Jonnesburg market

The values obtained from the analysis were similar to many other works done on heavy metals in plants in Nigeria and some parts of other continents. Mapanda et al. (2007) detected the concentration of Cu (1.45 – 19.78 mg kg^{-1}), Zn (9.89 –

89.78 mg kg^{-1}), Pb (0.60 – 6.32 mg kg^{-1}), Al (6.56 – 59.98 mg kg^{-1}), Mn (3.09 – 19.98 mg kg^{-1}), Cd (0.51 – 3.12 mg kg^{-1}), As (0.90 – 4.18 mg kg^{-1}), Se (0.41 – 1.93 mg kg^{-1}) and Cr (2.12 – 10.34 mg kg^{-1}) in tested vegetable samples in Harare Zimbabwe, while Orisakwe et al. (2012) also found presence of Cd, Pb and Mn in the vegetables collected in Owerri South Eastern Nigeria. The cabbage samples seem to contain high concentration of the selected metals. This trend is corresponding to the results reported by Harmanescu et al. (2011) in the vegetables sold in Romania markets, Dogheim et al. (2004) in the vegetable sold in Egypt market.

5.3.2 Concentration of metals in raw beef meat

Beef meat samples (n_s= 10) collected at Johannesburg market were analysed and nine metals were quantified. In beef meat, the highest concentration was detected for Al and Zn, 8.34 mg kg^{-1} and 15.78 mg kg^{-1}, respectively. While the concentration of other metals was lower than 1 mg kg^{-1} (Figure 5.5). Concentration of these elements detected in the current study are quite high for Zn, Pb, Cd and Mn, while Cd concentration was lower than the result reported by Dalia et al. (2015) in the analysis of beef meat sold in Egypt markets. Kramer et al. (2007) determined Cr concentrations as higher as 166 mg kg^{-1}in bovine meat in sold in Pretoria town market. This value is much higher than that obtained in this investigation. The concentration of Mn in current study was similar with that reported by Iwegbue et al. (2008) in food collected in Nigeria market.

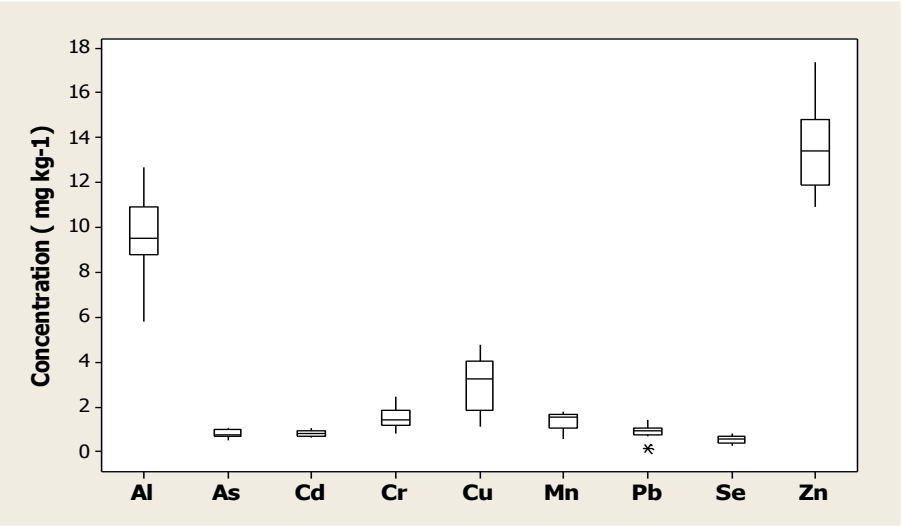

Figure 5.5 Concentration in mg kg^{-1} dry weight of metals detected in beef meat samples

5.3.3 Concentration of metals in raw fish

The analysis of fish samples collected in Johannesburg revealed that in overall potentially toxic elements can be quantified in the following sequence: Zn > As > Cu > Al >Mn > Cr> Pb> Cd > Hg> Se (based on mean results, n_s =10). The highest concentration of metals was detected for Zn, As, Cu, Al and Hg. Cu, Cr, Mn, Cd, Pb and Se were detected in lower concentration (Figure 5.6). The concentration of Zn was detected to range from 40.08 – 61.23 mg kg^{-1} (mean 50.70 mg kg^{-1}). These results were below the 75 mg kg^{-1} recommended limit for Zn in fish and fish products. This implies fish do not pose immediate hazards to human consuming the fish from Johannesburg market. Lower concentration of Zn in fish was corresponding to those reported by Oguzie et al. (2009) in fish collected in Nigeria River.

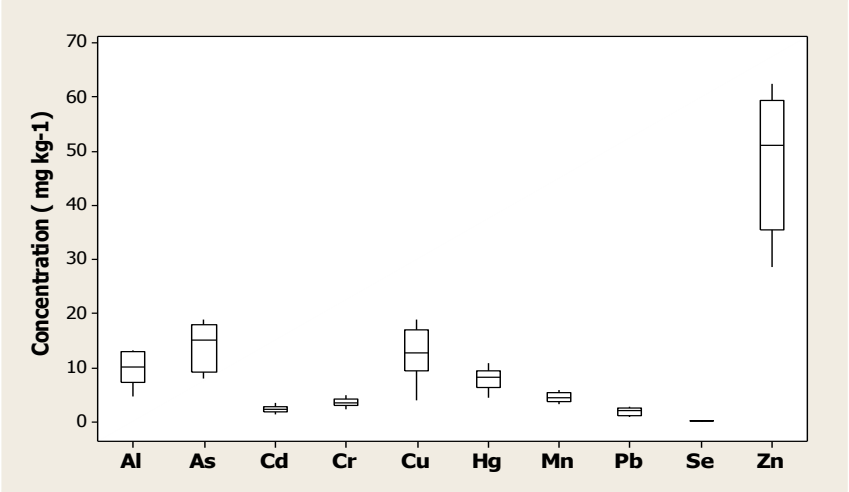

Figure 5.6 Concentration in mg kg^{-1} dry weight of metals detected in fish samples

As concentrations in fish were found to range from 7.89 – 18.78 mg kg^{-1} with a mean value of 9.45 mg kg^{-1}. The levels of As found in the current investigation were higher than the permissible limit of 0.4 mg kg^{-1} set by FAO/WHO (2000). The levels of Mn were found to range from 3.74 -5.9 mg kg^{-1} (mean 4.84 mg kg^{-1}). In all the fish samples the concentration was found to exceed 2.50 mg kg^{-1}, the WHO recommended limit for Mn in fish and fish products indicating that, the fish are not fit for human consumption with respect to Mn (FAO/WHO, 2000).

The concentrations of Cr were detected in the range of 2.38 to 4.87 mg kg^{-1} with the of mean 3.57 mg kg^{-1}. Concentrations recorded were above than the recommended limit of 0.15 mg kg^{-1} for Cr in fish and fish products (FAO/WHO, 2000). This means that consumption of fish from Johannesburg market pose immediate threat to the population consuming fish as far as Cr concentration is concerned. However, heavy metals are known to bioaccumulate in aquatic life and therefore need constant assessment. Higher concentrations for Cr reported in the current study have been reported in various studies in Ghana and Bangladesh (Gilbert et al., 2006; Ahmad et al., 2010).

The concentration of Cd was found in the range of 1.37 to 3.46 mg kg^{-1} with a mean value of 2.50 mg kg^{-1}. These concentrations are above the FAO/WHO

recommended limit of 2.0 mg kg^{-1} for fish and fish products (FAO/WHO, 2000). However, the consumption of fish should be cautious as cumulative effects might constitute health hazards to aquatic life and human who feeds on fish (Oronsaye et al., 2010).

The levels of Pb in fish were found in the range of 1.46 to 3.45 mg kg^{-1}. In some fish samples, Pb concentration was found higher than the permissible limit of 2.0 mg kg^{-1} in fish and fish products (FAO, 2003). The high concentrations could be attributed to discharge from industrial, farming and residential waste which may pollute water where the fish is grown.

Hg is a toxic metal element. It can cause many health problems such as kidney injury, brain damage and other injury (WHO/FAO, 2004). Hg contents were found in the range of 0.78 -0.91 mg kg^{-1} (mean of 0.86 mg kg^{-1}). The levels of Hg found in the present study are higher than the permissible limit set by FAO/WHO (2000). Compared to other studies in Hg content in fish, the results from the current study are higher than those reported by Akan et al. (2009) in fish collected in lake Tchad, Nigeria.

Total Se concentrations in fish muscle were detected in the range of 0.11 – 0.18 mg kg^{-1} with a mean of 0.17 mg kg^{-1}. Se concentration found in the present investigation were high than those reported by Gilbert et al., (2006). According to WHO/FAO (2000), the mean level of Se was higher than the permissible limit of 0.001 to 0.002 mg kg^{-1}.

5.4 Impact of environmental pollution on metals concentration in raw food from Kinshasa and Johannesburg

5.4.1 Vegetable foods

Knowing origin and applied agricultural practice in growth of vegetable samples such as beans, cabbage, cassava, it was possible to detect differences in element concentration for vegetables grown under the different agricultural conditions. Statistical analysis of the data by using student's t-test allowed comparison of vegetable samples from Kinshasa versus vegetable samples from Johannesburg.

The comparative mean levels of metals in vegetables collected in Kinshasa and Johannesburg are presented Table 5.1 to 5.3.

Table 5.4 comparative mean concentrations (mg kg^{-1} dry weight) of metals in beans collected in Kinshasa and Johannesburg

	Beans Kin n=10	**Beans JHB n=10**	**P-values**
Al	13.06±4.59	6.03±0.84	0.03
As	1.62±0.64	1.08±0.18	0.04
Cd	1.13±0.55	0.17±0.04	0.002
Cr	3.07±0.41	0.23±0.06	0.001
Cu	3.31±0.97	1.62±0.63	0.006
Mn	5.34±1.37	2.33±0.78	0.04
Pb	1.98±1.19	0.86±0.19	0.02
Se	0.64±0.16	0.18±0.06	0.009
Zn	19.05±6.50	7.66±2.04	0.007

$p > 0.05$ = no significant different
$p < 0.05$ = significant different

Table 5.5 comparative mean concentrations (mg kg^{-1}) of metals in cabbage collected in Kinshasa and Johannesburg

	Cabbage JHB n=10	**Cabbage Kin n=10**	**P-values**
Al	52.15±5.20	18.93±4.87	0.034
As	3.33±0.75	2.14±0.63	0.021
Cd	2.70±0.63	1.13±0.26	0.018
Cr	7.29±1.87	1.63±0.36	0.025
Cu	15.70±3.98	3.78±1.13	0.012
Mn	18.31±2.54	4.14±0.99	0.031
Pb	4.14±1.11	2.14±0.61	0.016
Se	1.41±0.43	0.18±0.04	0.039
Zn	75.12±11.08	27.93±6.10	0.016

$p > 0.05$ = no significant different
$p < 0.05$ = significant different

Table 5.6 Comparative mean concentrations (mg kg^{-1}) of metals in cassava collected in Kinshasa (Kin) and Johannesburg (JHB)

	Cassava JHB n=10	Cassava Kin n=10	P-values
Al	17.64±3.24	11.52±2.90	0.008
As	1.97±0.60	1.18±0.19	0.038
Cd	1.63±0.36	0.25±0.05	0.023
Cr	4.58±0.94	1.19±0.28	0.013
Cu	12.05±2.40	2.08±0.58	0.024
Mn	16.44±3.38	2.046±0.55	0.013
Pb	3.12±1.19	2.93±0.96	0.019
Se	1.03±0.18	0.15±0.03	0.031
Zn	28.39±3.51	9.85±2.24	0.028

$p > 0.05$ = no significant different
$p < 0.05$ = significant different

In overall, comparison of vegetable samples from Kinshasa markets versus vegetable samples from Johannesburg markets revealed that amounts of metals were significantly higher ($p< 0.05$) in the vegetables samples from Johannesburg markets. The results also shown that cabbage from Johannesburg contained a significantly ($p< 0.05$) higher amount of metals. The highest level of metals in vegetables collected in Johannesburg market can be due to the fact that the vegetables sold in this market from to some area where there are running mining activities. The vegetables are contaminated by metals accumulated in polluted soils. That explains the reason why these vegetables contained a higher amount of metals than those from DRC.

5.4.2 Beef meat

Quantitative analysis of beef meat samples from Kinshasa and Johannesburg was done to reveal the impact of agricultural practice on element concentration in beef meat. Table 5.7 shown the summary of statistical data of comparison mean concentration of metal in beef meat from Johannesburg and Kinshasa. Hg was not detected in any of meat beef samples. The level of metals in the beef meats collected in Johannesburg were significantly ($p < 0.05$) higher than those

collected in Kinshasa. The highest content of metals in the beef meats sample from Johannesburg can be due to high level of metals content in plants that are eaten by the animals.

Table 5.7 Comparative mean concentrations (mg kg^{-1}) of metals in beef meat collected in Kinshasa and Johannesburg

	Beef meat JHB n=10	Beef meat Kin n=10	P-values
Al	11.93±2.31	3.87±1.23	0.031
As	0.92±0.12	0.62±0.32	0.023
Cd	0.76±0.34	0.16±0.09	0.041
Cr	1.58±0.98	0.58±0.24	0.012
Cu	3.02±1.34	0.69±0.15	0.034
Mn	1.28±0.21	0.55±0.28	0.028
Pb	0.43±0.12	0.16±0.09	0.027
Se	0.49±0.29	0.29±0.08	0.017
Zn	14.80±3.45	4.47±1.83	0.029

$P > 0.05$ = no significant different
$P < 0.05$ = significant different

5.4.3 Fish

Table 5.8 presented the statistic data of the comparative mean concentration of metals in fish collected in Johannesburg and Kinshasa. The levels of metal were significantly ($p < 0.05$) higher in the fish muscle samples from Johannesburg compared to those collected in Kinshasa. Statistically there was significant difference in the fish muscle for the concentration of metals in the two counties.

The higher levels of metals detected in fish muscle samples from Johannesburg can be attributed to the fact that river where the fish growing are more polluted by the waste from the mining activities. Fish accumulated the metals in its different organs such as liver, kidney and muscle. Compared to Kinshasa, the rivers are more polluted the waste from the house and there are not mining activities in and around Kinshasa.

Table 5.8 Comparative mean concentrations (mg kg^{-1}) of metals in fish from Kinshasa and Johannesburg

	Fish JHB (mg kg^{-1})	Fish Kin (mg kg^{-1})	P-values
Al	9.10±4.26	2.63±1.65	0.030
As	12.04±5.88	3.48±0.89	0.042
Cd	2.50±1.05	0.64±0.11	0.039
Cr	3.56±1.24	0.37±0.08	0.01
Cu	11.75±2.34	7.33±1.83	0.043
Hg	9.19±1.46	2.94±0.26	0.002
Mn	4.84±1.08	0.51±0.08	0.002
Pb	2.54±1.02	0.39±0.16	0.023
Se	0.26±0.05	0.16±0.04	0.04
Zn	50.69±10.57	14.10±1.22	0.004

$P < 0.05$ = significant different
$P > 0.05$ = no significant different

5.5 Impact of cooking utensils on trace metal levels of processed food with traditional pot from Johannesburg markets and Kinshasa markets

The summary of mean concentrations of metals in the food cooked with traditional pot from Kinshasa (TPFK) and traditional pot from Johannesburg (TPFJ) are present in appendix V. The result revealed that food cooked with TPFK recorded more of metals than those cooked with TPFJ pot. The results shows that the high transfer rate of metals were attributed to the food cooked in TPFK compared to TPFJ. This is can be due to the presence of proactive layer in the CTPJ that prevent the contamination of food by the metals from the pots. Results obtained also indicated that Al was detected in high concentration in all the food analysed. This may be attributed to the fact that traditional utensil is a metal alloy that contain more than seventy percent (70%) of Al.

However, the levels of Al obtained in this study are above the recommended safe limit for the metal in food (30 mg kg^{-1}) by WHO/FAO (2000). This is in accord with Accominotti et al. (2000) who reported in his study that the amount of metals released into food during cooking or processing were still more than the daily intake of metals (DIM) recommended. The lowest concentrations of Al

recorded in the Control samples have shown that, some amounts of Al have been leached from the cooking utensil into the food items cooked. Thus, a periodic assessment of the level of this metal in human body is recommended to avoid bioaccumulation and its attendant's effects in future.

A range of 21.63 – 149.46 mg kg^{-1} was obtained for Al in this study with the highest level recorded in beans TPFK while the lowest concentration was obtained in beef meat cooked in TPFJ. The Results recorded for Al indicated that, the metal was leached from the cooking utensil into the food items cooked since high levels were obtained in samples cooked in TPFK. This is in covenant with Dobonne et al. (2010) that, the concentration of Al increased from 1.60 mg kg^{-1} in uncooked beans to 18.1mg kg^{-1} in beans cooked with traditional cooking pot made of Al during their research work in Cameroun.

Studies have also shown that, acidic foodstuffs and soft fruits most frequently take up more Al from the containers than their basic counterparts (Hughes, 1992). Greger et al. (2005) reported that the longer the cooking time, increase the level of metals in food. Based on results obtained, cooking of beans in traditional pot should be discouraged since the metals present are a risk for the consumers particularly in the prolonged exposure even at low concentrations. For example, several complications have been reported on toxicity of Al in human like Alzheimer's disease (Harrigton, 1994; Martyn et al., 1989), Neurons alteration diseases (Bharathi et al., 2008) among others.

Cu recorded a range of 6.90 – 37.06 mg kg^{-1}. In the current study the highest level of Cu was detected in beans cooked with TPFK while cabbage showed the lowest Cu concentration.

Zn recorded a range of 88.82 – 13.98 mg kg^{-1} in this study. The highest level of Zn was found in beans cooked in TPFK and the lowest was detected in beef meat cooked in TPFJ. Selenium was detected in higher level in beans cooked in TPFK (9.35 mg kg^{-1}) and the lowest was detected in beef meat cooked in TPFJ (1.08 mg kg^{-1}).

The obtained results also indicated that, levels of Cu in all the food samples cooked with TPFK were higher than their similar food samples cooked in TPFJ.

The high Cu, Zn, Se levels reported for beans in this research is in covenant with results reported by Onianwa et al. (2000) in beans 36.87mg kg^{-1} for Cu, 87.89 mg kg^{-1} for Zn and 9.31mg kg^{-1} for Se. On comparing the results obtained for those metals with the provisionally maximum tolerable daily intake (PMTDI) for the metal (0.5 mg kg^{-1} for Cu, 5.2 mg kg^{-1} for Zn and 0.002 mg kg^{-1} for Se) set by WHO/FAO (2000). It was notice that the levels of metals obtained in this study in cooked food samples process with the traditional utensils were higher than the standard required by the human body.

This work recorded a range of 1.75 – 43.91 mg kg^{-1} for Cr and 1.32 -20.22 mg kg^{-1} for Mn. The highest Cr and Mn level was obtained in beans cooked with TPFK while the lowest value was indicated by beef meat cooked with TPFJ. Results obtained indicated that, food samples cooked with TPFK recorded higher Cr and Mn levels than those cooked with TPJ. This revealed that some quantity of Cr and Mn may have been leached from traditional pot cooking utensil into foodstuffs cooked with it. These results showed that consuming foodstuff cooked with traditional pots cook wares may cause Cr and Mn toxicity and its attendant's health implications in human.

In this research work, Cd and Pb indicated a range of 0.91 – 16.91mg kg^{-1} and 0.43 -10.94 mg kg^{-1}, respectively. The highest level recorded in beans cooked with TPFK while the lowest level was obtained in beef meat cooked with TPFJ. This high Cd and Pb content in cooked beans may be attributed to the long cooking times for beans in pot compared to other food samples.

5.6 Determination of organochlorine Pesticides in raw food collected in Kinshasa and Johannesburg

5.6.1 Quality assurance of the method applied

The mean recoveries of the residues in beans, cassava, cabbage, beef meat and fish ranged from 80.20± 1.34 to 89.90 ± 3.23%, 80.41± 2.42 to 90.10±2.53%, 80.72±2.20 to 89.45±4.32%, 80.12±1.03 to 87.26±2.97% and 80.24±1.76 to 87.82±2.45%, respectively. The higher recovery values and good precision indicate accuracy of the procedure used in this investigation. The summary of

the recoveries are presented in appendix VI. The detection limits ranged from 1.37 to 37.65 ng g^{-1} showing the higher sensitivity of the Gas Chromatography – ECD at the operating conditions. Analysis of pesticide standard has shown a linear calibration curves with a good regression line ($r > 0.999$). The summary of the detection limits are in the appendix VII.

5.6.2 Levels of organochlorine pesticides in raw food from Kinshasa

Forty- six samples from Kinshasa were analysed for organochlorine pesticides. The following organochlorine pesticides were identified; BHC group, the cyclodiens aldrin, heptachlor, dieldrin, endrin and the DDT group of compounds. The mean organochlorine pesticide residues in food collected in Kinshasa markets are shown in Table 5.9. In all the food samples analysis DDT compounds and lindane were found in the highest concentration.

High residue levels were detected in beef meat followed by fish, beans and cassava. This could be attributed to the lipid content of the food since organochlorine pesticides are lipophilic. They tend to accumulate in tissues with high fat content that experience low turn-over rates, Borrell and Aguilar (2007).

Table 5.9: Mean concentration of Organochlorine pesticide levels (µg kg^{-1}) ±SD in the food samples from Kinshasa

	Cassava	Beans	Cabbage	Beef meat	Fish
α - BHC	ND	38.54±7.46	62.06±13.16	102.28±10.11	83.19±9.86
γ - BHC	5.21±1.89	40.83±5.67	56.05±4.23	103.28±8.93	72.53±6.83
β - BHC	ND	ND	ND	ND	ND
4,4' DDE	45.06±2.86	56.57±8.93	81.05±7.23	154.15±4.34	90.09±3.82
4,4' - DDD'	23.23±1.73	49.50±3.51	61.05±2.15	97.65±1.67	73.52±5.67
4,4' DDT	78.45±7.34	96.70±2.34	106.93±2.91	145.12±6.71	63.72±7.82
Dieldrin	ND	ND	ND	ND	10.39±1.12

ND = no detected

The levels of organochlorines were detected in the range of 5.21±1.89 to 154.15±4.34 µg kg^{-1}. The highest concentration was recorded for 4, 4'-DDE (154.15±4.34 µg kg^{-1}) in beef meat. The lowest concentration was detected for γ

– BHC (5.21±1.89 µg kg^{-1}) in cassava. Furthermore, results from the residue analysed confirm the previous finding on the use of pesticides in Bukavu (DR Congo) by Muyisa et al. (2014). They found 20 different pesticides were used, with organochlorine (DDT) being the most widely distributed and applied pesticides in Bukavu.

The concentrations of organochlorine pesticides measured in this work were high to those of similar works done in Ghana. For example, Essumang et al (2008) reported that the levels of organochlorine pesticides residues in food sold from Ghana markets ranged between 45.67 to 109.42 µg kg^{-1} and DDT was found in highest amount in all food sample analysed. Mwevura et al., (2000) reported lower concentration of organochlorine pesticide residues of 4, 4'-DDT (103.02 µg kg^{-1}); 4, 4'-DDE (115.67 µg kg^{-1}) in food samples from Dar es Salaam markets. According to the permissible limit set by WHO/FAO (2000) for organochlorine pesticides, the level of organochlorine pesticides in the food samples were below the permissible limit.

5.6.3 Levels of organochlorine pesticides in food from Johannesburg market

The mean levels of organochlorine pesticide residues were summary in Table 5.10. DDT and its metabolites, α – BHC and γ – BHC had the highest frequency of detection in all samples analysed. DDT group were found in higher concentration in the range of 105.74±4.27 to 134.57±5.23 µg kg^{-1} in beef meat. The lowest concentration of DDT group was detected in cassava in the range of 57.56±9.34 - 86.67±6.23 µg kg^{-1}. In overall, the concentrations of pesticides in the food samples are presented in the following sequence: beef meat > fish > beans > cassava.

Table 5.10: Mean concentration of Organochlorine pesticide levels (µg kg^{-1}) ±SD in the food samples from Johannesburg

	Cassava	Beans	Cabbage	Beef meat	Fish
α - BHC	12.39±3.45	58.53±2.19	79.51±4.12	159.74±9.56	108.95±7.62
γ - BHC	15.98±5.67	78.95±6.23	99.67±3.23	132.82±8.12	105.78±5.23
β - BHC	ND	85.76±3.83	45.83±6.52	101.97±7.21	99.76±5.72
Heptachlor	ND	35.78±3.48	ND	95.62±2.71	ND
Aldrin	ND	53.45±3.29	65.89±4.23	107.45±5.91	95.45±5.61
Heptachlor epoxide	16.95±1.56	ND	78.93±8.12	ND	78.56±5.20
4,4' DDE	85.78±8.92	99.67±7.41	106.65±5.63	253.58±6.23	125.78±7.23
4,4' DDD'	57.56±9.34	75.76±3.74	95.67±2.10	113.64±2.91	105.74±4.27
4,4' DDT	86.67±6.23	109.67±6.92	125.87±5.97	167.89±1.45	134.57±5.23
Endrin	23.78±3.91	ND	56.78±7.34	97.94±3.82	13.49±1.23
Dieldrin	ND	48.85±2.75	ND	25.89±2.31	75.78±3.61

ND = no detected

The results of the present investigation are comparable with other studies done in the world. For example, Dako and Acquaah (2007) found that the levels of organochlorine pesticide residues in meat from Kumasi and Buoho abattoirs were lower than the maximum limits set by FAO/WHO. Adeyemi et al. (2008) found that residue levels of organochlorine pesticide residues in food from Lagos markets were generally low and none were above the FAO maximum residue limits. In a comparable study, Usman et al. (2009) reported that all the marketed vegetables samples from Lahore, Pakistan had lower levels of organochlorine pesticides than the maximum residue limit (MRL) set by FAO/WHO.

The results of the study shown lower concentration than those found in vegetables from Ghana markets (Amaoh et al., 2006) which exceeded the MRLs for consumption. In a similar study by Odhiambo et al., (2007), the organochlorine residues detected in vegetables exceeded Chinese maximum Residues Limit of 50ng g^{-1} for DDT in vegetables.

5.7 Comparative levels of organochlorine pesticides in food from Kinshasa and Johannesburg

Appendix VIII shown the statistic data of the comparative mean concentrations of organochlorine pesticides in food collected in Johannesburg and Kinshasa markets. Statistical analysis shows that there were significant differences in the mean concentration of all the organochlorine residues collected in Kinshasa and Johannesburg. The levels of organochlorine pesticide in food samples from Johannesburg residues were significantly ($p < 0.05$) higher compared to those collected in Kinshasa. This could be due to the fact that South Africa use to much organochlorine pesticides in agriculture practices than Kinshasa.

5.8 Impact of cooking on pesticide residues in food samples

Organochlorine pesticide residues can be evaporated, hydrolysed and thermal degraded during cooking. However, the processes and condition used in food process are highly varied (Zabik et al., 2000). In overall, volatilisation of residues and the rate of degradation are increased by the heat involved in cooking.

In the present study the effect of heat processing on the organochlorine pesticides residues were assessed in beef meat, cassava, cabbage, beans and fish. The results of percentage of reduction for each pesticide after cooking the food samples are presented in Table 5.11.

Table 5.11 Percentage of reduction (%) of amount of organochlorine pesticides in food after cooking

	αBHC	**γ BHC**	**β BHC**	**4,4'DDE**	**4,4'DDD'**	**4,4'DDT**
Cassava	86,23	80,34	0,0	82,78	85,63	83,80
Beans	92,07	98,52	91,94	97,82	98,08	96,02
Cabbage	86,13	76,83	82,30	84,23	81,42	82,89
Beef meat	70,03	72,85	81,5	73,5	82,54	79,12
Fish	80,81	75,12	80,92	79,41	82,32	81,09

The results of this study show that the cooking process decreases the amount of the pesticides. The significant percentages of reduction for all organochlorine pesticides examined were found in all the food samples. The highest percentages of reduction were found in beans sample, followed by cassava, cabbage and fish.

The highest percentage of reduction of organochlorine pesticides in beans. It can be due to higher temperature and times used in cooking food. The results found in the present investigation were comparable to those reported by Chavarri et al. (2005). Other studies reported also a significant reduction of organochlorine pesticides after cooking the food **(**Fernandez-Cruz et al., 2006; Rasmussen et al., 2003; Holden et al., 2001).

5.9 Risk assessment

Daily intake of metal (DIM) calculated in food cooked in the traditional pots are above the acceptable Daily intake of metal for Al, Cd , Cr, Cu , Hg , Mn, Pb, Se and Zn set by FAO/WHO (2000). The summary of daily intake of metal in food samples and acceptable daily intake are presented in appendix IV. Daily intake of mercury was calculated in fish sample after cooking because this metal was found only in fish samples.

The highest DIM values were found in beans cooked in both traditional pots and the lowest were found in beef meat. The DIM values were observed in the following sequences in all food samples: beans > Cassava > cabbage > fish > beef meat. The consumption of the food cooked in these utensils exposed the consumers to consumption risk for metal elements. The statistical comparison of DIM obtained from food cooked in traditional pots from Kinshasa and Johannesburg shows that food cooked in traditional pots from Kinshasa contained higher ($p < 0.05$) amount of metals than food cooked in the traditional pots from Johannesburg. It is mean that Kinshasa population are more expose to toxic effect of metal elements, because the traditional pots are used every day in the kitchen. The calculated Hazard Indice for Hg in fish samples indicated that the fish collected in Johannesburg market are more pollute to Hg than the fish collected in Kinshasa markets.

Figure 5.7 below shows the hazard indices of food samples assayed, results obtained indicated that all the food cooked in the traditional pots had Hazard indices greater than 1. But the Hazards indices from the food cooked in the traditional pot Kinshasa were significantly ($p < 0.05$) high than those cooked in traditional pots purchase in Johannesburg.

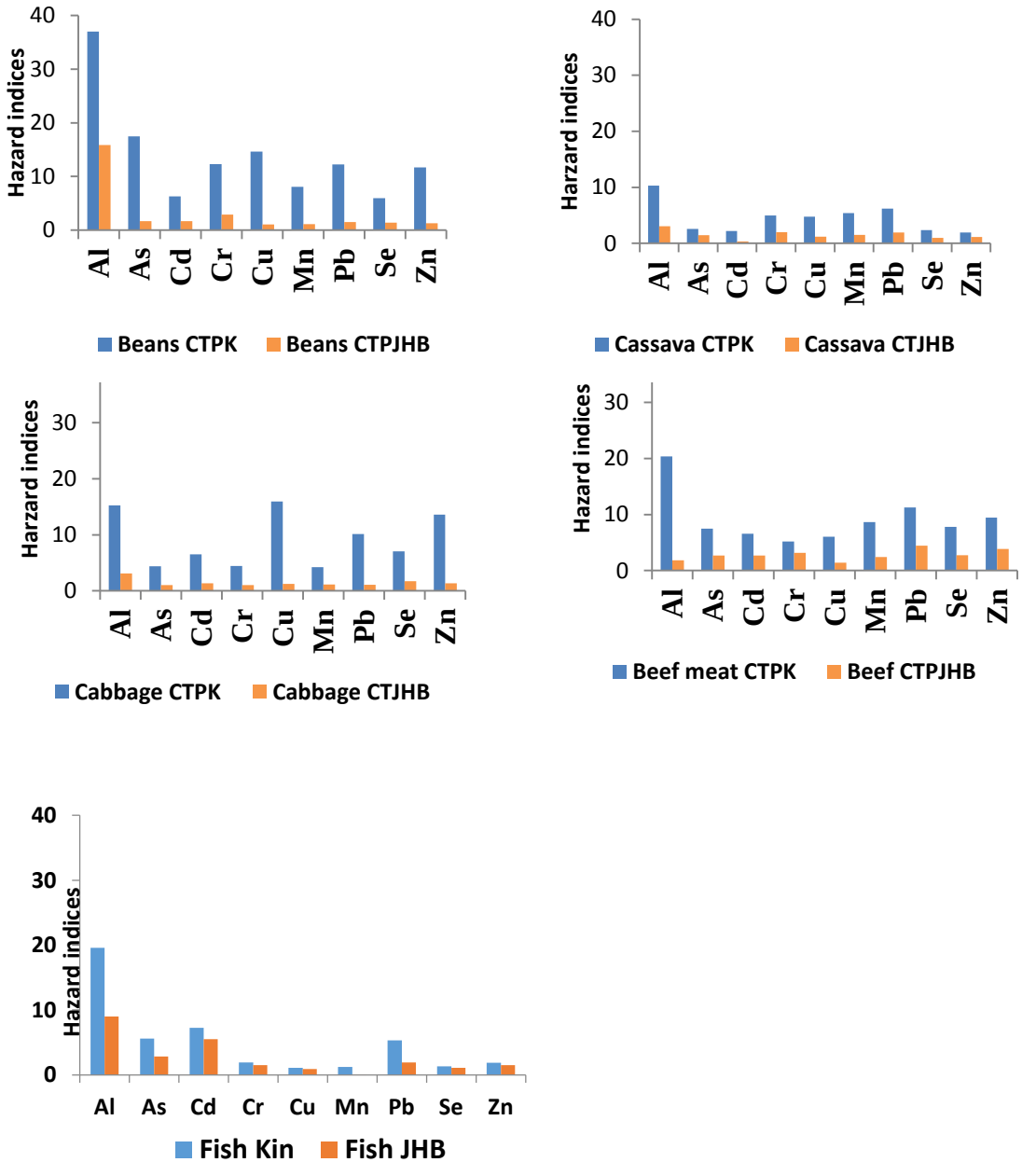

Figure 5.7 Hazards indices of cooked samples

Chapter 6: Conclusions and Recommendations

6.1 Conclusions

The concentration of metals such as Al, As, Cd, Cr, Cu, Mn, Pb, Se and Zn were high in the raw food from Johannesburg compared to Kinshasa. There was significant difference in the raw food for all the metals assessed in two cities since $p < 0.05$. The highest levels of selected metals in the raw food from Johannesburg can be attributed to the higher agricultural and mining activities carried out around Johannesburg compared to Kinshasa.

The results obtained in the current study have revealed that different food crops have different potential of accumulating trace metals. In addition to supplying valuable information about the trace metal levels in food crop commonly in South Africa and DR Congo. It has also shown that cooking utensils like traditional pots which are usually used in food processing have a positive impact on the metal transfer in foodstuffs. Although, the levels of metals in cooked with both pots were above the safe limits.

The DIM and HI values for the metals indicated that the population are more exposed to the toxic substance present in food substances which are frequently eaten by the population. This is because the values obtained from these indices with reference to the food samples exceeded the permissible limit set by WHO/FAO (2000). The assessments of metals in the food cooked with traditional pots confirm that those pots are a potential source of metals contamination in the food and constituted a real danger for people who used it daily. Based on results obtained, cooking food with traditional pots should be discouraged since this metal present a risk for the consumers particularly in the prolonged exposure even at low concentration.

In this study, the organochlorine pesticide residues were significantly ($p < 0.05$) high in the food sample from Johannesburg markets compare to Kinshasa markets. The amount of organochlorines in this study could be due to the fact that Johannesburg (RSA) is a high potential area of agriculture practice and there is a

possibility of organochlorine use in the past and Kinshasa is a malaria zone, there is also a possibility of organochlorine use in the past to kill the mosquitoes.

High residue levels were detected in beef meat in both counties followed by the fish, cabbage, beans and cassava. This could be attributed to the lipid content of the organs since organochlorine pesticides are more accumulated to the lipid tissue. But cooking process a have a positive effect on reducing dramatically the amount of pesticides in the food. One has to be careful about making conclusions that the cooking process reduces the risk from exposure to pesticides. The pesticides may have been transformed during the cooking process to some products and we do not known anything about the risk they may pose to human health. Those transformation products might be even more toxic than the original substance.

According to the public health, the concentration of organochlorine pesticide residues detected in all food samples are below compared to the permissible limit set by FAO/WHO and therefore do not pose a health risk to the population consuming food from Kinshasa and Johannesburg.

6.2 Recommendations

- Future studies should be done on determination of levels of heavy metals, organochlorines residues and other pesticides in human serum (blood and urine) who use the traditional pots daily in order to evaluate the possible risk to human. This is important since some levels of studies chemicals were above the allowed limits
- Similar studies may be performed to check contamination with other toxic metals such as Nickel, Uranium, Cobalt and antimony in the food sold in food sold in African markets.
- It is important to expand similar study to cover wide metals sources in various foods range in other cities in Africa.

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Appendices

Appendix I: The requirements of intake of essential elements for adult human organism (FNIC, S.A.; FSA, 2006; Iom, S.A., Schrauzer, 2002; White and Broadley, 2005)

USA guidance			**UK guidance**	
Element	**Recommended daily allowance (RDA)**	**Tolerable upper intake level (UL)**	**Daily reference nutrient intake (RNI)**	**Safe upper levels (SUL)**
Macro-elements, mg/day				
Ca	1000 - 1200	2500	700	1500
Cl	750 -3400	n	2500	N
K	1600 - 3500	n	3500	3700
Mg	310 - 420	350	300	400
Na	500 - 2400	< 2400	1600	N
P	700	4000	550	250
S	n	n	n	N
Micro-elements, mg/day				
B	n	20.0	n	9.6
Cu	0.9	10.0	1.2	10.0
F	3.0 – 4.0	10.0	n	N
Fe	8.0 – 1.8.0	45.0	11.4	17.0
Mn	1.8 – 2.3	11.0	> 1.4	4.0
Si	n	n	n	1500
Zn	8.0 – 11.0	40.0	9.5	25.0
Co	2.4	-	-	-
Cr	25 - 35	n	> 25	N
I	150	1100	140	500
Se	55	400	75	450

Appendix II: Food packaging materials and possible element leaching into food (Conti, 1997; Forrest, 2009; Rial-Otero et al. 2009)

Material	Properties leading to food contamination	Element of concern	Use in contact with food
Glass	➢ Low risk of contamination only if raw material contain metals	Pb, B	Bottles, jars mainly used for liquid food, kitchen utensils, dishes
Metals	➢ Corrosion ➢ Migration from coatings	Al, Cr, Cu, Fe, Ni, Sn, Zn	Cans, laminate metalizes films used for liquid food and powdered food, kitchen utensils
Plastics	Migrations of components to food, especially due poor thermal stability	Cd, Pb	Epoxy/phenolic resins, polypropylene, polyvinylchloride etc. used or miscellaneous food, kitchen utensils, disposable dishes
Paper	➢ Very hydroscopic ➢ Poor barrier properties lead to food contamination from environment not usable for liquid contact	Cd, Cr, Pb	Paper and paperboard boxes, wrapping used predominantly for dry food and for short term storage

Appendix III: Estimated element bioavailability from oral intake associated with gastrointestinal (GIT) absorption in human body (ATSDR, S.A.; WHO, S.A.)

Element	Bioavailability (BA),%	Remarks
Ag	21% (Ag acetate)	• Apart from GIT absorption, Ag compounds, including Ag salts and protein colloids, are absorbed across mouth and nasal mucous membranes • High intake of Se may lead to increase deposition of insoluble Ag salts in body tissues in the form of Ag selenide
Al	• 0.1 - 0.4 % • 0.5 –5% (BA forms, e.g, Al citrate) • ≤ 0.01% (Al hydroxide which is widely used as antacid)	• Absorption of Al varies depending mainly on type of anion and the concurrent exposure to dietary chelators such as citric acid, ascorbic acid, lactic acid • Al lactate has higher BA than other Al compounds found in water and food
Am	< 0.1 % Up to 0.5% (in infants)	• Absorption increases with duration of exposure • Absorption, distribution and excretion from organism is dependent on age

Element	Bioavailability (BA),%	Remarks
As	• > 95% (inorganic As) • 75 – 85% (organic As compounds such as MMA and DMA)	• Inorganic As (V) compounds are readily reduced to inorganic As (III) compounds which are taken up by the cell • If highly insoluble forms are imgested, BA and absorption will be much lower
B	81 -92 % 93.9 % (boric acid)	Apart from high BA, essentiality of B is established for plants and some animals, but not for humans
Ba	• < 5 – 30 % • With food: 1-15% (mean 6%) • 20 % (in adults) • 30 % (in children) • 60 % (in infants	• Ba of Ba is significantly compound dependent • Absorption of Ba sulphate is very low, higher Bahas acid soluble Ba compounds (Ba chloride, Ba carbonate) • Absorption of Ba compounds is influenced by age
Cd	• 1.1-10.6 % • 3-5 % (in health non-smokers)	• Absorption can be significantly affected by protein complexes • Cd is more readily found in free ionic form in water, while • In food cd ion generally exists in a complex with variety of • ligands, including proteins such as metallothionein • Cd in cigarette smoke has higher absorption efficiency due to its small particle size • Female organisms generally absorb greater amounts of cd than male

Appendix IV: Daily oral intake of metals (for adult human with body mass of 70 kg) and values of oral minimal risk level (ATSDR, S.A.)

Element	Predominant source of oral intake	Estimated average daily oral intake	Oral minimal risk level (MRL)
Ag	Drinking water and food	• 70-88 μg day^{-1} • food: 4.5 μg day^{-1} • water: 20-100 μg/day	No oral MRLs
Al	Food, pharmaceuticals, used as food additive	• 0.10-0.12 mg kg^{-1} day^{-1} • With pharmaceuticals at recommended dosages: 12-71 mg^{-1} ^{kg} μg day^{-1} • ^{day} (with antacid/ anti-ulcer products); 2-10 mg/kg/day (with buffered analgestics)	Aluminum: • 1 mg/kg/day
As	Food, drinking water, some pharmaceuticals	• 50.6 μg day^{-1} ♀ • 58.5 μg day^{-1} ♂ • grains: 20 μg day^{-1}	Inorganic arsenic: • 0.005 mg kg^{-1} day^{-1} (acute) • 0.0003 mg kg^{-1} day^{-1} (chronic) organic arsenic compounds: • 0.1 mg MMA kg^{-1} day^{-1}
B	Food, beverages, drinking water, pharmaceuticals	• 1.0 mg day^{-1} ♀ • 1.28 mg day^{-1} ♂	Boron and its compounds: • 0.2 mg kg^{-1} day^{-1}

Element	Predominant source of oral intake	Estimated average daily oral intake	Oral minimal risk level (MRL)
Ba	Food and drinking water	drinking water: 0.86 μg kg^{-1} day^{-1} • food: 9.30-25.30 μg kg^{-1} day^{-1}	Barium and its soluble salts: • 0.2 mg $kg^{-1}day^{-1}$ (intermediate) • 0.2 mg $kg^{-1}day^{-1}$ (chronic)
Cd	Food	• 0.30 mg $kg^{-1}day^{-1}$ ♀ • 0.35 mg kg^{-1} day^{-1} ♂	Cadmium: • 0.0005 mg $kg^{-1}day^{-1}$ (intermediate) • 0.0001 mg $kg^{-1}day^{-1}$ (chronic)
Cr	Food and drinking water	• 52-943 μg day^{-1} • With selected diets (25 and 43 % of fat): 25-224 (mean 76) μg day^{-1}	Chromium (VI): • 0.005 mg $kg^{-1}day^{-1}$ (intermediate) • 0.0009 mg $kg^{-1}day^{-1}$ (chronic)
Co	Food and drinking water	• 12-15 μg day^{-1}♀ • 9-10 μg day^{-1}♂	Cobalt: • 0.01 mg $kg^{-1}day^{-1}$ (intermediate)
Cu	food and drinking water	• 1.0-1.3 mg day^{-1} (0.014-0.019 mg $kg^{-1}day^{-1}$)	Copper: • 0.01 mg $kg^{-1}day^{-1}$ (acute) • 0.01 mg $kg^{-1}day^{-1}$ (intermediate)
F	Food, drinking water, beverages, dental products	• 0.35-3.03 (mean 1.76) mg day^{-1}	Fluoride: • 0.05 mg $kg^{-1}day^{-1}$ (chronic)
Hg	Food (fish and fish products), dental amalgams	• For methylmercury compounds with food 2.3-2.4 μg day^{-1} with fish Elemental Hg vapour daily	Mercury (II) chloride: • 0.007 mg $kg^{-1}day^{-1}$ (acute) • 0.002 mg $kg^{-1}day^{-1}$ (intermediate) Methylmercury: • 0.0003 mg $kg^{-1}day^{-}$

Element	Predominant source of oral intake	Estimated average daily oral intake	Oral minimal risk level (MRL)
I	Food and drinking water	• 0.064-0.379 mg day^{-1}	iodide: • 0.01 mg kg^{-1} day^{-1} (acute) • 0.01 mg kg^{-1} day^{-1} (chronic)
Li	Food, certain pharmaceuticals	• 0.65-3.10 mg day^{-1}	No oral MRLs
Mn	Food	• 0.7-10.9 mg day^{-1} (higher for vegetarians)	No oral MRLs
Ni	Food and drinking water, cigarette smoke	• With food: 69-162 μg day^{-1} • With water: 8 μg/day	No oral MRLs
Pb	Food and drinking water	• 24-56.5 μg day^{-1}	No oral MRLs
Se	Food and drinking water	• 1-2 μg $kg^{-1}day^{-1}$	No oral MRLs
Sn	Food, dental products	1-38 mg day^{-1} (higher from canned food)	Tin, dibutyl-, dichloride: • 0.005 mg $kg^{-1}day^{-1}$ (intermediate) inorganic Tin: • 0.3 mg $kg^{-1}day^{-1}$ (intermediate) Tin, tributyl-, oxide: • 0.0003 mg $kg^{-1}day^{-1}$ (intermediate) • 0.0003 mg $kg^{-1}day^{-1}$ (chronic)

Appendix V: Concentration (mg kg^{-1}) of metals in food after cooked in the traditional utensils

	Al	As	Cd	Cr	Cu	Hg	Mn	Pb	Se	Zn
Bean										
Cooked TRPK	149.46±7.98	6.87±5.98	10.59±3.64	43.91±2.89	37.06±1.45	0.67±0.32	18.97±2.06	16.91±2.97	9.35±1.76	88.8±3.41
Cooked TRPJ	43.26±3.89	2.09±0.72	2.89±1.23	1.75±0.76	7.17±1.09	0.40±0.12	13.23±4.56	3.45±1.23	3.45±1.24	50.34±5.67
Cabbage										
Cooked TRPK	56.6±7.1	8.90±2.04	5.57±0.6	10.60±0.58	15.48±6.8	0.39±0.12	20.22±0.05	4.60±1.98	4.30±1.02	43.9± 3.41
Cooked TRPJ	37.54±3.45	3.78±1.42	0.78±0.37	3.67±1.45	6.90±1.63	0.15±0.10	8.84±2.12	1.09±0.84	1.96±0.45	62.97±3.91
Cassava										
Cooked TRPK	45.47±3.45	4.90±1.74	3.38±2.1	8.58±3.2	15.16±1.34		20.75±2.03	6.3±2.73	10.89±1.21	15.9±1.34
Cooked TRPJ	37.89±4.92	3.06±1.73	2.96±1.34	8.09±2.95	12.65±5.91		16.42±2.31	3.21±1.07	5.93±1.95	13.07±2.34
Beef meat										
Cooked TRPK	79±6.45	4.78±1.9	2.04±0.09	2.072±0.65	32.206±1.13	0.19±0.07	2.944±0.75	3.71±1.76	2.413±0.39	8.18±0.011
Cooked TRPJ	21.63±2.41	1.67±0.85	0.43±0.12	1.84±0.93	16.96±1.23	0.12±0.08				
Fish (tilapia)										
Cooked TRPK	64.56±8.15	9.25±0.013	3.98±0.74	5.2±0.41	24.15±2.6	3.09±1.4	6.89±1.03	3.6±.29	3.09±0.70	75.234±0.07
Cooked TRPJ	29.89±0.54	4.67±1.32	3.04±2.31	4.08±2.09	19.96±1.27	9.20.96±0.34	5.04±0.21	3.05±3.21	2.45±1.90	67.98±1.21

Appendix VI: Recoveries (Average Recovery (%) ± RSD)

Cassava			
	Spike level		
	1 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}
α – BHC	85.4± 0.38	86.1±0.49	80.3±0.16
γ – BHC	80.9±0.33	81.2±1.02	81.5±0.36
β – BHC	81,1±0,66	80.6±0.06	81.7±0.19
Heptachlor	86.8±0.37	86.2±0.11	84.7±0.09
δ-BHC	80.5±0.38	80.2±0.26	81.3±0.10
Aldrin	81.8±0.23	81.3±0.20	82.8±0.26
Heptachlor epoxide	90.1±0.25	90±0.36	81.4±0.10
Endosulfan	80.4±0.42	80.2±0.31	80.5±0.26
4,4' DDE	87.2±0.26	87.3±0.25	83.6±0.09
Endrin	80.6±0.23	80.3±0.21	80.3±0.27
Dieldrin	87.4±0.14	87.1±0.16	84.5±0.15
4,4' - DDD'	84.4±0.34	84.1±0.09	80.7±0.21
Endosulfan II	85.7±0.17	85.6±0.095	83.7±0.15
4,4' DDT	81.7±0.18	81.7±0.21	81.8±0.15
Endrin aldehyde	81.5±0.39	81.1±0.15	80.8±0.18
Endosulan sulfate	89.2±0.17	89.2±0.19	83.7±0.15
Methoxylchlor	83.7±0.18	83.5±0.22	81.7±0.23

Beam			
	Spike level		
	1 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}
α - BHC	80.8±0.12	80.6±0.31	80.2±0.17
γ - BHC	81.5±0.22	81.3±0.65	81.8±0.25
β - BHC	80.7±0.23	80.4±0.10	81.9±0.15
Heptachlor	89.6±0.34	89.6±0.43	84.5±0.25
δ-BHC	80.2±0.06	80.2±0.1	81.3±0.25
Aldrin	80.5±0.18	80.6±0.10	82.7±0.21
Heptachlor epoxide	85.6±0.13	85.7±0.25	81.6±0.15
Endosulfan	83±2.21	81.5±0.17	80.4±0.11
4,4' DDE	86.3±0.49	85.9±0.16	83.5±0.11
Endrin	83.3±0.27	83.1±0.69	80.2±0.10
Dieldrin	89.4±0.16	89.3±0.24	84.4±0.25
4,4' - DDD'	81.7±0.21	81.7±0.09	80.6±0.12
Endosulfan II	84,.8±0.14	84.7±0.09	83.6±0.18
4,4' DDT	81.5±0.32	81.1±0.04	81.4±0.11
Endrin aldehyde	81.8±0.15	81.9±0.11	80.7±0.31
Endosulan sulfate	86.6±0.29	86.6±0.28	83.7±0.29
Methoxylchlor	82.7±0.13	82.6±0.09	81.7±0.30

Cabbage			
	Spike level		
	1 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}
α – BHC	82.7±0.42	83.4±0.43	84.4±0.17
γ – BHC	81.8±0.18	81.1±0.55	80.8±0.13
β – BHC	81.3±0.55	80.6±0.23	81.6±0.28
Heptachlor	85.8±0.17	85.3±0.28	87.8±0.17
δ-BHC	81.9±0.08	81.5±0.27	82.1±0.56
Aldrin	80.9±0.05	80.7±0.18	80.9±0.63
Heptachlor epoxide	87.9±0.08	87.5±0.38	88.7±0.21
Endosulfan	81.3±0.56	80.9±0.06	80.7±0.26
4,4' DDE	85.8±0.082	85.5±0.09	84.8±0.17
Endrin	81.8±0.13	81.8±0.06	81.2±0.19
Dieldrin	89.9±0.57	89.8±0.29	88.8±0.16
4,4' - DDD'	83.6±0.42	83.8±0.15	82.2±0.18
Endosulfan II	82.7±0.13	82.6±0.15	84.2±0.13
4,4' DDT	82.5±0.18	82.4±0.06	80.2±0.13
Endrin aldehyde	80.7±0.22	80.6±0.12	81.9±0.22
Endosulan sulfate	87.8±0.17	87.5±0.11	85.8±0.17
Methoxylchlor	82.7±0.26	82.4±0.09	87.6±0.34

Beef meat			
	Spike level		
	1 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}
α - BHC	82.8±0.19	82.8±0.17	81.3±0.25
γ - BHC	81.9±0.15	81.6±0.17	82.7±0.21
β - BHC	80.9±0.08	80.8±0.21	81.6±0.15
Heptachlor	85.6±0.31	85.5±0,19	80.4±0.11
δ-BHC	80.7±0.15	80.7±0.27	80.3±0.27
Aldrin	81.8±0.20	81.9±0.23	84.5±0.15
Heptachlor epoxide	87.2±0.20	87.2±0.11	80.7±0.21
Endosulfan	80.8±0.21	80.7±0.25	83.7±0.15
4,4' DDE	84.4±0.12	84.5±0.20	81.8±0.15
Endrin	82.2±0.17	82.2±0.11	80.8±0.18
Dieldrin	86.3±0.14	86.1±0.09	83.7±0.15
4,4' - DDD'	83.1±0.11	83.2±0.15	81.8±0.23
Endosulfan II	84.7±0.35	84.8±0.09	87.6±0.081
4,4' DDT	80.1±0.10	80.1±0.12	81.5±0.08
Endrin aldehyde	80.5±0.17	80.7±0.15	80.7±0.18
Endosulan sulfate	86.6±0.36	86.4±0.11	84.8±0.8
Methoxylchlor	82.3±0.14	82.4±0.25	81.1±0.06

Fish			
	Spike level		
	1 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}
α – BHC	83.8±0.19	83.7±0.15	84.6±0.25
γ – BHC	82.8±0.14	82.7±0.21	80.8±0.10
β – BHC	80.1±0.16	80.1±0.10	81.8±0.23
Heptachlor	86.7±0.23	86.7±0.19	87.6±0.081
δ-BHC	81.9±0.13	81.8±0.32	81.5±0.08
Aldrin	83.5±0.05	83.5±0.34	80.7±0.18
Heptachlor epoxide	86.7±0.29	86.6±0.28	88.6±0.08
Endosulfan	82.2±0.12	82.1±0.06	80.4±0.09
4,4' DDE	85.2±0.17	85.3±0.09	84.8±0.8
Endrin	80.2±0.17	80.3±0.26	81.1±0.06
Dieldrin	85.8±0.25	85.9±0.16	88.8±0.16
4,4' - DDD'	82.7±0.28	82.5±0.19	82.4±0.09
Endosulfan II	83.8±0.21	83.7±0.15	84.2±0.53
4,4' DDT	82.4±0.11	82.4±0.15	80.2±0.09
Endrin aldehyde	80.3±0.27	80.4±0.21	81.6±0.09
Endosulan sulfate	87.8±0.17	87.7±0.29	85.6±0.21
Methoxylchlor	84.9±0.12	84.7±0.20	87.3±0.12

Appendix VII: Limit of detection

	R^2	LOD (µg kg^{-1})
α – BHC	0.99	2.33
γ – BHC	0.99	3.09
β – BHC	0.99	8.91
Heptachlor	0.99	5.87
δ-BHC	0.99	19.29
Aldrin	0.99	8.50
Heptachlor epoxide	0.99	1.37
Endosulfan	0.99	9.78
4,4' DDE	0.99	4.56
Endrin	0.99	23.12
Dieldrin	0.99	5.87
4,4' - DDD'	0.99	20.23
Endosulfan II	0.99	37.65
4,4' DDT	0.99	4.12
Endrin aldehyde	0.99	2.33
Endosulan sulfate	0.99	3.09
Methoxylchlor	0.99	8.91

Appendix VIII: Comparative levels of organochlorine pesticides in food from Kinshasa and Johannesburg

	Bean Kin (µg kg^{-1})	Bean JHB (µg kg^{-1})	P-values
α – BHC	62.06±13.16	79.51±4.12	0.039
γ – BHC	56.05±4.23	99.67±3.23	0.024
β – BHC	0	45.83±6.52	0.009
Aldrin	0	65.89±4.23	0.003
Heptachlor epoxide	4.55±1.96	78.93±8.12	0.012
4,4' DDE	81.05±7.23	106.65±5.63	0.028
4,4' DDD'	61.05±2.15	95.67±2.10	0.015
4,4' DDT	106.93±2.91	125.87±5.97	0.041
Endrin	0	56.78±7.34	0.005

	Cabbage Kin (µg kg^{-1})	Cabbage JHB (µg kg^{-1})	P-values
α – BHC	38.54±7.46	58.53±2.19	0.032
γ – BHC	40.83±5.67	78.95±6.23	0.041
β – BHC	0	85.76±3.83	0.002
Heptachlor	13.88±3.71	35.783.48	0.012
Aldrin	0	53.45±3.29	0.0023
4,4' DDE	56.57±8.93	99.67±7.41	0.036
4,4' DDD'	49.50±3.51	75.76±3.74	0.019
4,4' DDT	96.70±2.34	109.67±6.92	0.035
Dieldrin	0	48.85±2.75	0.006

	Cassava Kin (µg kg^{-1})	Cassava JHB (µg kg^{-1})	P-value
α – BHC	0	12.39±3.45	0.009
γ – BHC	5.21±1.89	15.98±5.67	0.03
Heptachlor epoxide	0	16.95±1.56	0.007
4,4' DDE	45.06±2.86	85.78±8.92	0.02
4,4' DDD'	23.23±1.73	57.56±9.34	0.01
4,4' DDT	78.45±7.34	86.67±6.23	0.04
Endrin	0	23.78±3.91	0.008

	Beef meat Kin (µg kg^{-1})	Beef meat JHB (µg kg^{-1})	P-values
α – BHC	102.28±10.11	159.74±9.56	0.031
γ – BHC	103.28±8.93	132.82±8.12	0.029
β – BHC	0	101.97±7.21	0.004
Heptachlor	0	95.62±2.71	0.0023
Aldrin	0	107.45±5.91	0.006
Heptachlor epoxide	0	91.89±5.93	0.001
4,4' DDE	154.15±4.34	253.58±6.23	0.028
4,4' DDD'	97.65±1.67	113.64±2.91	0.039
4,4' DDT	145.12±6.71	167.89±1.45	0.043
Endrin	0	97.94±3.82	0.001
Dieldrin	0	25.89±2.31	0.008

	Fish Kin (µg kg^{-1})	Fish JHB (µg kg^{-1})	P-values
α – BHC	83.19±9.86	108.95±7.62	0.029
γ – BHC	72.53±6.83	105.78±5.23	0.034
β – BHC	ND	99.76±5.72	0.008
Aldrin	ND	95.45±5.61	0.005
Heptachlor epoxide	32.68±3.41	78.56±5.20	0.018
4,4' DDE	90.09±3.82	125.78±7.23	0.041
4,4' DDD'	73.52±5.67	105.74±4.27	0.029
4,4' DDT	63.72±7.82	134.57±5.23	0.036
Endrin	ND	13.49±1.23	0.007
Dieldrin	10.39±1.12	75.78±3.61	0.028