RISK ASSESSMENT OF ENDOCRINE DISRUPTING COMPOUNDS IN SELECTED FOOD ITEMS SOLD IN OPEN MARKETS IN ZAMBIA; A COMPARISON OF KITWE, KABWE AND LUSAKA

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THE UNIVERSITY OF ZAMBIA

LUSAKA

DECLARATION

I, Foster Miyanza do hereby declare that the contents of the thesis being submitted herein are my original work and they have not been previously submitted to any University for the award of a degree or any other qualification.

Signature……..Date……

CERTIFICATE OF APPROVAL

This thesis submitted by Foster Miyanza is approved as fulfilling the requirements for the award of the degree of Master of Science in Integrated Water Resources Management.

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ABSTRACT

Endocrine disrupting compounds (EDCs) are receiving increased attention in the environment due to their link to lifestyles diseases like hypertension, heart disease, type 2 diabetes and some cancers. This research aimed at assessing the presence and quantities of heavy metals and organic EDCs in the selected raw food items sold on open markets in the two cities and one town in Zambia. Fifty samples including Kapenta, fish and vegetables were collected from major open markets of Kitwe, Kabwe and Lusaka. Quick Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) sample extraction technique was optimised for extraction of selected organic EDCs, with primary secondary amine (PSA) as clean-up sorbents. Microwave assisted digestion was used to extract metals. Optimised QuEChERS parameters used are 75 mg clean-up sorbent, 5 min extraction time, 400 rpm separation speed, 0.5 g salt (NaCl), 6 mL methanol as extraction solvent. Detection was done using gas chromatography-mass spectrometry and inductively coupled plasma-optic emission spectroscopy for organic EDCs and heavy metals, respectively. The results showed that DDT metabolites, including 4-nonylphenol, were not detected in all the foodstuffs. The mean concentrations of dimethyl phthalate (DMP) ranged from 91.05 to 101.76 μg/kg, 77.14 to 123.82 μg/kg, and 85.65 to 98.55 μg/kg for samples from Kitwe, Kabwe and Lusaka, respectively. The mean concentrations of diethyl phthalate (DEP) ranged from 21.46 to 80.69 μg/kg, 63.93 to 161.67 μg/kg and 23.22 to 46.01 μg/kg for samples from Kitwe, Kabwe and Lusaka, respectively. There was no significant difference in the mean concentrations of DMP in all the samples from markets Kabwe, Kitwe and Lusaka, $P>0.05$. The mean concentrations of DEP showed no significant difference for samples from Kitwe and Lusaka, $P>0.05$. However, a significant difference, $P>0.0167$, in the mean concentrations was found between samples from Kitwe and Kabwe, and samples from Kabwe and Lusaka with samples from Kabwe Town having higher concentrations, upto 123.82 μg/kg compared to 101.76 μg/kg and 98.55 μg/kg, in both cases. The health risk analysis of DMP and DEP recorded the hazard index that is less than one, which indicates that the consumers are safe from the health effects that result from exposure to these phthalates through consumption of food. The investigation revealed that the mean level of trace elements ranged: Cd 3.0±0.33 to Al 3472.3±25 mg/kg for Kitwe; Cd 2.0±0.67 to Al 1698.7±17 mg/kg for Lusaka; Cd 1.9±0.31 to Al 662.7±6.01 mg/kg for Kabwe. The Hazard Index (HI) for all metals in all samples from all markets in Kabwe, Kitwe and Lusaka were greater than 1 indicating possible non-carcinogenic risk. The Carcinogenic Risk (CR) for cadmium was higher than 10^{-4} in all samples

from all markets in Kabwe, Kitwe and Lusaka. This indicates possible carcinogenic risk from prolonged consumption of foods under study. However, it is recommended that further studies need to be done to assess total phthalate exposure in order to make wholesome conclusions with regards to health risk through consumption of food from open markets.

DEDICATION

I sincerely dedicate this work to the Lord my God for His continued grace and sustenance during the research process.

I also dedicate the work to my son, Azariah Miyanza and my wife, Martha Simukumbwa, for the zeal and determination they inspired me with for me to remain focused during the study period.

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ABBREVIATIONS

CR : Carcinogenic risk

DDD : Dichlorodiphenyldichloroethane

DDE : Dichlorodiphenyldichloroethylene

DDT : Dichlorodiphenyltrichloroethane

DEP : Diethyl phthalate

DMP : Dimethyl phthalate

EDC : Endocrine disrupting compound

EDI : Estimated daily intake

FAO : Food and agriculture organization

GC-ECD : Gas chromatography-Electron capture detection

GC-MS : Gas Chromatography-Mass spectroscopy

GCXGC : Gas chromatography coupled with secondary Gas chromatography

HI : Hazard index

ICP-OES : Inductively coupled plasma optical emission spectrometry

LOD : Limit of detection

LOQ : Limit of quantification

PSA : Primary secondary amine

QuECHERS: Quick, Easy, Cheap, Effective, Rugged and Safe

THQ : Target hazard quotient

TOF : Time of flight

WHO : World health organization

CHAPTER ONE

1.0 INTRODUCTION

The introduction provides the background of this study, statement of the problem, rationale of the research, research objectives and the scope of the study.

1.1 Background

Endocrine system (ES) is a chemical messenger system consisting of hormones, the group of glands of an organism that secrete those hormones directly into the circulatory system to regulate the function of distant target organs and the feedback loops, which modulate hormone release so that homeostasis is maintained. Hormones are natural chemicals produced in cells within endocrine glands, which are located throughout the body. In humans, the major endocrine glands include the hypothalamus, pituitary gland, pineal gland, thyroid gland, parathyroid, thymus, pancreas, adrenal glands, kidneys and testes (male) or ovaries (female). The ES controls growth, development, metabolism, circadian cycles, electrolyte balance, glucose levels, sex hormones, T-cell development, calcium levels, puberty, a woman's menstrual cycle, bone growth and many other functions (Sever and Glass 2013; Encarnação et al. 2019). Some chemicals may interfere with any of the endocrine functions. Such chemicals are known as endocrine disrupting compounds (EDCs).

Different authors define EDCs differently. EDCs are superfluous natural or man-made chemicals that can change the functioning of the endocrine system in both humans and animals by either mimicking or blocking endocrine actions and have injurious impact on health as a consequence (Diamanti-Kandarakis et al. 2009; Sweeney 2002; Sanderson 2006). The Endocrine Society (2014) simply defines EDCs as "an exogenous (non-natural) chemical, or mixture of chemicals, that interferes with any aspect of hormone action." The World Health Organization (WHO) defines an endocrine disrupter as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" (WHO/IPCS 2002). Among the different definitions given, the WHO/IPCS definition of endocrine disrupters is widely regarded as a useful basis for dealing with endocrine disrupters. Specifically, EDCs can interfere with receptor binding, steroidogenesis and metabolism of hormones (Sanderson 2006).

Other chemicals are categorized as potential EDCs. WHO defines "a potential EDC as an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations" (WHO/IPCS 2002). A recent development is the introduction of a definition for a possible EDC at the Organization for Econommic Co-operation and Development (OECD) endocrine disrupters testing and assessment (EDTA) meeting in April 2011. A possible EDC is a chemical that is able to alter the functioning of the endocrine system but for which information about possible adverse consequences of that alteration in an intact organism is uncertain (OECD 2011).

Diseases that are cancer related have been increasing since the 1970s (WHO 2012). This is in some way attributed to the increased presence of man-made chemicals in the environment that find their way into humans through drinking contaminated water and eating food that contain such compounds. One of the groups of chemicals that are receiving attention is endocrine disrupting chemicals (EDCs) and potential EDCs. There are two classes of substances that can cause endocrine disruption and these are natural substances including hormones found naturally in the body of humans and animals and phyto-estrogens found in some plants and man-made substances. The group of man-made substances comprise of synthetically produced hormones designed intentionally to interfere with the endocrine system, like oral contraceptives and man-made chemicals designed for use industry, agriculture and consumer goods that may have unforeseen adverse or synergistic effects. Manufactured chemicals also include chemicals unintentionally formed or produced as a by-product of industrial analysis or combustion (Petrovic et al. 2002). Some of the deleterious health effects of EDCs on organisms, wildlife and humans are that, the disruptors may be associated with altered reproductive function in males and females, abnormal growth patterns and neurodevelopmental delays in children. In addition, changes in immune function, the development of learning disabilities, severe attention deficit/hyperactivity disorder, cognitive and brain development problems constitute effects of EDCs. EDCs can also lead to dermal toxicity, deformations of the body (including limbs), breast cancer, prostate cancer, thyroid and other cancers, sexual development problems such as feminizing of males or masculinizing effects on females (Sharma et al. 2016; Monneret 2017). Still other effects include, immunotoxicity, neurobehavioural abnormalities, altered or reduced sexual behaviour, altered thyroid and adrenal cortical function, pathological changes to the spleen, damaged digestive

systems, amongst others (Game et al. 2006; Dmitruk et al. 2008). EDCs comprise of a broad range of contaminants.

EDCs consists of more than 800 different chemical compounds including natural compounds such as phytoestrogens present in a wide variety of plants (e.g., soybean genistein, mycotoxins such as zearalenone) and synthetic compounds. EDCs include plasticizers [bisphenol A (BPA), phthalates], plasticizer alternatives (di-(2-ethylhexyl) phthalate and bisphenol A alternatives), surfactants (alkylphenols), heavy metals (arsenic, lead and mercury). Preservatives for cosmetic and pharmaceutical products (parabens), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, insecticides (e.g. DDT and its metabolites), dioxins, fungicides, various pharmaceuticals and perfluorinated compounds are also classified as EDCs (Grzeskowiak et al. 2016; Coster et al. 2012; Scognamiglio et al. 2016; Azzouz et al. 2019). Some pesticide classes including organochlorines, organophosphates, carbamates, pyrethroids, triazines, carbamic and ureic compounds have also been recognized as potential EDCs (Scognamiglio et al. 2016).

EDCs exposure is often behind corticoid and/or thyroid dysfunction and behind adverse effects on the reproductive and neurological systems (Gore et al. 2015). Hauser et al. (2007) and Duty et al. (2005) found an association between DNA damage in sperm and environmental exposure to phthalates. In animal experiments, it was demonstrated that heavy metals such as cadmium (Cd), arsenic (As), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) may exhibit endocrine-disruption (Georgescu et al. 2011). Many investigations have confirmed that heavy metals accumulate in fatty tissues and then affect the functions of nervous system, endocrine system, immune system and other body systems (Li et al. 2013; Wang 2013). Ma and Singhirunnusorn (2012), reported that the toxicity of Zn and copper (Cu) can change the function of the human central nervous system and respiratory system and disrupt the endocrine system.

Prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (WHO 1992; Jarup 2003). Chromium, for example, is toxic or carcinogenic even at low concentrations when people are exposed for a long time (Zhang et al. 2015). Heavy metals such as cadmium (Cd) and lead (Pb) have been shown to have carcinogenic effects (Trichopoulos 1997).

High concentrations of heavy metals (Cu, Cd and Pb) in fruits and vegetables were related to high prevalence of upper gastrointestinal cancer (Turkdogan et al. 2002).

Due to an unprecedented increase in the production and use of industrial and agricultural chemicals during the last decades, humans are speculated to be routinely exposed to a wide variety of endocrine disrupting chemicals (Diamanti-Kandarakis et al. 2014). Some typical EDCs such as metals like mercury, arsenic, lead, copper, zinc, arsenic and organic compounds like chlorpyrifos, malathion, diazinon, dimethyl phthalate, diethyl phthalate, dibutylphthalate and polybrominated diphenyls have aroused a considerable amount of attention in the past two decades. According to Mill and Chichester (2005), EDCs present a potential threat to aquatic organisms even at low concentrations of 0.1–10 ng/L. These compounds tend to accumulate more in fish than in the water; thus, they can affect the health of human beings via the food chain (Diao et al. 2017). The occurrence and distribution of EDCs in edible fish species are therefore, important for both ecology and human health. This is especially true for Zambia whose population consume a lot of fish and other food stuffs from open markets. No proper quality assurance is given to such foods in as regards to environmental chemicals is concerned.

1.2 Statement of the problem and rationale of the research

Despite that the effects of EDCs and their mechanisms of action have been extensively studied as given in the background above, there are currently, large gaps, which exist in the status of endocrine disrupting compounds in food related materials. While in many developed countries, such data is available, very little exist in Zambia and yet the majority of population in cities / towns depend on food items sold on open market whose quality and level of EDCs are not known. The levels of heavy metals in vegetables, edible termites and fish were determined (Kapungwe 2013; Kaile and Nyirenda 2016; Mbewe et al. 2016; Siame et al. 2016; Kapaale et al. 2021; Hasimuna et al. 2022) not from open markets. For organic pollutants, levels of dichlorvos in vegetables grown around Lusaka (Sinyangwe et al. 2016) and Monze district (Mwanja et al. 2017) were determined, levels of Malathion pesticide in vegetables and fruits in Kasama were determined (Macha 2022) and the levels of DDT and its metabolites were determined in lechwe (Sichilongo & Torto 2006). Up to date, there is no study dedicated to assessing EDCs in open markets in Zambia. This is challenging for policy makers to come up with appropriate policies and regulatory framework of these compounds in food stuffs particularly those sourced from open markets and consumed. There

is need for background data on EDCs using cheap extraction methods. Empirical evidence gathered using cheap extraction methods is essential to influence policy and regulatory framework development of policies in Zambia.

1.3 Research objectives

The main objective is to assess the levels of EDCs in selected food items from open markets in two cities (Kitwe and Lusaka) and one town (Kabwe) in Zambia with the following specific objectives:

(i) To assess the presence of potential organic EDCs in the selected food items from open markets in Kabwe, Kitwe and Lusaka;
(ii) To determine the quantities of the selected organic EDCs in these food items;
(iii) To determine the quantities of heavy metals in the selected food items; and
(iv) To assess the health risk posed by both heavy metals and organic EDCs.
(v) To compare levels of quantified EDCs among the three towns

1.4 Thesis organization

The first part of Chapter 1 is the introduction on EDCs. The second part is the state of the problem and the rationale of the study. The study sites are described in part 3. Part 4 outlines the research objectives while part 5 outlines the scope of the study. Chapter 2 consists of literature review of both organic and metallic EDCs. The first and second parts describe how the endocrine system and EDCs work, respectively. Part 3 discusses the classes of organic EDCs. The fourth and fifth parts discuss the sources and effects organic EDCs, respectively. Part 6 descusses sample preparation prior to analysis of EDCs. The seventh part describes the methods used for extraction of EDCs. Part 8 describes sample concentration and separation techniques. Part 9 discusses the the techniques used to detect and quantify EDCs. Chapter 3 describes the methodology of the study. Methodology includes desktop study, fieldwork and laboratory work. Results and discussion are presented in the fourth Chapter. Chapter 4 is in two parts, analysis of organic EDCs and analysis of metallic EDCs. Health risk assessments are also presented in Chapter 4. Chapter 5 presents the conclusions and the recommendations of the study. References come at the end.

CHAPTER TWO

2.0 LITERATURE REVIEW

This Chapter is a review of available literature on endocrine disrupting compounds (EDCs). The Chapter reviews the determination of EDCs in Zambia, how the endocrine system works, and how EDCs work. The Chapter provides a review of the classification, sources and effects of EDCs. The Chapter continues by reviewing the methods used for extraction, detection and quantitation of EDCs.

2.1 Determination of EDCs in Zambia and available gaps

The assessment of EDCs, both organic and inorganic, in food, environmental and biological matrices is very important as EDCs are responsible for the development of a number of diseases. As a result, a considerable amount of effort has been expended in trying to know the levels heavy metals and organic pollutants with endocrine disruption and other health implications. Herein is outlined the studies done in Zambia in different matrices, and the knowledge gaps that exists.

Heavy metals in soil: All crops grow in soil where they obtain nutrients including heavy metals. Soil contamination by heavy metals contaminates crops in turn. Tembo et al. (2004) determined the levels of heavy metals, limited to only four heavy metals, in soils around Kabwe town. Their results ranged between 0.08 and 28 mg/kg (Cd); 0.20 and 0.61 mg/kg (Cu); 0.10 and 758 mg/kg (Pb) and 0.40 and 234 mg/kg (Zn) suggesting high precipitation of metals from the core mining activities. However, their research only covered up to 20 km in each direction from the lead and zinc mining area. Therefore, little to nothing is known about the levels these metals after the research distance. In trying to distinguish lithogenic sources of metals from anthropogenic contamination of soils caused by fallout of dust from mining operations, flotation ore treatment plants, tailings dams, smelters and slag dumping grounds, Kříbek et al. (2010) determined the levels of metals (Cu, Co Zn, Pb, As and Hg) in topsoil in the central-northern part of the Copperbelt Province. Their results show that there was more of Cu and Co than other metals. However, they do confirm that the contents of As, Zn, Pb, Cr and Ni are usually higher in soils over areas underlain by the Katanga Supergroup even via natural enhanced concentrations derived from the bedrock and transfer by weathering into the overlying soils. Such studies are lacking in major agricultural

areas of Zambia; hence nothing is known about the levels of heavy metals in those soils and how much is usually contained in agricultural produce.

Heavy metals in water and sediments: Fish is an important delicacy that stays in water. Contamination of water and its sediments increases the risk of fish contamination by metals. Sracek et al. (2012) investigated the contamination of surface water and sediments of the Kafue River network in the Copperbelt. They reported that the total metal levels in stream sediments show that the Kafue River and especially its tributaries downstream from the main contamination sources are highly enriched with respect to Cu and exceed the Canadian limit for fresh water sediments. Other researchers assessed the levels of heavy metals in sediments of Kafue River at different points of contamination. They determined the levels of Cu, Cd, Pb, Ni, Mn, Cr, and Fe in sediments at Kafue Bridge, Hippo Dam, Mpongwe, Ithezi Tezhi Dam and Kafue Town along Kafue River. They reported the highest Pb and Fe concentrations recorded at Hippo Dam (36.2 mg/kg) and (733 mg/kg) at Kafue Town, respectively. Other notably high metal concentrations were Cr (42.5 mg/kg) at Kafue Bridge, Cu (233 mg/kg) at Mpongwe, and Mn was 133 mg/kg at Kafue Town while it was highest at Ithezi Tezhi Dam (166 mg/kg) (Mbewe et al. 2016). These values are high suggesting a possible contamination of aquatic life in Kafue River. Hasimuna et al. (2021) investigated the level of some heavy metals (Cu, Co, Fe, Mn, Pb and Zn) in the Kifubwa and Solwezi Rivers. Six sites known to be associated with heavy metal anthropogenic activities were sampled. Their results showed little contamination in the surface sediments of the named rivers. In Zambia, many rivers and lakes exist whose water and sediment quality are not known.

Organic EDCs in water and soil: From 2000 to 2010, a Zambian private mining company reintroduced the use of DDT for malaria control without studying its fate in the environment. To this effect, Munyinda et al. (2015) assessed the presence of DDT and its metabolites in the soil and water around communities (Chongwe, Mongu and Chawama) that were exposed to DDT around the said period.

Heavy metals in foodstuffs: Kapaale et al. (2022) examined the heavy metal (Cu, Co, Pb, Mn, Ni, Zn and Fe) content in selected edible termites in Magoye and Mazabuka district. Hasimuna et al. (2022) investigated the contamination of heavy metals (Fe, Zn and Cu) in the Largescale Yellowfish (Labeobarbus marequensis) from the Solwezi River in North-western Zambia.

Evaluation of the levels of the seven heavy metals (Cu, Cd, Pb, Ni, Mn, Cr and Fe) in three fish species including three-spot bream (Tilapia andersonii), red-breasted bream (T. rendalli) and nile tilapia (Oreochromis niloticus) at different locations in the Kafue River (Mbewe et al. 2016). The levels of six heavy metals (As, Co, Cu, Hg, Pb and Z) in leaves and tubers of cassava growing on uncontaminated and contaminated soils of the Copperbelt mining district were analyzed. The levels of copper in cassava leaves from contaminated soils reached as high as 612 mg/kg as compared to 252 mg/kg for leaves from uncontaminated soils. The levels of Co, As and Zn reached as high as 78 mg/kg, 8mg/kg and 231mg/kg, respectively, in leaves of cassava leaves from contaminated soils (Kríbek et al. 2014).

When investigating the levels of heavy metal contamination in vegetables irrigated using wastewater, the researcher also investigated the contamination of wastewater and soils from the farming zones. The vegetables included Chinese cabbage, pumpkin leaves, tomato, swiss chard leaves, bean leaves, okra fruits, sugarcane stem and rape. The study sites were New Farm Extension in Mufulira Town in the Copperbelt Province and Chilumba Gardens in Kafue Town in Lusaka Province. The heavy metals investigated were Pb, Cu, Co, Ni and Cr. The study indicated that the levels of heavy metals were higher than acceptable limits in wastewater used to irrigate crops and that there are potential health risks associated with consumption of crops grown using contaminated wastewater (Kapungwe 2013). Siame et al. (2016) analyzed the heavy metal concentrations of Cu, Ni, Zn, Co, Pb and Fe in fruits (eggplant, lemon and tomato) and green leafy vegetables (pumpkin leaves and rape) purchased from four different markets (Chamboli, Chisokone, Chimwemwe and Chipata markets) in Kitwe. Their results were higher than the permissible limits by WHO/FAO. To the best of my knowledge, this is the only research conducted on open markets, though limited with the number of vegetables analyzed.

Organic EDCs in foodstuffs: Sichilongo and Torto, (2006) determined the levels of organic EDCs (deltamethrin, aldrin, endosulfan, dieldrin, DDD, heptachlor, d-t-allethrin, DDE, endrin and DDT) in serum, whole blood and liver tissue samples from Kafue lechwe (Marsh Antelope) of Lochinvar National Park, a park that was originally a ranch using pesticides. Their results indicated that the levels of most of the EDCs far exceeded the maximum residue limits set by the Codex Alimentarius of the United Nations (UN), Food and Agricultural Organization (FAO). Mwanja et al. (2017) investigated the levels of dichlorvos in vegetables (cabbage, rape and tomatoes) and

fruits (oranges) from Monze District, with some samples exceeding the codex Alimentarius maximum residual limit. Macha (2022) assessed the levels clorpyrifos, dichlorvos and malathion in vegetables and fruits from Kasama. Sinyangwe et al. (2016) determined the levels of dichlorvos in three vegetables (rape, lettuce and cabbage) supplied at various markets around Lusaka. The results indicated that the average levels of dichlorvos were significantly above the maximum accepted limit as set by Zambian Food and Drugs Act on vegetables. This is the second study that focused on open markets even though limited to only three vegetable types and to one EDC type.

2.2 Classification of EDCs

There are two broad categories of EDCs, natural and synthetic. Natural EDCs include naturally occurring androgens and estrogens, phytoestrogens and some heavy metals. Synthetic EDCs include artificial androgens and estrogens and other industrial compounds (Liu et al. 2009; Grzeskowiak et al. 2016). Industrial EDCs belong to different classes of chemical compounds, which were designed to perform a certain kind of action but later on it was realized that they have functional properties that can result in disruption of endocrine functions.

Synthetic EDCs include pesticides, personal care products, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), polycyclic aromatic hydrocarbons (PAHs), flame retardants, bisphenols, phthalates, surfactants, parabens, dioxins, pharmaceuticals and furans among others (Grzeskowiak et al. 2016; Coster et al. 2012; Scognamiglio et al. 2016; Azzouz et al. 2019; Sangeetha et al. 2021; Jackson and Sutton 2008). The detailed discussion of the classes of synthetic EDCs and their uses is limited to the ones that were of interest to my study that include pesticides, phthalates and alkylphenol polyethoxylates and alkyl ethoxylates that include nonylphenols as discussed in the following paragraphs;

(i) Pesticides

Pesticides are used to kill unwanted organisms in crops, public areas, homes and gardens, and parasites in medicine. Pesticides are classified based on their action with different substances. Pesticide classes include insecticides, germicides, fungicides, herbicides, rodenticides, avicides, larvicides, and acaricides (Rudel and Perovich 2009; Sangeetha et al. 2021). Another classification of pesticides is based on the chemical nature of compounds and includes organochlorines,

organophosphates, carbamates, pyrethroids, triazines, carbamic and ureic compounds (Scognamiglio et al. 2016). Organochlorines include dichlorodiphenyltrichloroethane (DDT), lindane, endosulfan, aldrin, dieldrin, chlordane and methoxychlor. Organophosphates include parathion, malathion, diaznon and glyphosate; carbamates include carbaryl, carbofuran and aminocarb; pyrethroids include permethrin, cypermethrin and deltamethrin; and triazines that include atrazine. DDT and its metabolites belong to organochlorides. Although the global use of DDT has decreased, its persistence in the environment has resulted in continued human exposure (Strong et al. 2015). In addition, DDT is still used in many parts of the world, especially where the risk of contracting malaria is present (Patisaul and Adewale 2009). DDT is known to negatively influence reproductive development through disruption of multiple endocrine pathways (Holm et al. 2006). The primary metabolite of DDT, DDE (Dichlorodiphenyldichloroethane), is far more persistent than the parent compound and thus still found in the environment at low levels. These metabolites, DDE and DDD, have comparable physical and chemical properties with DDT (Guan et al. 2009).

Pesticides have widespread applications in the agriculture sector to grow crops and different foodstuff. Through food chain, these pesticides find their way to human body and wild animals. Volatile pesticides may be present in outdoor environments and thus accumulate with various materials. Pesticides are added to carpets, paints and building materials, this makes indoor environment suspected for their presence and accumulation on stuffs like toys, carpets and any other material (Rudel and Perovich 2009).

(ii) Phthalates

Phthalates are diesters of phthalic acid (1,2-benzenedicarboxylic acid) and an alcohol moiety. The phthalate products display different properties, which depend on the length and degree of the branching of the side chain. Phthalates are common plasticizers added to polymeric materials to improve their flexibility and workability (Jeddi et al. 2016). Phthalates are in two categories, short-chain and long-chain phthalates. Dimethyl phthalate (DMP), diethyl phthalate (DEP) and dibutyl phthalate (DBP) constitute short-chain phthalates. Long-chain phthalates include butyl-benzyl phthalate (BBP), di-n-hexyl phthalate (DNHP), di-2-ethylhexyl phthalate (DEHP), di-n-octyl phthalate (DNOP), di-iso-nonyl phthalate (DINP) and di-iso-decyl phthalate (DIDP) (Sangeetha

et al. 2021). Phthalates have been widely used in numerous consumer products, including cosmetics, food packaging, building materials, medical supplies, home furnishings, toys due to their characteristic properties, such as their good insulation, high strength, excellent corrosion resistance, low cost and ease of fabrication (He et al. 2019; Chi et al. 2017: Fan et al. 2017). Phthalates are structurally similar to fats and have a high binding affinity toward them; hence, fatty foods wrapped in plastics are most vulnerable to contamination by phthalates (Sangeetha et al. 2021).

Phthalates that have high-molecular weight, like butylbenzyl phthalate (BBzP), di-(2-ethylhexyl) phthalate (DEHP) and mixtures of di-n-octyl phthalates (DnOP) are most well-known for their use as plasticizers in polyvinyl chloride (PVC) materials such as food packaging, flooring and medical devices toys and fuels to enhance performance (Serrano et al. 2014; Zota et al. 2014). Long-chain phthalates are also used in furniture manufacturing industries, clothing, building materials, insecticide carriers, chloride resins, adhesives and cellulose film coatings (Sheikh et al. 2016). On the other hand, low-molecular weight phthalates, dimethyl phthalate (DMP), diethyl phthalate (DEP) and dibutyl phthalate (DBP) are primarily added to cosmetics and personal care products (perfume, deodorants, hair sprays, skin cleansers) as solvents, fixatives and adhesives to retain colour or fragrance (Serrano et al. 2014; Sathyanarayana 2008). Due to non-covalent bonds between the phthalate chemicals and their parent materials, there can be significant leaching and volatilization leading to environmental contamination and thus ubiquitous exposures in the general population (Cao 2010; Serrano et al. 2014; Josh et al. 2015). Human exposure to phthalates occurs through ingestion, inhalation and dermal adsorption from air, food, water, dust and soil (Net et al. 2015; Encarnação et al. 2019).

(iii) Alkylphenol polyethoxylates (APEOs) and alkyl ethoxylates (AEOs) metabolites

These are nonionic and are used as detergents, emulsifiers, humidifiers, stabilizers, skimmers and dispersing agents in industrial, agricultural and household applications. They are intermediates in the synthesis of various industrial products. Phenols are defined as hydroxyl derivatives of benzene and its condensed nuclei. Phenol and its derivatives are aromatic molecules containing hydroxyl, methyl, amide or sulphonic groups attached to the benzenoid ring structure. These compounds are

toxic, persistent and very difficult to remove from the environment (Toniolo et al. 2007; Huang et al. 2007; Chaliha et al. 2008).

The presence of phenol and phenolic compounds in the environment has been of great public concern due to their high toxicity, persistence and prevalent use in large quantities in the production of plastics, plasticizers, drugs, dyestuffs, explosives, pesticides, detergents, stabilizers and antioxidants. They are among the most frequent contaminants in food, water and hazardous waste sites (Liao et al. 2006; Suliman et al. 2006; Gabriel et al. 2007). The EDCs alkylphenols, alkylphenol monoethoxylates and alkylphenol diethoxylates are as a result of alkylphenol polyethoxylates (APEOs) and alkyl ethoxylates (AEOs) degradation. APEOs are broken down into nonylphenols (NPs) or octylphenols (OPs) to a lesser extent. These two compounds are estrogen active, toxic and persistent in the environment (Sangeetha et al. 2021; Sajid et al. 2016; Guenther et al. 2002). NPs are organic compounds with a nine-carbon alkyl chain bound to a phenol ring. NP is commonly used in pesticides, lubricating oils and laundry or dish washing detergents. NP is a mixture of more than 100 isomers, but 4-NP makes up over 90% of the NP (Lu and Gan 2014).

Phenylphenols such as 2-phenylphenol (2-PH) and 4-phenylphenol (4-PH) possess fungicidal properties and are used as sanitizers and disinfectants in industrial and household products and also as post-harvest fungicides for fruits (EPA 2006). Alkylphenols such as nonylphenol (NP) and 4-*tert*-octylphenol (4-OP) are widely used as ingredients of domestic products such as surfactants and food packaging films, as well as in industrial chemicals for rubber production (Li et al. 2013).

(iv) Metallic EDCs

There is a lot of evidence that heavy metals can serve as EDCs in humans and animals (Yan et al. 2023; Macedo et al, 2023). Among the known classes of EDCs, heavy metals have a major impact on endocrine health. The heavy metals best characterized as EDCs include cadmium (Cd), lead (Pb), arsenic (As), copper (Cu), mercury (Hg), nickel (Ni), manganese (Mn) and zinc (Zn). Other metals like aluminium (Al) are potential EDCs (Paschoalini et al. 2019).

2.3 Sources of EDCs

The sources of EDCs are presented in the next subsections as follows:

2.3.1 Sources of organic EDCs

Human exposure to EDCs occurs through dermal absorption, consumption of pharmaceuticals and personal care products, direct contact with household goods, consumption of contaminated water and food, and inhalation of polluted air and soil particles, especially children. In developing countries, careless dumping of garbage and untreated industrial effluent adds to the high levels of EDCs in the environment (Olujimi et al. 2010). EDCs come into the aquatic environment through the discharge of residual chemicals by households, agricultural, manufacturing industries and through the discharge of inadequately treated wastewater (Fawell and Ong 2012; Qin et al. 2012). Wastewater treatment plants (WWTPs) and untreated urban wastewater are the major routes through which EDCs are released into several water sources. Due to the incomplete removal of these contaminants by WWTPs, many of the contaminants like alkylphenols (e.g. nonylphenol) survive and escape from the sewage treatment plants into surface water. In addition, effluents from sewage treatment plants, surface water run-off from settlements, road system, leakage from septic tanks and landfill sites contribute to the burden of ogarnic EDCs in surface water (Harrison et al. 2006).

Faecal and urinary deposits produced during animal husbandry contribute large amounts of steroidal hormones. However, the amount and species of manure-borne estrogens from livestock waste depend on the species, sex, age, hormonal status, among other traits of the animal (Hanselman et al. 2003). Food contact materials are a source of EDCs such as Bisphenol A, which is used in many containers mainly in epoxy-based lining canned foods. The lining is used to give protection from pathogens. However, since the chemicals are in direct contact with the food, they may leach into food and finally get to humans. Because of the hazardous effects on humans, bisphenol A is no longer used in baby bottles (Gore et al. 2015).

Pharmaceuticals include prescription drugs. After these drugs are consumed, they enter the wastewater stream as a result of partial metabolism and excretion. In addition, pharmaceuticals originate from people flushing unused medicines and from agricultural runoff, as farmers use pharmaceuticals in fish farming or to help prevent livestock diseases or to promote an increase in animal size. Livestock faeces and residual animal medicines are among the EDCs sources (Boxall et al. 2004). EDCs also enter into the groundwater through leaching and leaking of poorly designed sewer and landfills (Fawell and Ong 2012). Personal care products enter into the aquatic

environments through recreational activities such as swimming and also through showering and bathing as well as other technological process (Larsson et al. 2007). The human body is exposed to triclosan daily through direct contact with personal care and household products as well as exposure to the whole ecosystem including the water, soil and other organisms. Ingestion and dermal absorption are the key routes of human absorption of triclosan (Sandborgh-Englund et al. 2006).

2.3.2 Sources and uses of metallic EDCs

Heavy metals are present in our environment as they formed during the earth's birth. Their increased dispersal is a function of their usefulness during our growing dependence on industrial modification and manipulation of our environment (Colborn et al. 1993; Jarup 2003).

Cadmium (Cd) is a heavy metal widely dispersed throughout environmental matrices. Elevated concentrations of this metal in soil and reservoirs are the result of heavy emissions from different sources of pollution (Bhattacharyya et al. 2000). The extraction, foundry, metallurgical and electroplating industries are the main sources of occupational exposure to Cd, while exposure in the general population occurs through the ingestion of contaminated foods (meat, fish, fruit) or contact with consumer products such as nickel/cadmium batteries, pigments (Cd yellow), paints, and plastic products (Zadorozhnaja et al. 2000). Tobacco smoke is one of the most common sources of Cd exposure because the tobacco plant concentrates Cd. Smoking one pack of cigarettes a day results in a dose of about 1 mg Cd/year (Klaassen 2006). Fodder plants are the main constituents through which Cd enters the food chain.

Mercury (Hg) is a metal that is extensively used in melting, mining and manufacturing industries and is a component in a number of electrical instruments and medical products such as thermostats, thermometers, switches, dental amalgams and batteries. Occupational exposure occurs mainly among those working in the manufacture of paints, precision instruments, Hg vapour lamps, fluorescent bulbs and batteries (Gochfeld 2003). Hg is very little distributed in nature and rarely exists in the pure metal state. Minerals that commonly contain Hg are mercury sulphide, chlorosulphuric mercury ore and thiosulphuric antimony mercury ore. Organic mercury fungicides are widely used in agriculture. In addition, mercury is used as a catalyzer in industrial processes (Clarkson 2002). The general population is exposed to Hg primarily through the ingestion of

contaminated foods, particularly fish, where Hg accumulates in the form of methylmercury (MeHg). Mercury exposure also occurs in the general population through dental amalgams that release this metal during chewing. Fish contaminated with Hg poses a serious health risk to pregnant women and their babies. Methyl mercury bioaccumulates and biomagnifies in muscles of predatory fish that are at the top of the food chain, such as albacore (Gochfeld 2003; Bayen et al. 2005; Bhan and Sarkar 2005).

Arsenic (AS) is widely distributed in the natural environment, both as pure element and within complex compounds. As represents the twentieth most prevalent metal in the earth crust, with a soil concentration of 1-2 ppm. The predominant source of environmental As pollution include the use of As in the foundry industry, agricultural products such as herbicides and fungicides, and combustion of fossil fuels (Vahidnia et al. 2007). Exposure of the general population occurs mostly through the ingestion of contaminated food and water. Occupational exposure to As occurs in workers in the pesticide, ceramics, paint, insecticide and wood preservatives industries. This heavy metal is part of a large number of pesticides broadly used in rigorous agriculture (Ratnaike 2003).

Lead (Pb) is largely found in nature, in rocks, under several combinations. The terrestrial crust contains on average 1-2 ppm lead. Most of the lead (Pb) that is present in environmental matrices originates from anthropogenic sources. Lead is used in the production of batteries, cables, pigments and chemical additives and was employed in petrol products. The main sources of environmental Pb pollution are from foundry and mining industries, refineries, waste disposal and Pb-recycling industries (WHO 1995). Due to human activities, lead is largely spread in the environment in water, air, soil, flora and fauna. The general population is exposed to Pb via the ingestion of contaminated food and water and inhalation of airborne Pb (WHO 1995).

Manganese (Mn) forms about 0.1% of the Earth's crust. Mn is ranked as the 12th most abundant element present naturally in rocks, water, soil and organisms. There are inorganic and organic manganese compounds, with the inorganic forms being the most common in the environment. Mn is used in the manufacture of dry-cell batteries, in iron and steel production and the production of potassium permanganate. Other Mn chemicals are used as oxidants in production of hydroquinone, manufacture of glass, textile bleaching, asmoxidizing agent for electrode coating in welding rods, in matches and fireworks and in the tanning of leather. Mn produces neurotoxicity, and its toxicity has been observed primarily in occupational environments such as Mn mining and smelting,

battery manufacturing and steel production (Santamaria et al. 2007). Mn is essential to various functions, like the control of metabolism, reproductive processes and as an enzymes co-factor. However, depending on the concentration, Mn can be toxic to fish and some aquatic organisms (Correia et al. 2021).

Zinc (Zn) is a chalcophilic element and a trace constituent in most rocks. Zinc rarely occurs naturally in its metallic state, but many minerals contain Zn as a major component from which the metal may be economically recovered (WHO 2001). Zinc is mainly used as a protective coating of other metals, such as iron and steel, but further important applications are in dye casting, the construction industry and other alloys. This metal is a widely used catalyst and its inorganic compounds have various applications for automotive equipment, storage and dry-cell batteries, and organ pipes. Moreover, Zn chloride, sulphide and sulphate have dental, medical and household applications. Zinc contamination results from industrial smoke, with the most relevant compounds represented by Zn chloride, Zn chromate, Zn phosphur, Zn sulphate and Zn oxide. Contamination is also possible by use of zincate containers to heat milk and foods. Moreover, Zn is an important substance used in the fabrication process of several pesticides (WHO 2001).

Nickel (Ni) originates from natural and artificial sources and can be found practically in all environmental compartments that include water, air, soil and living organisms. In the air, Ni is distributed in the form of aerosols that contain various Ni concentrations, depending on the primary source of metal contamination. Transportation and distribution of Ni particles between different compartments of the environment is strongly dependent on the particles size and the climatic conditions. Generally, the particles originating from artificial resources are smaller compared to those derived from natural sources. Water contamination is as a result of sedimentation of metal particles form the atmosphere, of residual industrial and city waste in addition to soil and natural rocks erosion (Dormer et al. 1973).

Aluminum (Al) is the most abundant metal and the third most abundant element in the earth's crust. Al is not found free in nature and is found in mostly in igneous rocks as aluminosilicate minerals. Al is also present in air, water and many foods. Al enters environmental media naturally through the weathering of rocks and minerals. Through anthropogenic activities, Al is released in form of air emissions, wastewater effluents and solid waste primarily associated with industrial

processes (Keith et al. 2009). Al is widely used in industry, cooking utensils, civil construction, food packaging, personal care products and pharmaceuticals (Fernandez-Davila et al. 2012).

Copper (Cu) in the soil originates from the primary and secondary minerals (chalcopyrite, malachite, azurite, cuprite, atacamite). With decomposition of these minerals, copper oxidizes and goes into the divalent state. The divalent copper ion (Cu^{2+}) creates different forms in soil, which are of different solubility and accessibility for plants (Doner and Ege 2005).

2.4 How endocrine disrupting compounds work

EDCs exert their endocrine action by binding to hormone receptors of different glands acting as agonists or antagonists. For example, bisphenol A (BPA) has been reported to act as an agonist when bound to estrogen receptors (ERs) and an antagonist when bound to androgen receptors (AR). EDCs interact with membrane-bound receptors and nuclear receptors. EDCs that bind membrane receptors can disrupt non-genomic signaling pathways while those that bind to nuclear receptors can disrupt genomic pathways. Primarily, EDCs may interfere with hormone synthesis, secretion, transport, binding, metabolism and elimination resulting in various effects (Casals-Casas and Desvergne 2011; Yilmaz et al. 2020). The effects may include, for example in females, morphological and functional alterations of the reproductive system, which can result in infertility, irregular menstrual cycles, endometriosis, uterine fibroids, precocious puberty or premature ovarian insufficiency (POI), gynecological cancers and polycystic ovary syndrome (PCOS) (Rattan et al. 2017).

Nuclear receptors includes androgen (AR) where testosterone binds for male sexual development, estrogen (ERα, ERβ) whose ligand is estradiol for female sexual development and thyroid hormone receptor (TRα, TRβ) where thyroid hormone binds for metabolic and heart rate control. Progesterone receptor (PR) whose ligand is progesterone for female sexual development and glucocordicoid (GRα, GRβ) with cortisol as ligand and involved in stress response, metabolism and development. Other nuclear receptors are peroxisome proliferator-activated (PPARα,β,λ) whose ligands are lipids/fatty acids for lipid homeostasis, and arylhydrocarbon receptor (AhR) with unknown ligand. AhR is involved in stress response, organ development, neurogenesis, metabolism and circadian rhythm. More nuclear receptors exist. Estradiol can also bind to non-nuclear estrogen receptor (GPR30) (Moral et al. 2008; Yilmaz et al. 2020).

EDCs are also capable of acting through transcriptional coactivators, enzymatic pathways involved in steroid biosynthesis and/or metabolism and numerous other mechanisms that converge upon endocrine and reproductive systems. Other possible modes of actions include genetic susceptibility, oxidative stress and epigenetic modifications like DNA methylation. These effects are worrisome since changes in genetic make-up in the early stages of development may have significant effects in later years and may as well lead to transgenerational inheritance of disease (Moral et al. 2008; Anway and Skinner 2008; Diamanti-Kandarakis et al. 2010; Skinner 2011).

2.5 Effects of organic EDCs

The effects of the selected organic EDCs on the health of animals and humans are discussed as follows:

(i) Pesticides

Human beings are exposed to pesticides due to their occupations or through dietary and environmental exposure (water, soil, air). The endocrine effects of pesticides are numerous due to numerous types of pesticides. The effects include; disruption of hormone expression in the hypothalamus, alteration of thyroid hormone dependent gene expression, inhibition of 17β-estradiol and progesterone activity, and increase as well as decrease of estrogen production and aromatase activity. Some pesticides like carbofuran increase progesterone, cortisol and estradiol level and decrease the level of testosterone. Other pesticides like dimethoate increase insulin level in blood, decrease luteinizing hormone level in blood. Malathion inhibits catecholamine secretion (Cocco 2002). Lindane reduces oestrous cycles and luteal progesterone levels, increases insulin and estradiol blood serum levels, decreases thyroxine levels and bind competitively to AR, ER and PR receptors. Some pesticides like molinate damage the reproductive tract and reduces fertility. Parathion inhibits catecholamine secretion, increases melatonin synthesis and inhibits gonadotrophic hormone. Trichlorfon and other pesticides achieve alteration of thyroid function (Rawlings et al. 1998). Pesticide exposure influences the risk of breast cancer (Cohn 2011). Thyroid hormone production can be inhibited by some ten pesticides (amitrole, cyhalothrin, fipronil, ioxynil, maneb, mancozeb, pentachloronitrobenzene, prodiamine, pyrimethanil, thiazopyr, ziram and zineb). Cryptorchidism and hypospadias are other effects of pesticides (Cocco 2002; Sugiyama et al. 2005; Carbone et al. 2006).

At the human level, endocrine disruptor pesticides have also been shown to disrupt reproductive and sexual development and these effects seem to depend on several factors, including gender, age, diet and occupation. Infants are extremely vulnerable to pre and postnatal exposure to endocrine disruptor pesticides, resulting in a wide range of adverse health effects including possible long-term impacts on intellectual function and delayed effects on the central nervous system functioning. Pesticide exposure may affect spermatogenesis leading to poor semen quality and reduced male fertility (Ribas-Fito et al. 2003; Eskenazi et al. 2006).

Organochlorine pesticides such as DDT and its metabolites are estrogen mimetics. They bind and activate the estrogen receptors (ER) and produce estrogen-like effects. DDT and its metabolites show adverse effects on wildlife like birds and reptiles by changing endogenous hormone levels, increasing levels of proteins regulated by estrogen and reduction of male phallus size. These compounds could change various effects that are estrogen regulated in humans such as reduction of bone mineral density, reduced sperm count, spontaneous abortion, endometriosis, breast cancer, nervous system, immune system and metabolism (Burgos-Aceves et al. 2021; Frigo et al. 2002). DDE induces changes in the balance of male reproductive hormones with an extreme estrogen synthesis. DDE as well induces reproductive dysfunctions and prostate cancer progression in humans (Burgos-Aceves et al. 2021). Through their anti-androgenic effect, DDT and its metabolites target aromatase the main enzyme responsible for converting androgens to estradiol in placenta. Decreased aromatase activity alter steroid hormone secretion in human placenta (Wojtowicz et al. 2007). Recently, Wojciechowska et al. (2017) demonstrated that DDT and DDE could change the secretion of hormones by increasing the synthesis and secretion of oxytocin, estradiol and progesterone from the bovine placentome.

The vascular endothelial growth factor and insulin-like growth factor are vital for development of ovarian follicles and the corpus luteum function. However, through changes in the expression of the vascular endothelial growth factor and insulin-like growth factor by interaction with DDE, animals as well as humans can develop polycystic ovaries and impaired fertility (Burgos-Aceves et al. 2021). It has been reported that exposure to DDT and metabolites can cause miscarriages, premature rupture of fetal membranes, preterm birth, early onset of puberty and menopause (Wojtowicz et al. 2007).

DDT can interfere with thyroid, estrogen, androgen, rennin-angiotensin, insulin and neuroendocrine systems which can directly influence the reproductive, cardiovascular and metabolic systems of human body (Gore et al. 2014).

(ii) Phthalates

The ED potential of phthalates is known via animal experiments. Phthalates can cause oxidative stress, problems in embryonic development, impairments in reproduction and effects in neuro-behaviour. Prenatal and infant exposure to phthalates and their metabolites through breast milk is linked to loss of pregnancy, behavioral, mental and cognitive effects like lower IQ indices, hyperactivity, attention problems, social communication problems along with negative effects in the standard development of reproductive organs (Su et al. 2014; Ejaredar et al. 2015). Besides, research shows that male infants are more susceptible to phthalates than female infants. Low sperm quality is also linked to phthalate exposure. Phthalate exposure can lead to decreased sex and thyroid hormones concentrations, obesity, allergies, early puberty, fibroids, asthma and breast cancer (Pak et al. 2011; Huang et al. 2012).

Phthalates can significantly affect thyroid function in exposed subjects. Interestingly, phthalates may behave as both a thyroid receptor (TR) agonist and a TR antagonist (Ibhazehiebo and Koibuchi 2011). Some studies have described a negative association between urinary phthalates and serum-free and total T4 (Meeker et al. 2007). Other studies have indicated that urinary phthalates are positively associated with serum thyroid-stimulating hormone (TSH), while another study found a positive correlation between phthalate intake and serum TSH in Taiwanese children (Wu et al. 2013). Phthalates have anti-androgenic and estrogenic effects in testis. Some studies have confirmed a decrease in androgen levels and a reduced semen quality after phthalate exposure. Phthalates cause reduced sperm motility in humans (Wang et al. 2015). It was proposed that anogenital distance (AGD) was inversely related to urinary levels of phthalate metabolites in 134 newborn humans (Swan et al. 2015).

A mixture of phthalates (MEP, MEHP, MBP, MiBP, MiNP and MBzP) affects cell cycle regulators, apoptotic factors and several receptors and receptor-associated genes inhibiting growth of antral follicles in mouse. Similarly, prenatal exposure to a mixture of phthalate (DEP, DEHP,

DBP, DNOP, DIBP and BBP) induced increased uterine weight, cystic ovaries, disrupted the estrous cycle and reduced fertility in female rats (Zhou et al. 2017).

(iii) Alkylphenol polyethoxylates (APEOs) and alkyl ethoxylates (AEOs) metabolites

Nonylphenol and octylphenol have estrogenic potential to interfere with the function and metabolism of estrogen. Nonylphenol and octylphenol bind to estrogen receptors and can block or alter endogenous estrogen functions in various reproductive and developmental stages. Xenoestrogens mimic the estrogen pathway and affect the normal function of female sex hormones. This mechanism is suspected to lead to carcinogenesis in women including the development of endometrial, breast and ovarian cancers as well as the development of prostate cancer in men (Wen et al. 2020). However, alkylphenols are 100 to 10000 times less estrogenic than 17β-estradiol. Nevertheless, their extensive use and capacity to bioaccumulate in fat depots entails considerable contribution to the environmental estrogen pool by these chemicals (Hejmej et al. 2011). 4-tert-octylphenol (4-t-OP) is an estrogen receptor agonist with equal affinities for the two isomeric estrogen receptors (ERα and ERβ). The interaction of 4-t-OP with estrogen receptors is thought to account for some of its adverse effects that include effect on aromatase activity as well as on endogenous level of estrogen. Effects on the endogenous level of estrogen affects hormone production, induction of reproduction toxicities involving spermatogenesis in mammals, reduced egg production in the female fish, induction of vitellogenin in male fish and amphibians (Hejmej et al. 2011; Olaniyan et al. 2020). 4-t-OP disturbs thyroid homeostasis as evidenced in delayed metamorphosis in amphibians, a process normally regulated by the thyroid hormones (Croteau et al. 2008). 4-n-nonylphenol (4-n-NP) has potential to interfere with estrogenic, androgenic and thyroid endocrine systems. At low concentrations, 4-n-NP causes early onset of puberty, gain in uterine weight and thyroid dysfunction due to changed levels of thyroid-stimulating hormone, tetraiodothyronine (FT) 3 and 4 in rats (Ji et al. 2019).

Alkylphenols may as well regulate the functions of non-estrogen receptors directly or indirectly. In this regard, nonylphenol has been shown to affect immune responses. In a study conducted by Iwata et al. (2004), 4-n-nonylphenol suppressed T helper-type 1 (Th1) development and enhanced T helper-type 2 (Th2) development whereas estrogen by itself failed to affect Th1/Th2 development. 4-n-Octylphenol (4-n-OP) elicited similar effects but 4-nonylphenol and 4-t-OP elicited much weaker effects. Their conclusion is that 4-n-NP and 4-n-OP directly suppressed Th1

development and enhanced Th2 development through mechanisms that are independent of estrogen receptors. Khan et al. (2003) investigated the effects alkylphenols on the Ca^{2+} channels located on the endoplasmic reticulum membrane. The channels used are inositol-1,4,5-trisphosphate (IP3)-sensitive Ca^{2+} channels from porcine cerebellum and rat testicular membranes. All alkylphenols inhibited the extent of IP3-induced Ca^{2+} release from both cerebellar and testicular microsomes with 4-n-NP being the most potent. These results illustrate another mechanism by which alkylphenols can disrupt endocrine function independent of estrogen receptors (Khan et al. 2003). Exposure to 4-n-NP appears to affect the thyroid and pituitary hormones more than the steroidal hormones (Bandiera 2006).

(iv) Metallic EDCs

The first metal to be linked with endocrine disruption is arsenic (As). As can alter reproduction and sex differentiation by interfering with sex hormones and steroidogenesis by adrenal cortex. As binds to the glucocorticoid receptor and interferes with glucocorticoid hormones activity. As inhibits glucocorticoid receptor-mediated gene activation. As also interferes with the estrogen receptor. Davey et al. (2007) reported that As significantly suppressed estrogen receptor-dependent gene transcription of the 17ß-estradiol-inducible vitellogenin gene in chicken embryo liver. The same authors reported that non-cytotoxic concentrations of As of 2-225 ppb in cell cultures, significantly inhibited estradiol receptor-regulated effects in human breast cancer cell line known as Michigan Cancer Foundation-7 (MCF-7).

A number of studies show that Cd interferes with the activity of steroid hormones in both male and female reproductive organs even at low-concentration exposure. Cd disrupts steroidogenesis by interfering with the biosynthesis of estrogens, androgens and progesterone in vivo and in vitro experiments. The effect is disturbed sex differentiation and altered gametogenesis. However, it may bind both the estrogen and androgen receptor (Masufumi and Schin'Ichi 2006). Strumylaite et al. (2011) brought out some significant evidence of higher Cd levels in breast tissue and biological media from women with breast cancer compared to controls. This suggests that exposure to Cd could be interpreted as a potential risk factor for breast cancer. Cd can cause impaired expression of hypothalamic genes, high concentrations of leutinizing hormone (LH) and low concentrations of anti-Müllerian hormone (AMH) in plasma and abnormal follicular growth with associated reduction of antral follicles. The main results of these effects are alteration of the

estrus cycle, premature ovary failure and polycystic ovary syndrome (Zhang et al. 2017; da Costa et al. 2021). Cd exposure is linked to numerous human health problems, including an increased incidence of renal pathologies, osteoporosis, leukemia and hypertension and is involved in the onset of lung cancer. Cd can prevent the binding of the estrogen receptor to the DNA-hormone responsive elements in the cell nucleus by replacing the Zn atom in the Zn fingers of the estrogen receptor (Denier et al. 2009).

Most available data indicate that Hg may act as a major EDC (Darbre 2006). Both organic and inorganic Hg compounds highly accumulate in major endocrine glands like hypothalamus, the pituitary gland, the thyroid, the testes, the ovaries and the adrenal cortex. Hg-based compounds disrupt steroidogenesis, including sex hormones synthesis, male and female fertility as well as the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-adrenal axis (Tan and Mahaffey 2003). Studies have shown that it can increase the incidence of spontaneous abortion, preterm birth and congenital malformation. Additionally, a 2019 study carried out on rats revealed that Hg can also impair the estrous cycle and follicular growth with decreased antral follicles and increased follicular atrophy, as well as increased cystic ovarian follicles. High levels of Hg have been linked to infertility with alterations of sperm count, motility and morphology due to detrimental effects on steroid hormone production and directly on Leydig cells (Merlo et al. 2019).

Lead is a powerful disruptor of adrenal and ovarian steroidogenesis, inhibiting synthesis and activity of progesterone, 17-hydroxyprogesterone, 17,20-dihydroxyprogesterone, deoxycorticosterone, corticosterone and 21-deoxycortisol in a dose-dependent manner. Interestingly, its effects on 17β-estradiol, testosterone and cortisol are biphasic with stimulatory effects after low-levels exposure and inhibitory effects after high-level exposure. Pd exposure results in disturbed fertility in females, as revealed by an in vitro study that examined the consequences of Pb exposure on cytochrome P-450 aromatase (P-450 ARO) and ß-estradiol receptors, two key proteins in the function of the pituitary-ovarian axis. It was shown that the activity of both P-450 ARO and ER-β in the granulosa cells of the ovarian follicles was strongly inhibited in women exposed to Pb. Pb contamination may modify endocrine-controlled processes such as longevity, development, sexual receptivity, fertility and locomotion (Chaube et al. 2010; Taupeau et al. 2003). Pd has been reported to increase aldosterone synthesis, upregulating the 11β-hydroxylase 2. Pb may also be involved in alteration of fertility in men because of a reduction in

seminal parameters. In females, Pb affects the morphology of the ovary and reduces the number of primary follicles, interfering with follicular growth (Qu et al. 2021). Effects on the female reproductive system (alterations in pregnancy) and male reproductive system (morphological alterations in spermatozoa and the sperm count) have also been linked to Pb exposure. One of the main causes of Pb-related reproductive toxicity stems from the way this metal affects the endocrine system (WHO 1995).

Nickel, in animal experiments, can prevent the binding of the estrogen receptor to the DNA-hormone responsive elements in the cell nucleus by replacing the Zn atom in the Zn fingers of the estrogen receptor (Denier et al. 2009). Ni may inhibit the release of prolactin probably due to agonist effect on hypothalamic dopamine. Ni inhibits secretion of growth hormone though with demonstratable growth hormone release stimulatory effects from the pituitary gland. Nickel significantly decreases iodine uptake by the thyroid (Dormer et al. 1973).

Copper plays a major role in growth and development, cell function and is a co-factor for various metabolic enzymes in living organisms. Nonetheless, extreme amount of Cu can cause hostile effects on the growth and metabolism, antioxidant and genetic parameters, histological structure, immunological activity, egg development and hatching in aquatic organisms (Correia et al. 2021). Copper can prevent the binding of the estrogen receptor to the DNA-hormone responsive elements in the cell nucleus by replacing the Zn atom in the Zn fingers of the estrogen receptor (Denier et al. 2009).

Zinc can alter reproduction and sex differentiation by interfering with sex hormones and steroidogenesis by adrenal cortex (Denier et al. 2009). Zn, however, improves male fecundity by wielding a positive influence on spermatogenesis. It does so because it is able to preserve sperm viability through inhibition of DNAases, it is able to stabilize membranes and has antioxidant activity against extreme superoxide anions formed by faulty spermatozoa and/or leukocytes (Gavella and Lipovac 1998).

Exposure to manganese, as demonstrated in animal studies, may affect the normal function of the endocrine system by altering the production and secretion of sexual hormones like stimulated LH release and gonadotropin secretion in the hypothalamus (Pine et al. 2005).

As of 2009, a number of toxicity studies on aluminium did not show any effects suggesting endocrine disruption (Keith et al. 2009). However, a 2010 study on mature Nile tilapia females considered Al as an EDC because it showed decreased plasma levels of 17 α-OHP. Al exposure showed to be deleterious to reproduction by accelerating lipid mobilization and deposition from the liver and in the ovaries, respectively, and decreasing protein deposition in eggs (Correia et al. 2010). Correia et al. (2021) reported reduced relative fecundity in *Astyanax altiparanae* (fish) females suggesting permanent impairment in reproduction.

2.6 Methods of extraction of EDCs

This section discusses the methods of extraction for both organic and metallic EDCs.

2.6.1 Common methods of extraction of organic EDCs

The high toxicity of EDCs and other pollutants at low-level concentrations has promoted the development of effective techniques for their separation and detection in various types of matrices (Azzouz et al. 2020; Tang and Weng 2013). The development of miniaturized extraction procedures has become a trend in recent years. The main idea behind miniaturized extraction techniques is development of methods that can easily extract target compounds at trace level concentration in different matrices by employing minimum amount of sorbents, samples and solvents, but still retain their accuracy, precision and reproducibility (Sajid et al. 2016). Solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are the traditional and among most commonly used extraction methods for EDCs in complex environmental and biological samples (Albero et al. 2017; Blasco et al. 2002).

(i) Solid phase extraction (SPE)

SPE process is based on distribution of analytes between solid sorbent packed in a cartridge and liquid sample, which moves through the solid phase. Solid phase usually consists of small porous particles of silica with or without bonded organic phase, organic polymers and ion exchangers. Mechanisms of extractions are based on adsorption, partitioning or ion exchange according to kind of solid phase. SPE is used for extraction of both the organic and inorganic compounds. The solid sorbent captures a particular analyte when liquid or gas sample is passed through it. A solvent is then used to wash any unwanted constituents captured with target analyte and finally target analyte

is eluted by using a suitable organic solvent or mixture of organic solvents. The choice of a suitable combination of sorbent material and solvents for extraction of particular class of target compounds is critical because it controls selectivity, affinity and capacity. High recoveries are obtained when analytes are strongly retained by the sorbent in the presence of water and have a subsequent low retention during elution by organic solvents. The sorbent is normally packed into small tubes or cartridges for suction or pressure generation to make the elution through the column a faster process (Pichon 2000; Kortenkamp et al. 2011).

SPE offers a number of advantages over liquid-liquid extraction (LLE) including good selectivity and specificity, high reproducibility, high recoveries, lower organic solvent consumption, the possibility of automation, concentration effect and is effective with various matrices. Either SPE can be online where the extraction is directly integrated into the system of the quantitative analysis or it may be off-line where the extraction column is not connected in any way with the gas or liquid chromatograph. In on-line SPE, full automation of the process occurs and the method is characterized with ease of application of samples with minimum sample manipulation, a large throughput and cost-efficiency. However, despite the higher costs of off-line SPE, it is often used because when combined with gas chromatography (GC), water must be removed completely prior to eluting analytes. However, SPE is highly complex and difficult to master, requires lengthy method development and is costly. Clogging the pores of the solid phase by large biomolecules, oily materials and fine solids in the sample, time consuming due to several steps of operation including conditioning, sample loading and elution are among the limitations of SPE. SPE is especially labourious and time consuming if the sample volumes are large (100-1000 mL per sample). Co-extraction of unwanted matrix components with the analytes in matrices such as wastewater, is common with SPE (Pichon 2000; Kortenkamp et al. 2011).

(ii) Liquid-liquid extraction (LLE)

LLE is a versatile classical sample preparation technique. LLE is based on establishment of distribution equilibrium of the analytes between two immiscible phases, an aqueous and an organic phase. Apparatus for LLE is a separating funnel. If distribution constant is large enough, a quantitative extraction of the analytes can be achieved in one-step. However, most of the LLEs are multistep. LLE process is an important method to separate constituents (solutes) of homogeneous liquid solutions. In this separation process, second liquid solvent is added, which is immiscible or

partially miscible with the feed and the solutes of the mixture are distributed between the two phases. Both liquids have to be thoroughly contacted and subsequently separated from each other again. To achieve high purities and yields, it is necessary to operate with multiple stages (Wei et al. 2014). LLE commonly is used for extraction of both organic and inorganic compounds. Ease of operation and simplicity of the method are advantages of LLE. However, LLE is expensive, tedious, labour intensive, time consuming, requires large volumes of organic solvents, forms emulsions, difficulty to automate, difficult phase separations, low concentration factor, unfriendly to the environment with a potential of sample contamination when trace determinations are necessary and it is not applicable to hydrophilic compounds (Zhang et al. 2009).

Due to various drawbacks associated with the conventional methods, other methods were introduced. SPE miniaturization gave birth to solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE). Miniaturization of LLE, by decreasing volume of the extracting solvent at microliter scale, gave birth to liquid phase microextraction (LPME). Modified forms of LPME like single drop microextraction (SDME), dispersive liquid-liquid microextraction (DLLME), hollow fiber based supported liquid membrane microextraction and liquid phase microextraction based on solidification of floating organic drop (LPME-SFO) were developed. Other methods developed include pressurized liquid extraction (PLE) and pressurized hot water extraction (PHWE) (Sajid et al. 2016). Other methods include ultrasonic-assisted extraction (UAE), Soxhlet extraction and supercritical fluid (SFE) extraction (Ramalepe et al. 2022). Combinations of preparation techniques (LLE with SPE and Soxhlet extraction with SPE) have been used to extract various types of analytes from various matrices. Combined preparation techniques increase the sample preparation time and possible error (Sarraf et al. 2020). PLE, microwave assisted extraction, Soxhlet extraction and sonication are used for extraction of solid samples. For extraction of liquid samples, the most applied method is SPE, although LLE is reported as well (Gatidou et al. 2007).

(iii) Salting-out assisted liquid-liquid extraction (SALLE)

Between the two conventional methods, solid phase extraction (SPE) and liquid-liquid extraction (LLE), LLE is the most extensively used sample pre-treatment technique owing to its short method development time, easy with which inorganic salts are removed and low cost (Sarraf et al. 2020). In conventional LLE, organic solvents, which are water-miscible in all proportions, cannot be used

as extracting solvents. However, phase separation of some water-miscible organic solvents from the aqueous solution can easily be induced by addition of high concentration of salts, properly mixed, into aqueous sample solutions (Rustum 1989). As a result, SALLE was introduced for sample treatment (Song et al. 2013). The salts help the polar analytes to move, selectively, into the polar organic phase from the aqueous phase. In SALLE, water-miscible organic solvents are used as the extractants (Liu et al. 2010). SALLE method was established as a sample preparation method based on a number of advantages such as straightforwardness, easy purification and quick partition equilibrium. For several separation processes, this method has been used in extraction of phthalate esters, removal of metal chelates, determination of carbonyl compounds and pharmaceutical compounds (Razmara et al. 2011; Cai et al. 2007; Gupta et al. 2009).

(iv) Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)

Due to a growing demand for high-throughput multiresidue methods (MRMs), which should be easy to perform, quick and cheap, requiring small volumes of solvents, providing a high selectivity without complicated clean-up solutions and allowing analysis of a broad range of analytes, QuEChERS extraction method, being a variant of the SALLE technique was developed. QuEChERS as a multiresidue analytical method was presented at the 4th European Pesticide Residue Workshop in Rome by Anastassiades et al. (2002) for the first time and the detailed method was published in 2003 (Rejczak and Tuzimski 2015). QuEChERS extraction method, being a variant of the SALLE technique, was developed to add a dispersive solid-phase extraction (dSPE) cleanup step after the partitioning of the organic phase and aqueous phase in the presence of salts. The QuEChERS method was primarily developed for determination of pesticide residues in vegetables and fruits (Anastassiades et al. 2003). The procedure involves a single-phase extraction of the analyte with acetonitrile, following liquid-liquid partitioning, accomplished by the addition of anhydrous $MgSO_4$ and NaCl. Residual water is removed and clean-up is simultaneously done by using dSPE (Sarraf et al. 2020).

Owing to the various modifications the technique affords, QuECHERS has a wide range of applications. QuEChERS approach is useful for analysis of, among others pesticides, veterinary drugs and other pharmaceuticals, mycotoxins, polycyclic aromatic hydrocarbons (PAHs), dyes, acrylamide, synthetic musks and UV flters, bisphenols, polybrominated diphenyl ethers and other flame retardants, endocrine disruptors, and other chemical compounds (Rejczak and Tuzimski

2015). The method has been applied in different matrices including various fruits, various teas, herbs, water, meat, eggs, cereals, animal feeds and various vegetables (Cunha et al. 2007; Lozano et al. 2012; Huertas-Pérez et al. 2016; Sinha et al. 2012; Wilkowska and Biziuk 2011; Nuapia et al. 2016). The modifications include the use of different extraction solvent, salt formulation, buffer additions for salting-out step and the application of various adsorbents for clean-up step (Rejczak and Tuzimski 2015).

Because selectivity of the dSPE clean-up step is crucial for obtaining satisfactory and accurate results, more than 50 SPE sorbents and freezing-out were tested in terms of their selectivity and applicability for removal of co-extractives depending on the different sample type. It was then established that different sorbents have significant influences on the purity and recovery of analytes (Anastassiades et al. 2003). Among amino-sorbents and alumina (e.g. aminopropyl, PSA), primary secondary amine (PSA) is the most commonly used and suitable for variety of plant-based commodities (Anastassiades et al. 2003). However, PSA sorbent is not suitable for samples with high contents of pigments (e.g. carotenoids or chlorophyll) and fat and is not capable of adsorbing non-polar pigments. Small amounts of graphitized carbon black (GCB) in combination with PSA proved to be the best solution in handling with such matrices, but due to high affinity of planar analytes towards GCB (e.g. cyprodinil, hexachlorobenzene, chlorothalonil, thiabendazole) it shows significant limitations.

Carbon-based sorbent, GCB and octadecyl silica (C18) are used when high pigmented and high fat samples are being considered. However, carbon-based sorbents have a disadvantage of losses of planar analytes (Anastassiades et al. 2003). To resolve this issue, United Chemical Technologies (UCT) developed a polymeric sorbent (ChloroFiltr®) for efficient removal of chlorophyll without loss of planar analytes (UCT QuEChERS informational booklet 2011; Wang and King 2013). Reversed-phase system sorbents, such as C18 are good at purifying samples with significant fat and waxes content but recoveries of the more lipophilic analytes may be difficult (Rejczak and Tuzimski 2015). C18 had greater impact than PSA for the matrix cleanup and improving performance, without negatively effecting recoveries. New available sorbents are zirconium based, Z-Sep and Z-Sep Plus, offered by Supelco. The Z-Sep is a sorbent based on modified silica gel with zirconium oxide and the Z-Sep Plus sorbent consists of both zirconia and C18 dual bonded on the same silica particles (Sigma-Aldrich website).

In 2013, Deng et al. tested amine-functionalised magnetic nanoparticles and multiwalled carbon nanotubes (MNPs/MWCNTs) composites as dSPE sorbents. Due to their weak anion exchange property amine functionalized MNPs may promote strong interaction with various polar organic acids and high content of pigments and sterols could be adsorbed by MWCNTs. Comparisons with commercial adsorbents including C18 and PSA/GCB were performed. The results showed that recoveries obtained by MNPs/MWCNTs composites, C18 and PSA/GCB were above 85%. However, the extracts obtained by using MNPs/MWCNTs are cleaner than those obtained by using C18 (Deng et al. 2013). In the same year, Guan et al. evaluated the ability of amine-modified graphene to cleanup fatty acids and other interfering substances from acetonitrile extracts of oil crops. The authors conducted experiments to compare amine-modified graphene (NH2-G, CH3NH-G, and nBuNH-G), PSA, MWCNTs and GCB. CH3NH-G sorbent achieved the greatest reduction of fatty acids among the sorbents with average recoveries between 70.5 and 100%. CH 3NH-G proved to be a new type of reverse-phase dSPE sorbent (Guan et al. 2013).

In 2014, chitin obtained from shrimp shell waste was applied as d-SPE sorbent in methodology elaborated for organic contaminants analysis in drinking water treatment sludge (Cerqueira et al. 2014). Satisfactory recovery rates in comparison to other sorbents such as C18, PSA, C18 and GCB were obtained. The advantage of chitin as a dSPE clean-up sorbet is the significant reduction of the method cost (Cerqueira et al. 2014). In the same year, Hou et al. (2014) proposed the multi-walled carbon nanotubes (MWCNTs) as dSPE adsorbents. It was demonstrated that MWCNTs mixed with PSA resulted in further improvement of the performance of dSPE step (Hou et al. 2014). Although bio sorbents are receiving much attention in solid-liquid extraction, little has been reported in the QuEChERS technique. However, it is anticipated that some biosorbents will be alternatives as commercial clean-up sorbents in the QuEChERS technique (Ramalepe et al. 2022; Billiard et al. 2020). A comparison of PSA with Moringa Oleifera seed (MOS) protein as a clean-up sorbent for the extraction of EDCs was performed by Ramalepe et al. (2022). MOS protein gave better selectivity with recoveries, 76.2% to 105.2%, comparable to PSA and even gave cleaner extracts in cases. The study has shown that MOS protein is a promising alternative to PSA with the advantages of MOS protein being cheaper and greener. The advantages and disadvantages of the selected sample preparation methods for organic EDCs are summerized (Table 1).

Table 1: Advantages and disadvantages of selected sample preparation methods for organic EDCs

Method	Advantage	Disadvantages
Solid-phase extraction (SPE)	Good selectivity and specificity, high reproducibility, high recoveries, lower organic solvent consumption, possibility of automation, concentration effect, is effective with various matrices.	Highly complex and difficult to master, requires lengthy method development, is costly, clogging of pores of solid phase, co-extraction of unwanted matrix components
Liquid-liquid extraction	Ease of operation and simplicity, short method development time, versatility, low cost	Tedious, labour intensive, time consuming, requires large volumes of organic solvents, forms emulsions, difficulty to automate, difficult phase separations, low concentration factor, unfriendly to the environment with a potential of sample contamination when trace determinations are necessary, not applicable to hydrophilic compounds, water-miscible organic solvents cannot be used for extraction
Salting-out assisted liquid-liquid extraction	Water-miscible organic solvents are used extractants, straightforward, easy purification, quick partition equilibrium	Relatively poor extraction of polar and charged analytes due to low dielectric constant of water immiscible organic solvents
QuEChERS	Easy to perform, easy to modify, quick, cheap, requires small volumes of solvents, provides high selectivity, no complicated clean-up solutions, allows analysis of a broad range of analytes, it is green	No one sorbent is applicable to all sample matrices

2.6.2 Sample preparation and extraction methods for metallic EDCs

The determination of metals in complex matrices, such as food, often require extensive sample preparation and/or extraction systems preceding instrumental analysis. However, depending on the physical state of the sample, analysis may be direct or may require preparation. Samples can be in liquid or solid form. Liquid samples include aqueous solutions (e.g. beverages, water, blood plasma and urine) and other liquid forms, which include fuels, oils and organic solvents. Solid samples include sediments, soils, plastics, animal tissues and plants (Bader 2011).

Liquid samples generally can be introduced directly for analysis without any prior treatment. For measurement with atomic spectroscopy, there are no precautions to be considered except sample collection and storage. As long as possible interferences are under control, concentrations levels meet the dynamic range and sensitivity of the spectroscopic method used, the analysis of solutions may be performed automatically with all modern atomic spectroscopic systems (Anwar et al. 2004). Transparent and colourless samples with a turbidity of less than one nephelometric turbidity unit (NTU), without odour and with single phase may be directly analyzed, or after enrichment, for metals without digestion. However, comparison of digested and undigested samples should be performed for verification or if changes in existing matrices are encountered (Eaton et al. 2005).

For non-aqueous samples, analysis significantly depends on their viscosity. In flame atomic absorption spectroscopy (FAAS) analysis, for instance, the viscosity should be similar to that of water for which most nebulizers are designed. Only some organic solvents, such as ethanol or methyl isobutyl ketone, fulfill this condition and are usually utilized for dilution of organic liquids. The major problem faced with these procedures is the dilution factor, which reduces the metal content per unit volume. Notwithstanding, standards can be prepared in the pure solvent and the analytical response is comparable to that given by the same element in aqueous solution (Anwar et al. 2004; Bader 2011).

Solid samples could have high amounts of organic or inorganic matter. Analysis of solid samples using spectroscopic techniques requires that samples be transformed into a solution through suitable dissolution methods (Bader 2011). It involves steps from simple dilution to partial or total dissolution. The methods include dry or wet decomposition of the samples in open or closed systems. These systems may use ultrasonic, thermal or radiant energy. Ultraviolet, infrared and microwaves constitutes the radiant energy (Iyengar et al. 1997). Closed systems improve the efficiency of oxidation and reduce digestion time by allowing high pressures build-up above atmospheric pressure. The effect is of this is high boiling temperatures that leads to quick completion of sample dissolution. Unlike open systems, closed system do not allow elements to volatilize (As, Cd, Pb, Se, Zn and Hg), reduced quantity of reagents used and do not occasion contamination from outside sources (Bader 2011). However, few techniques allow the direct introduction of the sample without any preparation. Such techniques include neutron activation, X-ray fluorescence, thermogravimetry and spectrographic techniques. The major problem with

such methods is the lack of reliable calibrations. Conversely, sample preparation makes possible the separation and/or pre-concentration of analytes, which allows the use of a number of determination techniques that include gravimetry, titrimetry, electrochemical analysis, chromatography and spectrometry (Iyengar et al. 1997).

Traditional methods for sample preparation, such as Soxhlet extraction, dry-ashing and wet-digestion are time consuming because they require long and tedious work and require large amounts of reagents, which are expensive, generate hazardous waste and might contaminate the sample with the analytes (Sneddon et al. 2006; Arruda 2007). The time is reduced significantly by using microwave digestion and can be up to 20-60 times shorter (Siitonen and Thompson 1998) than conventional methods, which is widely accepted by various scientists (Mester et al. 1999).

Metal analysis by spectroscopic methods requires that all analytes from the samples be converted from the solid phase into solution, since all spectrometers are based on an analysis of the solution. Because of that, digestion is used. Digestion is the melting of sample using some melting agent. Reagents for digestion can be a base or acid. In order to prepare the best sample possible, it is necessary to add a sufficient amount of reagents and bring enough energy to break bonds in the crystal lattices. For this purpose there are two basic methods generally used and include dry-ashing and wet-ashing (Bock 1979). However, these sample preparation methods are usually labour intensive and time consuming and they are the major source of errors, mostly due to analyte loss and/or contamination. Sample digestion might also include health hazards for the laboratory personnel and it usually produces a lot of corrosive and/or toxic waste. Finally yet importantly, any digestion or dissolution is inevitably associated with a dilution of the analyte content, making solution analysis not necessarily suitable for determination of the lowest trace concentrations (Arruda 2007). Direct solid sample analysis and slurry sample preparation are alternatives to avoiding the aforementioned limitations (Vale et al. 2006).

(i) Dry-ashing (digestion) technique

Dry-ashing is used to digest samples with a large amount of organic matter and involve the ignition of the sample in a stream of air or oxygen. This type of digestion is used to prepare samples of food, plant and biological material. It is suitable when nonvolatile components are to be analyzed. The method involves burning the sample at high temperatures (300-1000°C) in an open vessel

using a muffle furnace or microwave heating and removing the organic matter from the samples by thermal decomposition, usually in the presence of a melting agent (Buldini et al. 2002). The sample is mixed with a melting agent leading to products that are easily dissolved in water or in diluted acid. Oxidizing reagents, like pure magnesium nitrate [$Mg(NO_3)_2$] and magnesium oxide (MgO) may be used as melting agents in order to prevent the volatilization of analytes such as As, Cd, Hg and Pb and to speed up the digestion process (Buldini et al. 2002).

The dry-ashing method is simple and allows large quantities of samples to be treated at the same time. This method permits the pre-concentration of metals in the final solution, which is necessary when very low concentrations are involved. The ash comes out completely free of organic matter and that is necessary for some analytical techniques. Dry-ashing requires little or no reagents (Buldini et al. 2002). On the other hand, the addition of melting agents significantly increases the content of inorganic salts or electrolytes, which might be a problem during analysis especially in the case of some analytical spectroscopic techniques, and it might contribute to contamination and losses in evaporation. In that case, careful blank control is necessary (Buldini et al. 2002).

(ii) Wet-ashing (digestion) technique

Wet-digestion is a method of converting the components in the complex matrices to the simple chemical form. The digestion is performed by applying energy or heat, by application of chemical reagents, or by a combination of these two methods. The type of reagent used will depend on the type of sample and various oxidizing agents that enable the determination of metal content or extract metals from inorganic materials are commonly used. Normally, a combination of concentrated acid and heating is used, and important factors to take into account are the strength of the acid, its oxidizing power, boiling point, solubility of obtained salt and cleanliness. The advantage of wet-digestion is that it is equally effective for both organic and inorganic materials, which often leads to complete destruction of the sample matrix and enables the elimination of some interference (Yaman et al. 2005; Doner and Ege 2004).

The wet methods may be performed using various forms of energy including thermal, ultrasonic energy and radiation. The techniques are carried out in open vessels, in tubes, on a hot plate or in an aluminum heating block or in closed vessels at higher pressure with thermal or microwave energy. Extreme care must be applied when using sealed pressure vessels in order to avoid vessels

rupturing during conventional or microwave-assisted digestion of organic materials. The applicability of this method is dependent on the type of food because carbohydrates are easily mineralized with nitric acid at 180^0C, whereas fats, proteins and amino acids cause incomplete digestion due to the relatively low oxidation potential of nitric acid (HNO_3) at 200^0C. The presence of fats, proteins and amino acids require the addition of sulfuric or perchloric acid (Yaman et al. 2005; Doner and Ege 2004).

The type of acid used in the preparation procedure can have significant consequences in the analysis step. It is known that HNO_3 is the most desirable reagent in all atomic spectrometric techniques. Regardless of occasionally observed signal suppression (e.g. in ICP-OES) in the presence of HNO_3. However, in practice, there are no severe analytical problems encountered with HNO_3 at concentrations up to 10%, at times higher, in all atomic spectrometric techniques. Hydrogen peroxide (H_2O_2), added in most mineralization procedures, is rarely responsible for analytical problems (Arruda 2007). The hydrochloric acid (HCl) presence is not undesirable in ICP-OES analysis. However, it is exclusively prohibited in GFAAS analysis because of the possible formation of volatile and difficult-to-dissociate analyte chlorides that could cause vapor phase and/or spectral interference (Welz and Sperling 1999). Use of sulfuric acid is avoided despite its efficiency in digestion of organic matrices because of its high viscosity. Its presence in analytical techniques where the sample introduction is by nebulization (FAAS, ICP-OES, and ICP-MS) is undesirable. Microwave-assisted digestion is an attractive method, especially for small samples (Arruda 2007).

(iii) Microwave-assisted digestion

Microwave (MW) technology has significantly improved some traditional operations in chemistry and engineering and has introduced the acronym MEC, which means microwave-enhanced chemistry. The application of microwave has improved the efficiency and speed of digestion for samples that are difficult to dissolve. It is used for digestion of geological, biological, food samples, sludge and ash. Two different MW-assisted digestion systems are available, pressurized closed-vessel systems and open focused-MW systems that work under atmospheric pressure (Arruda 2007).

The main characteristics of commercially available focused-MW technology include safety work at atmospheric pressure, handling of large samples that can generate a large amount of gas, mainly when working with organic materials and the use of different types of materials for the construction of the reaction vessel [borosilicate glass, quartz and polytetrafluoroethylene (PTFE)]. The traditional microwave oven operates at high pressures that is dependent on the type of vessel in which the digestion is performed and can be used for samples weighing up to 10 g. For pressures up to seven MPa, PTFE sealed containers are used, while the quartz tube is used for pressures up to twelve MPa (especially when using sulphuric acid) (Yaman et al. 2005). Other characteristics are programmed addition of reagents (or samples) at any time during digestion, allowing for a gradual attack of acid and low-energy directional microwave field that can accelerate leaching of organometallic species without affecting the carbon-metal bond or can extract organic component. The consequence of the oriented nature of microwave energy is high efficiency and avoidance of application of high-power (Yaman et al. 2005; Arruda 2007).

By using focused microwave technique, the sample preparation for the analysis can be improved, because it allows better control of energy, that is, input into the sample. In addition, the use of closed containers enables achieving greater temperature by the increased pressure. Complete digestion of large amounts of samples or samples rich in organic components can be conducted in an open vessel at atmospheric pressure (Arruda 2007; Yaman et al. 2005).

MW-assisted digestion with HNO_3 and HCl acids without or with the addition of H_2O_2 is an extensively used procedure for the mineralization of food samples. The destruction of the plant material with a mixture of HNO_3 and H_2O_2 provides the fastest, safest and most precise analytical results with an accuracy of more than 5% for the determination of heavy metals in vegetable matrix. MW heating has several advantages over conventional heating (e.g. on a hot plate) as the energy is generated in the digestion mixture and not transferred by conduction. Among the key advantages of MW-assisted digestion are the much shorter digestion times and the reduced need for aggressive reagents to obtain complete digestion (Kingston and Jassie 1988; Yaman et al. 2005).

MW-assisted digestion in closed vessels has gained popularity as a simple and fast dissolution technique that minimizes risk of sample contamination, acid consumption and loss of volatile elements. One of the limitations is the time required for cooling before the vessels can be opened,

which may take hours, depending on the type of equipment used. The main advantages of focused-MW radiation are safety, versatility, control of MW energy released to the sample and the possibility for programmed addition of solutions during the digestion. However, in open-vessel digestion, loss of volatile elements is the major problem that affects the results for low-level elements due to the high amount of reagents used. There is also increased risk of sample contamination. This risk can be minimized by using vapour-phase acid digestion, which has proven to be very effective in minimizing the residual carbon content (Kingston and Jassie 1988; Yaman et al. 2005).

(iv) Ultrasonic-assisted exctraction

Ultrasonic-assisted extraction uses ultrasonic vibrations to extract sample with polar solvents in an ultrasonic bath. The method is used for chemical extraction from solid samples because it is simple. The ultrasonic field enables generation of microcavitations in the liquid surrounding the sample. The effects are mechanical disruption of the cell wall that makes it release its content, and local heating of the liquid, which increase the diffusion of extract. Following the collapse of cavitation bubbles at or near walls or interfaces, the kinetic energy is introduced in the whole sample volume. When this happens, the mass transfer across the solid-liquid interface improves. The mechanical effects of ultrasounds induce a greater penetration of solvent into cell membrane thus facilitating the release of cell contents and improving mass transfer (Alupului et al. 2009; Keil 2007).

Wet-digestion assisted by ultrasonic radiation is mainly used for low contamination of water matrices. Mostly it is performed with a small addition of hydrogen peroxide or nitric acid. Generally, probes or a water bath carries out ultrasonic digestion of samples, but more often an ultrasonic bath is used because they are cheaper, while probes require a shorter retention time than baths (Luque de Castro and Capote 2007).

Ultrasound has been employed for sample preparation in order to improve analytical throughput (Korn et al. 2006). The application of ultrasound respond to the need for quick preparation methods and low reagent consumption. Therefore the main advantages of ultrasound-assisted wet digestion are rapid digestion, high processing capacity of the sample treatment and small amounts of reagents. Also, digestion can be performed in an ultrasonic bath using a plastic container with a

screw (screw-top bottles) or polypropylene centrifuge tube, so the samples can be centrifuged rather than filtered (Korn et al. 2006). However, chemical information of samples surrendered to ultrasonic irradiation can be severely compromised since the collapse of cavitation bubble results in a strong local temperature increase and free radical production, which could provoke analyte loss and gross analytical errors (Luque de Castro and Capote 2007). Analyte losses are also observed for spectrometric determinations contrasting the results obtained for various metals pretreated with ultrasound devices with other sample preparation techniques (Nascentes et al. 2001; Manutsewee et al. 2007; Balarama and Arunachalam 2004).

In determining trace elements in solid substrates, efforts have been focused on avoiding mineralization procedures based on dry-ashing and ultrasound or microwave-assisted wet chemical digestion methods. Two analytical approaches, slurry sampling and direct solid sampling combined with either AAS or inductively coupled plasma (ICP)-based techniques have significantly decreased the number of preliminary operations in the analytical process for the handling of solid samples (Cal-Prieto et al. 2002; Ebdon et al. 1997; Santos and Nobrega 2006). The selected advantages and disadvantages of sample preparation methods for metallic EDCs are presented (Table 2).

Table 2: The advantages and disadvantages of selected sample preparation methods for metallic EDCs

Method	Advantage	Disadvantages
Dry-ashing	Simple, allows large quantities of samples to be treated at the same time, the ash comes out completely free of organic matter, requires little or no reagents	The addition of melting agents significantly increases the content of inorganic salts or electrolytes, which might be a problem during analysis especially in the case of some analytical spectroscopic techniques, and it might contribute to contamination
Wet-digestion	Effective for both organic and inorganic materials, which often leads to complete destruction of the sample matrix and enables the elimination of some interference	Use of reagents increases the probability of contamination
Microwave-assisted digestion	Quick sample digestion, high efficiency, avoidance of application of high-power, reduced need for aggressive reagents	Time required for cooling before the vessels can be opened which may take hours, depending on the type of equipment used, in open-vessel digestion, loss of volatile elements is the major problem and increased risk of sample contamination
Ultrasonic extraction	Rapid digestion, high processing capacity of the sample treatment, and small amounts of reagents	Chemical information can be severely compromised since the collapse of cavitation bubble results in a strong local temperature increase and free radical production, which could provoke analyte loss and gross analytical errors

2.7 Detection and quantitation techniques for EDCs

This section describes the methods used to detect and quantify both organic and metallic EDCs.

2.7.1 Detection and quantitation techniques for organic EDCs

Organic EDCs are a very diverse class of chemical compounds and hence large number of instrumental methods are used to analyse these compounds. The methods include liquid chromatography coupled with mass spectrometry (LC-MS), high-performance-liquid chromatography (HPLC), liquid chromatography coupled with electrochemical detection (LC-

ED), capillary electrophoresis (CE) and gas chromatography coupled with mass spectrometry (GC-MS) (Gatidou et al. 2007; Mottaleb et al. 2009). Other methods are photochemical fluorimetric method, electrochemical method, enzyme-linked immunal sorbent assays (ELISA), total organic carbon (TOC) sensors and biosensors (Samnani et al. 2015; Tijani et al. 2013). However, chromatographic methods, LC-MS/MS and GC-MS, are the best and most often utilized in the determination of organic EDCs. LC-MS/MS is typically used to determine more polar and less volatile compounds, while GC/MS or GC-MS/MS are used for volatile or volatizable compounds and metabolites. Derivatization can be used to broaden the applicability of GC/MS and GC-MS/MS analyses to more polar compounds (Pietrogrande and Basaglia 2007).

(i) Gas chromatography-mass spectrometry (GC-MS)

GC/MS is applied for the analysis of non-polar and volatile or volatizable compounds. However, the analysis of polar compounds requires a derivatization step (Fatta et al. 2007). In case of GC-MS analysis, the water matrix effects occur less frequently than with LC-MS/MS (Reddersen and Heberer 2003). In addition, GC/MS generally has lower LODs when compared with LC-MS/MS. The disadvantage is that it is complicated and more time-consuming and requires complex preparation of the sample in case of derivatization step (Díaz and Barceló 2005). GC is regularly adopted because of its advantages of high resolution, rapid separation and easy linkage with sensitive and selective detectors (Zhao et al. 2008).

Derivatization of polar components results in analogues that are less polar and more thermostable. It increases the sensitivity of analysis but also increases the loss of sample by performing additional operations (Gómez et al. 2007). A negative aspect of derivatization is the use of carcinogenic and toxic reagents and potentially explosive, such as diazomethane. An ideal derivatization reaction should allow the detection of analytes that have polar functional groups and it is effective when the reaction occurs in a given time with 90% efficiency. Derivatization involves optimization of many variables that include the derivatizing agent and its dose, the reaction temperature and duration (Pietrogrande and Basaglia 2007; Fatta et al. 2007). One of the risks with multi-compound methods involving derivatization is that harsh chemicals or the high temperatures of the derivatization procedure can result in thermal breakdown or conversion of underivatizable parent compounds. As such, one solution is to split samples into two fractions prior to the GC/MS

analysis, where one-half is submitted to derivatization for the analysis of polar target compounds and the other is directly analysed by GC (Pietrogrande and Basaglia 2007).

(ii) Liquid chromatography-mass spectrometry (LC-MS)

LC-MS is suitable for the determination of compounds that are polar, non-volatile or thermolabile substances, which cannot be analysed by GC-MS. For example, the detection of some pharmaceuticals, such as antibiotics, can only be achieved using LC-MS/MS (Díaz-Cruz and Barceló 2005). LC should preferably be applied for the analysis of organic micropollutants only when using tandem MS because this combination is able to produce fragment ions that are necessary for the explicit identification of the analytes. LC-MS/MS allows separation and detection of compounds that have identical molecular weight but dissimilar product ions, even if they co-elute. MS/MS detection is therefore preferred for increased analytical sensitivity and selectivity in complex water matrices (Díaz-Cruz and Barceló 2005). Atmospheric pressure chemical and electrospray ionization (APCI and ESI) are modes of ionization interfaces that are the most widely used with LC-MS/MS. Low or medium polar compounds are determined by APCI and the analysis of polar analytes like alkylphenols is conducted using ESI (Díaz-Cruz and Barceló 2005).

Matrix effects are one of the major drawbacks of LC-MS/MS, particularly when working in the ESI mode. Matrix effects can suppress or, less frequently, enhance analyte signals, consequently producing inaccurate results (Gros et al. 2006). When contaminated environmental samples are analyzed, for instance wastewater, it is essential to carry out proficient cleanup of samples if LC-MS/MS is to be applied (Ternes 2001). To obtain reliable results, both the LC separation and MS parameters require optimization. The process of optimizing the analytical methods like LC involves making a series of studies to determine the parameters that give the best results for all target analytes. Optimization of MS parameters, including cone voltage and collision energy, is performed through flow injection analysis (FIA) for each compound of interest (Gros et al. 2006).

The ion suppression/enhancement effects play an important role in LC-MS quantification. Therefore, the extent of these effects needs to be quantitatively assessed. To eliminate any possible variations during the ionization process and the mass analysis, the contamination of the ion source or the mobile phase, extraction losses, or any other unpredictable effects, an internal standard must

be used. Matrix effects might as well adversely affect important parameters like LOD, LOQ, the linearity, the accuracy, or the precision. Therefore, sample pretreatments involving isolation of the analytes, extracts purification and preconcentration are required (Mei et al. 2003; Vanatta and Coleman 2007; Dussault et al. 2009).

At present, a combination of LC/MS and GC/MS techniques appears to be the most powerful and comprehensive approach to multi-class compound analysis because the application of the two complementary methodologies widens the range of compounds that can be reliably measured (Pietrogrande and Basaglia 2007).

Advancements in chromatographic systems including use of narrow bored, short columns, high mobile phase flow rates with the help of ultra-high pressures have reduced the total chromatographic run time remarkably. Shortening of analysis time is a desirable step towards high throughput analysis, which is required by research and analysis laboratories (Petrovic et al. 2004; Samnani et al. 2015). Another advancement in chromatographic systems is the coupling of GC or LC with mass spectrometry (MS) detector. Compounds are separated by chromatographic methods and then identified qualitatively and quantitatively by the MS component (Frank 2000).

The MS detector can determine molecular structure with high sensitivity and selectivity. The use of selective ionization mode (SIM), coupling of quadruple with time of flight (TOF) enables the method to analyse and quantify target compounds from very complex matrices (Frank 2000). These highly efficient separation systems must be accompanied by suitable sample preparation procedures. Combining a liquid-liquid extraction and dispersive solid-phase extraction clean-up, QuEChERS provides a clean sample for analysis by gas chromatography or liquid chromatography and is therefore ideal for samples with complex matrices (Schmidt and Snow 2016). However, the existing techniques are not without challenges. Lack of portability and suitability during fieldwork constitute one challenge with chromatographic systems (Blazkova et al. 2009). Other challenges include the sophisticated and costly nature of the equipment and high energy demand coupled with the need of the derivatization of samples in the case of GC-MS prior to analysis. Some techniques can only detect or identify a small range of analytes at a time in the matrices. Sensors and biosensors are being developed as a replacement to the commonly used chromatographic methods.

(iii) Sensors and biosensors

Sensors and biosensors are reported to be more sensitive than chromatographic methods, ease to operate, eliminate pretreatment steps, reduce harsh sample preparation, lower energy demand, have higher sensitivity, have fast responses and are cheaper. Unlike chromatographic methods, sensors and biosensors can provide biological activity information such as toxicity and endocrine-disrupting effect (Tijani et al. 2013; Yin et al. 2009). However, despite the simplicity in the use of biosensors to detect EDCs, one major challenge is that biosensors are specific and can only sense a single analyte at a time. This means that a lot of biosensors need to be developed, which will require additional cost and time (Tijani et al. 2013). These drawbacks form the basis to develop more simple and robust instrumental techniques that are affordable, durable and easy to maintain which can screen, detect and quantify a large diversity of composites concurrently (Tijani et al. 2013; Samnani et al. 2015). Table 3 summarizes the advantages and disadvantages of quantitation methods for organic EDCs.

Table 3: The advantages and disadvantages of quantitation methods for organic EDCs LOD=limit of detection; LOQ=limit of quantification; ESI=electrospray ionization and LC-MS=liquid chromatography-mass spectrometry

Technique	Advantages	Disadvantages
Gas chromatography-mass spectrometry	Water matrix effects occur less frequent. Used to analyze non-polar and volatile compounds. High resolution and rapid separation. Easy linkage with sensitive and selective detectors.	Polar compounds requires a derivatization step which lengthens the analysis time, uses carcinogenic and toxic reagents, harsh chemicals and high temperatures which may result in thermal breakdown of underivatizable parent compounds. Costly. Not portable.
Liquid chromatography-mass spectrometry	Suitable for the determination of compounds that are polar, non-volatile or thermolabile. LC-MS/MS allows separation and detection of compounds that have identical molecular weight but dissimilar product ions, even if they co-elute.	Matrix effects particularly when working in the ESI mode. Matrix effects may affect important parameters like LOD, LOQ, linearity, accuracy or precision. Use of internal standard is a must. Costly. Not portable. Difficult linkage with sensitive and selective detectors.
Sensors and biosensors	More sensitive than chromatographic methods, ease to operate, eliminate pretreatment steps, reduce harsh sample preparation, lower energy demand, have higher sensitivity, have fast responses, are cheaper and portable. Can provide biological activity information such as toxicity and endocrine-disrupting effect.	They are specific and can only sense a single analyte at a time.

2.7.2 Detection and quantitation methods for metallic EDCs

The methods used to quantify metals are classified by the function of the technique used and include atomic fluorescence spectrometry (AFS), atomic absorption spectrometry (AAS), optical emission spectrometry (OES) and atomic mass spectrometry (MS). The merits and drawbacks of individual techniques are summarized in Table 4.

Table 4: The advantages and disadvantages of quantitation spectrometric techniques
AAS=atomic absorption spectroscopy; FAAS=flame atomic absorption spectroscopy; ETAAS=electrothermal atomic absorption spectroscopy; ICP=inductively coupled plasma; OES=optical emission spectroscopy and MS=mass spectrometry

Technique	**Advantages**	**Disadvantages**
Flame AAS	Simplicity, low operational cost	Low sensitivity, mainly relies on the nebulizer system that is characterized by only 5-10% efficiency, has a very short residence time for analysis, prone to interferences, poor reproducibility, poor detection limits
Graphite furnace AAS	More sensitive than FAAS, detection limit is about two orders of magnitude better than that of FAAS, accepts solutions, slurries, or solid samples, efficient atomizer than a FAAS	Difficult of operation, high energy demand, subject to more interferences than the FAAS, requires increased analysis time, lower precision due to high energy
Hydride generation AAS	Excellent low levels of detection in the ppb or ppt range, high sensitivity, determine levels of trace metals at wavelengths below 200 nm where there is considerable spectral interferences from radicals in conventional FAAS	Restricted only to volatile metals; results depend heavily on the acid concentration, valence state of the analyte, gas pressures, reaction time, and cell temperature.
Cold vapor AAS	Very sensitive and tunable sensitivity through variation of sample amount, has detection limits in the ppb or ppt range	Restricted to determination of mercury and cadmium
Optical emission spectroscopy	Necessitates simultaneous multi-element analysis due to its ability to measure emissions from several different elements concurrently	As the number of emission wavelengths increases, the probability for interferences that may arise from emission lines that are too close in wavelength to be measured separately also increases
Inductively coupled plasma-OES	Detection limits typically range from ppm to ppb, unlike conventional OES, ICP-OES provides higher reproducibility and quantitative linear range and reduces molecular interferences due to a higher temperature	More expensive than conventional OES, and in complex samples, the emission patterns can be difficult to interpret
Atomic fluorescence spectrometry	Fewer spectral interferences than in OES, greater sensitivity due to very low background radiation	It is difficult to detect a large number of elements in a single run using AFS
Inductively coupled plasma-MS	Extremely high sensitivity to a wide range of elements	More expensive to buy and maintain

Laser induced breakdown spectroscopy	The potential advantages include simplicity, lack of sample preparation for gases, liquids, and solids, simultaneous multi-element detection, ability to detect high and low z-elements, good sensitivity for many elements, only optical access to the target is needed, standoff analysis capabilities	Limitations include the power of the laser, sensitivity and wavelength range of the spectrometer, various difficulties arise for liquid samples due to the complex laser-plasma generation mechanisms, and splashing, waves, bubbles and aerosols caused by the shockwave accompanying the plasma formation affects precision and analytical performance
Anodic stripping voltammetry	Low cost, simplicity, high sensitivity with 10 to 100 times more than that of ETAAS for some metals, ease of operation, rapid analysis, portability and applicability for field monitoring of environmental samples	Manual anodic stripping voltammetry with conventional beaker-type electrochemical cells is relatively slow and labourious

(i) Atomic absorption spectrometry

Atomic absorption spectrometry (AAS) is a technique for measuring quantities of elements present in samples by measuring the absorbed radiation by the element of interest. AAS is a technique used mostly for determining the concentration of metals within samples such as aqueous solutions, metals and alloys, glass, food, drugs, environmental samples, biological samples, industrial wastes, among others. AAS can be used to analyze the concentration of over 62 different metals in a solution. In AAS, atoms in the form of atomic vapour are generated using a flame (FAAS) or an electrothermal system (ETAAS) or chemical vapour generation (CVG-AAS). Three steps are involved in turning a liquid sample into an atomic gas: desolvation (the liquid solvent is evaporated and the dry sample remains); vapourization (the solid sample vapourizes to a gas); and volatilization (the compounds that compose the sample are broken into free atoms). A portion of vapourized atoms is, then, thermally and collisionally excited to a higher electronic energy level before returning to their ground energy state by photon emission. The amount of the analyte is proportional to the amount of radiation absorbed by ground-state atoms regulated by Beer's Law (Christian 2003).

In FAAS, a liquid sample is aspirated into a flame through a nebulizer. The sample is converted to a mist that is composed of uniform droplets that are easily introduced into the flame. The flame desolvates and atomizes the sample providing a source of neutral atoms or molecules for analysis in the spectrophotometer. Although other flame types have been documented, the most commonly

used flame is an air–acetylene flame. Because the temperature of the air-acetylene flame is not sufficient to destroy oxides that might form or are present, a nitrous oxide-acetylene flame often is used, depending on the analyte and nature of the sample. The air-acetylene flame burns within a temperature range of 2125°C-2400°C, but the nitrous oxide-acetylene flame burns within a temperature range of 2650°C-2800°C. Flames can be optimized for a particular analysis by either decreasing or increasing the fuel to oxidant ratio. The fuel to oxidant ratio can be adjusted to be either rich or lean oxidizing or reducing, depending on the analyte of interest. It should be noted that only ground state atoms are involved in this process. Therefore, the ionization occurring due to high temperature of the flame needs to be kept to a minimum (Alves et al. 2001; Aleixo et al. 2004; Amorim and Ferreira 2005).

The flame AAS was the first variant of AAS to be introduced due to its inherent simplicity and low operational cost. However, it is characterized by a low sensitivity, mainly relies on the nebulizer system characterized by only 5-10% efficiency and has a very short residence time for analysis in the absorption volume (Aleixo et al. 2004; Karve and Rajgor 2007). Therefore, several methods are reported in the literature to improve the sensitivity of flame AAS. Pre-concentration of the elements prior to introducing the sample to flame, such as the beam injection flame furnace AAS is performed (Aleixo et al. 2004). The pre-concentration step can also be carried out by liquid-liquid extraction using dithizone as a complexant, as reported by Carasek et al. (2002) or Amorin and Ferreira (2005). However, the most widely pre-concentration method used is solid-phase extraction using various supports such as carbon, silica gel, cellulose and amberlite resin (Alves et al. 2001; Karve and Rajgor 2007). Another way to increase the sensitivity of flame AAS is Donnan dialysis, which involves the migration of the sample across a membrane barrier into a receiver volume, which is smaller in order to have a pre-concentration factor (Antonia and Allen 2001). A different method to increase the sensitivity of flame AAS involves the use of co-precipitation (i.e. with aluminium hydroxide followed by centrifugation and washing of the precipitate) (Doner and Ege 2005). FAAS is one of the commonest instrumental methods for analyzing metals and some metalloids. However, because of interferences, poor reproducibility and poor detection limits, alternative methods have been developed for some elements, mostly metalloids (Doner and Ege 2005).

Electrothermal AAS (ETAAS) is a good alternative for the detection of heavy metals at trace levels. It uses a modifier (e.g. $Pd(NO_3)_2$, Pd-$Mg(NO_3)_2$, $(NH_4)H_2PO_4$-$Mg(NO_3)_2$, Ir, W-Ir, W-Ru and W) that stabilizes the heavy metals, is more sensitive than flame AAS and allows the analysis of solid and semi-solid samples with minimal manipulation (Gonzalez et al. 2001; Korn et al. 2006; Farinas et al. 2007). It has a higher sensitivity than FAAS. However, it requires an efficient optimization of the temperature programme and choice of the chemical modifier to obtain valid results (Welz et al. 2014).

The graphite furnace AAS (GFAA) and flame AAS measurement principle is the same. The difference between these two techniques is the way the sample is introduced into the instrument. In GFAA analysis, an electrothermal graphite furnace is used instead of the standard burner head. A tube of graphite is placed inside a furnace with rigorous control of temperature, where it can be cooled by current inert gas. The sample is heated stepwise (up to 3000ºC) to dry the sample, ash organic matter and vaporize the analyte atoms. The atoms are then excited by absorption of radiation at distinguishing wavelengths. Samples can be deposited either directly onto the wall of the graphite furnace or onto a small graphite platform, which sits inside of the graphite furnace. Throughout the heating process, the graphite furnace is purged with an inert gas, usually nitrogen or argon. At the atomization step, the furnace is quickly heated to a high temperature usually to luminosity. The purge gas flow is stopped temporarily as a transient absorption signal produced by the atomized analyte is measured. The advantage of the graphite furnace is that the detection limit is about two orders of magnitude better than that of AAS (Ajtony et al. 2007).

The GFAAS has several advantages over a FAAS. It accepts solutions, slurries or solid samples, it is a much more efficient atomizer than a flame furnace and it can directly accept very small quantities of sample. It also provides a reducing environment for easily oxidized elements. Samples are placed directly into the graphite furnace. However, GFAAS is a difficult operation since the high energy that is provided to atomize the sample particles into ground state atoms might also excite the atomized particles into a higher energy level, thus lowering the precision. The GFAAS technique is used only at concentration levels below the optimum range of direct flame atomic absorption because it is subject to more interferences than the FAAS procedure and requires increased analysis time. The method of standard additions may be required to insure validity of data. Because of the high sensitivity of this technique, it is extremely susceptible to contamination;

extra care in sample handling and analysis may be required. GFAA has been the most common instrument used for analysis of lead (Pb). Countries where Pb is a criterion for pollution standards use AAS as a general technique in the reference methods to quantify it. For low-level determination of volatile elements such as As, Ge, Hg, Sb, and Se, hydride generation coupled with AAS provides lower detection limits (mg-μg range) (Butcher 2006; Hu et al. 2007).

Chemical vapour generation atomic absorption spectrometry (CVG-AAS) has excellent sensitivity, and, in the past, it has been restricted to some elements (arsenic, antimony, selenium, bismuth, cadmium and lead). Currently, many researchers have developed works to extend the applicability of this technique for the determination of other chemical elements (D'Ulivo 2016). Chemical vapour generation techniques include hydride generation atomic absorption spectroscopy (HGAAS) and cold vapour atomic absorption spectroscopy (CV-AAS).

The hydride generation technique makes use of the separation of the analyte from the matrix by conversion to the volatile hydrides and offers a pathway to trace analysis of elements, which cannot be determined by conventional methods. Excellent low levels of detection are attained when this separation method is combined with atomization of the hydride in a heated quartz tube. The sensitivity of this technique is due to its ability to determine the levels of these trace metals at wavelengths below 200nm, a region where there is considerable spectral interferences from radicals in conventional flame AAS (Campbell 1992).

In HGAAS, elements forming volatile hydrides (As, Pb, Sn, Bi, Sb, Te, Ge and Se) reacts with reducing agents in mid-acid, sodium borohydride and hydrochloric acid, to generate the hydride of the analyte of interest. The gaseous hydrides are transported by the stripping argon flow directly to an inert quartz cell on the central burner head and perfectly adjusted in the optical path of the AA spectrometer. The cell can be externally heated or an air-acetylene flame can heat it. The hydride gas is dissociated in the heated cell into free atoms for absorption process. The maximum absorption reading, peak height, or the integrated peak area is taken as the analytical signal. This is known as the direct-transfer mode of hydride generation (Campbell 1992; D'Ulivo 2016). Commercial direct-transfer hydride generators are available in two configurations that include continuous flow and flow injection. As with the cold vapour detection of mercury, hydride generation also can be very sensitive and has detection limits in the ppb or ppt range. The great advantage of this technique is the separation of specific elements as hydrides (pre-concentration

stage), eliminating the interference from the matrix. The major limitation to the hydride generation technique is that it is restricted primarily to the elements listed above and results depend heavily on the acid concentration, valence state of the analyte, gas pressures, reaction time and cell temperature. A number of common matrix components, leaving the technique subject to chemical interference, also suppresses the formation of the hydrides. Metalloids are routinely analyzed by HGAAS. Inductively coupled plasma (ICP) is also a powerful analytical, instrumental method for these elements but at this point, its higher cost limits its widespread use as compared to AAS or HGAAS (Campbell 1992; D'Ulivo 2016).

Cold vapour AAS has a restricted use for the determination of mercury and cadmium. It has an excellent sensitivity and it is one of the most recommended methodologies for the determination of mercury due to its chemical characteristic reduction to atomic state. This is achieved by a sample reaction with a strong reducing agent like tin chloride or sodium borohydride in a closed reaction system. The volatile free mercury is then driven from the reaction flask by air or argon bubbling through the solution, being carried in the light path of the AA spectrometer. In addition, all of the mercury in the sample solution placed in the reaction flask is chemically atomized and transported to the sample cell for measuring the absorbance. The advantage of this technique is that sensibility can be increased by simply addition of the amount of the sample. The technique is very sensitive and has detection limits that range from parts per billion (ppb) to parts per trillion (ppt) depending on the sample and the laboratory environment (Silva et al. 2014; D'Ulivo 2016).

(ii) Optical emission spectroscopy (OES)

In OES, the sample is subjected to higher temperatures, by the thermal energy of a flame, argon plasma or an electrical discharge that not only cause dissociation into atoms but also cause significant amounts of collisional excitation and/or ionization of the sample atoms to take place. Once the atoms or ions are in their excited states, they can decay to lower states through thermal or emission transitions. This is accompanied by the emission of electromagnetic radiation, normally in the form of light in the UV-Vis region. The wavelength of the emitted radiation and its intensity provide the qualitative and quantitative information about the analyte. In OES, the intensity of the light emitted at specific wavelengths is measured. The measured intensity is used to determine the concentrations of the elements of interest (Bernazzani and Paquin 2001).

The most significant benefits of OES results from the excitation properties of the high temperature sources used in OES. These thermal excitation sources can occupy a large number of different energy levels for several different elements at the same time. All of the excited atoms and ions can then emit their distinguishing radiation at nearly the same time. This results in the flexibility to choose from several different emission wavelengths for an element and the ability to measure emission from several different elements concurrently, which necessitates simultaneous multi-element analysis. It can be used for the analysis of major components of the sample as well as for trace analysis, because calibration curves are linear over several orders of magnitude. Because of these advantages OES technique are popular in analytical laboratories. However, a drawback associated with this feature is that, as the number of emission wavelengths increases, the probability for interferences that may arise from emission lines that are too close in wavelength to be measured separately also increases (Bernazzani and Paquin 2001; DeGraff et al. 2002).

Inductively coupled plasma optical emission spectrometry (ICP-OES) is a technique based on OES principles with a spectrometer for trace detection of metals in solution, in which a liquid sample is injected into argon gas plasma contained by a strong magnetic field. Just like in conventional OES techniques, analyte atoms in solution are aspirated into the excitation region where they are desolvated, vaporized and atomized. The elements in the sample become excited and the electrons emit energy at a distinguishing wavelength as they return to ground state. The emitted light is then measured by optical spectrometry. ICP Spectrometers can be used to analyze environmental samples, contaminants in food or water and metalloproteins in biological samples. Most ICP-OES instruments are designed to detect a single wavelength (monochromator) at a time. Since an element can emit at multiple wavelengths, it is sometimes desirable to detect more than one wavelength at a time. This can be done by sequential scanning or by using a spectrometer that is designed to capture emissions of several wavelengths (polychromator) simultaneously. Detection limits typically range from parts per million (ppm) to parts per billion (ppb), although depending on the element and instrument, it can sometimes achieve even less than ppb detection (Chen and Jiang 2009). Unlike conventional OES, it provides higher reproducibility and quantitative linear range and reduces molecular interferences due to a higher temperature (7000-8000 K) in the plasma excitation source. On the other hand, ICP-OES is more expensive than conventional OES, and in complex samples emission patters can be of difficult interpretation (Ibáñez and Cifuentes 2001; Luykx and van Ruth 2008).

The comparison of FAAS and ICP-OES reveals that they have a similar sensitivity, FAAS is a cheaper and the costs of gas consumption to employ FAAS are lower than using ICP OES, even when utilizing nitrous oxide, which is more expensive than acetylene. However, both methodologies have allowed the coupling with sequential injection analysis, flow injection analysis and others flow techniques. The comparison between electrothermal AAS (ETAAS) and inductively coupled plasma mass spectrometry (ICP-MS) shows well-matched results. The sensitivities are similar, but the gases consumption using ETAAS is much lower than ICP-MS and the maintenance of ETAAS is cheaper than ICP-MS. However, the optimization step of the experimental conditions for ETAAS, in some cases, can be more complicated when the element is volatile and the matrix is complex. The multi-element characteristic of the ICP-OES and ICP-MS has always been the respective principal advantage in relation to FAAS and ETAAS (Alexovic et al. 2017; Clavijo et al. 2015).

FAAS presents good selectivity and low cost operation whereas the dynamic range is typically within the mg/L range. ICP-OES has several advantages as simultaneous multi-element determination and low detection limits, which are typically better than those obtained by FAAS. ETAAS as well as ICP-OES and ICP-MS are detection techniques intrinsically characterized by remarkable sensitivity. However, ETAAS signals are susceptible to sample matrix composition as well as to other operational factors, whereas in ICP-OES and ICP-MS both high contents of dissolved salts and spectral matrix interferences can produce undesirable analytical responses (Butcher 2006).

Laser induced breakdown spectroscopy (LIBS) is a type of OES that uses a very energetic laser pulse as the excitation source. It involves analysis of atomic emission lines close to the surface of sample produced by laser pulse where the very high field intensity initiates an avalenche ionization of the sample elements, giving rise to the breakdown effect. Spectral and time-resolved analysis of this emission are appropriate to identify atomic species originally present at the sample surface and can determine various metals. The limitations include the power of laser, sensitivity and wavelength range of the spectrometer. Normally, this technique is used for solid samples (Barbini et al. 2000).

Using LIBS for liquid samples may cause various difficulties due to the complex laser-plasma generation mechanisms in liquids. In addition, splashing, waves, bubbles and aerosols caused by

the shockwave accompanying the plasma formation affects precision and analytical performance. In order to overcome these problems, there are various procedures for liquid samples like analyzing the surface of a stationary liquid body, the surface of a vertical flow of a liquid, the surface of a vertical flow of a liquid or of in falling droplets. The bulk of a liquid or dried sample of the liquid deposited on a solid substrate can also be analyzed (St-Onge et al. 2004). Though the results gotten are satisfactory, it is apparent that such experimental artifices challenge one of the most attractive advantages of LIBS, which is working on an unprepared sample that enable in-situ and real-time measurements (Charfi and Harith 2002). The results obtained by LIBS method showed accuracy with the results obtained by ICP in the range of 0.03-0.6 %. This shows that LIBS can be utilized for determination of heavy metals (Gondal and Hussain 2007).

The range of potential applications of LIBS is unmatched by any other analytical technique and is due to its various advantages that include simplicity, lack of sample preparation for gases, liquids, and solids, simultaneous multi-element detection and ability to detect high and low z-elements. Additionally, good sensitivity for many elements, only optical access to the target is needed and standoff analysis capabilities. These advantages permit application of LIBS to real-world analytical needs that cannot be addressed by conventional analytical methods (Gondal and Hussain 2007).

(iii) Atomic fluorescence spectrometry

Atomic fluorescence is a spectroscopic process based on the absorption of a certain wavelength of radiation by atomic vapours and subsequent radiational deactivation of the excited atoms toward the detection device. Both the absorption and subsequent atomic emission processes occur at wavelengths characteristic of the atomic species present (Meyers 2000). In AFS, a light source, such as that used for AAS, is used to excite atoms only of the element of interest. The selective excitation of the AFS technique can lead to fewer spectral interferences than in OES. The main advantage of fluorescence detection compared to absorption measurement is greater sensitivity, because the fluorescence signal has a very low background radiation. The resonant excitation provides selective excitation of the analyte, avoiding interference (Meyers 2000). However, it is difficult to detect a large number of elements in a single run using AFS. This is because the instrument limits the number of spectral excitation sources and detectors that can be used at one time (Bernazzani and Paquin 2001).

(iv) Atomic mass spectrometry

Atomic mass spectrometry is related to AAS, OES and atomic fluorescence spectrometry. Instead of measuring the absorption, emission or fluorescence of radiation from a high temperature source, such as a flame or plasma, mass spectrometry measures the number of singly charged ions from the elemental species within a sample. Similar to the function of a monochromator in emission/absorption spectrometry that separates light according to wavelength, a quadrupole mass spectrometer separates the ions of various elements according to their mass-to-charge ratio in atomic mass spectrometry (Jones 1992).

Inductively coupled plasma mass spectroscopy (ICP-MS) is one such atomic mass spectrometry technique. In this case, the atomization and ionization of the analyte is carried out using high temperature argon plasma (ICP). The ions are then separated and collected and analyzed based on their mass to charge ratio. Thus, the constituents of an unknown sample can then be identified and measured. ICP-MS offers extremely high sensitivity to a wide range of elements (Xiong and Hu 2007; Chen and Jiang 2009).

One of the primary uses of this technique is in the environmental field. Such applications include water testing, soil, food, herbal drug analysis for safety and other material analysis for industrial purposes. One of the largest application of ICP-MS is in the medical and forensic field, precisely, toxicology. A physician may order a metal analysis due to a number of reasons that include metabolic concerns, suspicion of heavy metal poisoning and even hepatological issues. Depending on the specific parameters unique to each patient's diagnostic plan, samples collected for analysis can range from whole blood, urine, plasma, serum, to even packed red blood cells. This technique is also widely used in the field of radiometric dating, in which it is used to analyze relative abundance of different isotopes (Vladimir et al. 2007; Eliott et al. 2007).

(v) Anodic stripping voltammetry (ASV)

Although there are methods with adequate sensitivity for the determination of heavy metals such as AAS, ICP-MS and ICP-OES, electrochemical methods are one of the most favourable techniques for determination of heavy metal ions because of their low cost and high sensitivity. The spectroscopic methods are highly sensitive and selective techniques. However, they require relatively expensive instruments, the application of complex operational procedures, long

detection times and are not suitable for field applications. On the other hand, electrochemical methods have the advantages of low cost, simplicity, high sensitivity, ease of operation, rapid analysis, portability and applicability for field monitoring of environmental samples (Cao et al. 2008; Arduini et al. 2010).

Among the electrochemical methods of analysis, voltammetry is the only method that has high sensitivity and can be applied for the in-situ identification and detection of heavy metal ions pollution. Among the voltammetric techniques, stripping voltammetry is considered the most sensitive and suitable for the determination at trace levels of many metals and compounds in environmental, clinical and industrial samples (Kokkinos et al. 2009).

Identification and detection of heavy metal ions is specifically achieved through anodic stripping voltammetry (ASV). ASV in connection to mercury film and hanging mercury drop electrodes has long been recognized as one of the most powerful methods for trace and ultra-trace heavy metal determination, particularly in aqueous samples (Hocevar et al. 2002). ASV is best used for metals that form amalgams with mercury, which include bismuth, gallium, indium, thallium, tin, cadmium, copper, lead and zinc. ASV is a technique that detects specific heavy metals in numerous sample matrices. ASV consists of three steps including electroplating of metals in solution onto an electrode that concentrates the metal, cessation of stirring and stripping off the metals on the electrode, which generates a measurable current. The generated current is specific for each metal, and by its magnitude, quantification can be done (Kokkinos et al. 2009; Arduini et al. 2010).

The sensitivity of ASV is 10 to 100 times more than that of ETAAS for some metals. Due to its low limit of detection, ASV may not require any preconcentration step. ASV also allows simultaneous determination of 4 to 6 metals with low-cost instrumentation. The stripping step can be either linear, staircase, square wave, or pulse (Bott 1992). Other advantages of ASV include requirement of cheaper equipment as compared to spectrometric methods and the easy of building portable systems for field measurements. Manual ASV with conventional beaker-type electrochemical cells is, however, relatively slow and labourious, particularly since environmental analysis usually requires repetitive measuring cycles on multiple samples (Intarakamhang et al. 2013).

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

This chapter describes the study methods used following the objectives given in Chapter 1. The research methods used included:

(i) Desktop study involving the search for and reading of published literature on related work in order to establish the methodologies used in a research of this nature;

(ii) Field work that involved collection of samples from large open markets in the three study areas;

(iii) Laboratory work involving the preparation of samples prior to analysis. Sample preparation involved drying, grinding, packaging, storage and acid digestion; and

(iv) Report writing included data processing and recording. It also involved a comparative study that compared the obtained findings with other similar findings within the Southern Africa region.

3.1 Study area

Three areas were selected, Kabwe Town, Kitwe City and Lusaka City. The study was conducted in these towns because that is where industries and traffic are pronounced. Kabwe Town was selected because of its mining activities history. Kitwe was chosen because it has the most mining industries in Zambia. Lusaka, on the other hand, was chosen on the basis that it is the largest city in Zambia with a lot of traffic, industrial businesses and that it has the largest population, which is more dependent on the foodstuffs sold, especially at Soweto Market. With increase in anthropogenic activities, there is increase in the amount of EDCs in the environment, especially if not controlled. The study areas are presented in Figure 1.

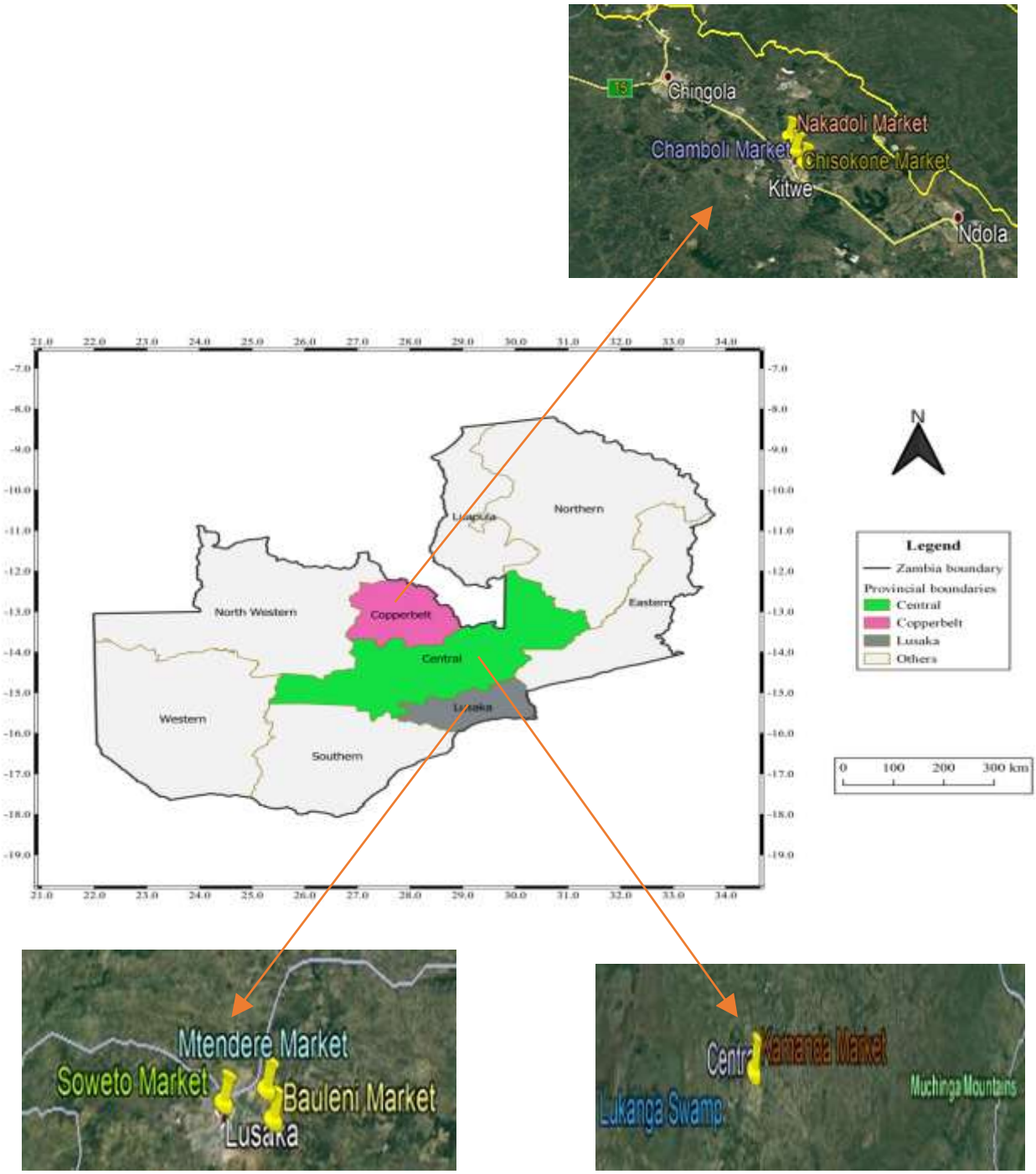


Figure 1: Map of Zambia showing the study cities, town and markets (Miyanza et al. 2023)

3.1.1 Lusaka City

Lusaka is the capital city of Zambia and the largest city in the country. It is the main centre of trade and commerce in the country. Most of the produce from various regions of the country are taken to Lusaka for marketing. Lusaka is located in South Central Zambia at 15°25'S 28°17'E, on a plateau at 1280m above sea level and covers an area of 360 km2. The city has a population of more than 3,079,964 with a growth rate of 2.9% according to 2022 census (ZSA 2022).

3.1.2 Kitwe City

Kitwe City is in the northern part of Zambia. It is the second largest city in the country and is the main industrial and commercial centre of the Copperbelt Province. The city lies between Latitude 12° and 13° South and Longitude 28° and 29° East. The city sits on a fairly flat land with an altitude of 1295 m above sea level and covers an area of 777 km². According to central statistics of 2022 census, the city has a population of 661,901 with an approximated 2.1% annual growth rate (ZSA 2022).

3.1.3 Kabwe Town

Kabwe is a town located in central Zambia north of Lusaka on the Great North Road. It is the provincial capital of the Central Province. The city is located at Latitude 14°27' South and Longitude 28°27' East. The town is an important transportation and former mining center known for its lead pollution as it was once the mine center for lead and zinc. The town has a population of about 299,206 with a growth rate of 3.3% according to 2022 census (ZSA 2022).

3.2 Desktop study

In the desktop study, literature review consisted of map collection, reading journal articles and books from Google Maps, Google Scholar, PubMed, Science Direct and Core.

3.3 Field work

This involved field visitation to the selected markets in Kabwe, Kitwe and Lusaka. It also involved the collection of fifty samples, which include Kapenta samples, tomato samples, fish (tilapia) samples and vegetable samples (cabbage, rape, spinach, cassava leaves, Chinese cabbage). These

were randomly bought from open markets of Kitwe, Kabwe and Lusaka. Samples were collected from Soweto Market (Figure 2) of Lusaka being the largest and central market in the city. The samples from Kitwe were collected from Chisokone and Chamboli markets (Figure 3) as they are the larger markets in the city. Samples were collected from Mine and Kamanda markets (Figure 4) of Kabwe because they are larger markets in the town.

Figure 2: Cabbage and tomato samples from Soweto Market of Lusaka City, Zambia

Figure 3: Sampling from Chamboli and Chisokone markets of Kitwe City, Zambia

Figure 4: Sampling from Mine and Kamanda markets of Kabwe Town, Zambia

Fresh fish, tomato and vegetable samples were collected in cooler boxes (Figure 5a) and transported to the lab where vegetables were air-dried (Figure 5b) at room temperature, pulverized using a domestic blender, then stored in polyethylene airtight bottles, fish and tomato samples were freeze-dried, pulverized using a domestic blender, then stored in polyethylene bottles. Kapenta samples were collected as dry samples. These were also pulverized using a domestic blender and stored in airtight polyethylene bottles (Figure 5c).

5a

5b

5c

Figure 5: Sample transportation, preparation and storage; 5a: cooler boxes used to carry fresh samples; 5b: air-dried vegetable samples and 5c: pulverized samples in polyethylene bottles

3.4 Laboratory work

Laboratory work involved qualitative and quantitative analysis of heavy metals and organic EDCs in the laboratory at the University of Witwatersrand, Johannesburg, South Africa.

3.4.1 Optimization of QuEChERS method using Response Surface Methodology (RSM)

Response Surface Methodology was used for the screening and optimization of the QuEChERS method. To get the best recovery in the application of real food samples, the sample mass, speed and time of centrifuge, amount of sodium chloride (NaCl) and magnesium sulphate ($MgSO_4$), solvent type and solvent extraction volume which affects the extraction efficiency of QuEChERS method was screened using L8 (3 levels with 7 factors, 6 responses and 21 runs) linear model. The selected parameters were optimized by a Central Composite Orthogonal (CCO) design which composed of a full or fractional factorial design (Quadratic model, 17 runs). The statistical tool used for the design of experiment is MODDE Pro 13.0.1 (Sartorius Stedim Biotech, Malmö, Sweden).

Three center points replicates were employed for the design and for each experimental run. The screened factors are shown in Table 5.

Table 5: Parameters screened using L8 linear model

QuEChERS parameter	Minimum	Maximum	Factor type
Centrifuge time	5 min	20 min	Quantitative
Centrifuge speed	3400 rpm	4000 rpm	Quantitative
Solvent volume	5 mL	10 mL	Quantitative
Solvent type	Methanol, acetone and acetonitrile		Qualitative
Amount of NaCl	1 g	3 g	Quantitative
Amount of $MgSO_4$	1 g	3 g	Quantitative
Sample mass	0.5 g	3 g	Quantitative

The QuEChERS method was carried out using the procedure by (Rawn et al. 2010) with modifications where necessary. The method was first performed following different screening conditions as displayed in Table 6 using a full factorial design as a screening tool. Each run was done following the combination shown in Table 7. Varying amounts of sample (Table 7) was weighed in a 50 mL centrifuge tube and spiked with 500 µL of 200 µg/L standard mixture to give

varying concentrations in µg/kg. The spiked samples were allowed to stand for 30 min to allow the standards to integrate into the samples. Varying volumes of solvent was added according to Table 6, and the sample was vortexed for 1 min. This was followed by an addition of NaCl and anhydrous $MgSO_4$ and the mixture was vortexed vigorously for 1 min and then centrifuged for varying minutes under different speeds (Table 7). After centrifuge, the supernatant was transferred into a second centrifuge tube for the clean-up process with anhydrous magnesium sulphate and PSA. The solution was further vortexed for 30 secs, and then centrifuged for 5 min under the same conditions and filtered using first a 0.45 um PTFE syringe filters followed by 0.22 um PTFE syringe filters and injected in the GC-ECD for analysis.

Table 6: Experimental responses of independent factors for screening

Run order	Sample mass	Centrifuge time	Centrifuge speed	NaCl mass	$MgSO_4$ mass	Solvent volume	Solvent type	DMP	DEP	4-NP	DDT	DDE	DDD
5	0.5	3400	5	1	1	5	methanol	44.99	123.3	66.88	23.42	123.4	132
8	0.5	3700	12.5	2	2	5	acetone	76.85	132.5	88.56	37.04	95.66	96.73
18	0.5	4000	20	3	3	5	acetonitrile	44.24	72.63	88.26	75.85	87.46	130.7
2	1.75	3400	5	2	2	5	acetonitrile	28.88	67.87	66.69	56.57	147.9	66.16
12	1.75	3700	12.5	3	3	5	methanol	32.65	45.65	85.46	43.22	78.66	101.7
15	1.75	4000	20	1	1	5	acetone	67.65	143.02	136.96	71.05	119.9	146.5
10	3	3400	12.5	1	3	5	acetone	98.89	107.9	123.52	13.66	174.7	65.65
21	3	3700	20	2	1	5	acetonitrile	11.92	63.98	108.59	65.95	67.32	127.3
16	3	4000	5	3	2	5	methanol	12.6	59.1	75.55	59.76	45.69	101.5
9	0.5	3400	20	3	2	10	acetone	45.68	56.96	48.59	88.73	48.51	77.97
20	0.5	3700	5	1	3	10	acetonitrile	47.06	65.46	32.79	79.41	119.9	68.55
1	0.5	4000	12.5	2	1	10	methanol	16.95	45.56	45.45	76.58	38.69	141.3
3	1.75	3400	12.5	3	1	10	acetonitrile	14.65	25.66	36.86	85.91	52.32	90.85
19	1.75	3700	20	1	2	10	methanol	22.89	45.36	104.55	79.89	71.32	73.09
14	1.75	4000	5	2	3	10	acetone	105.26	65.95	86.95	65.78	54.55	62.12
4	3	3400	20	2	3	10	methanol	26.68	20.65	102.33	65.66	68.99	46.69
7	3	3700	5	3	1	10	acetone	56.66	64.56	56.89	89.86	36.69	55.65
11	3	4000	12.5	1	2	10	acetonitrile	42.63	32.22	72.15	108.7	56.86	75.66
6	1.75	3700	12.5	2	2	7.5	methanol	23.36	48.96	66.96	56.99	39.46	46.69
17	1.75	3700	12.5	2	2	7.5	methanol	21.99	35.36	65.58	56.52	29.83	85.96
13	1.75	3700	12.5	2	2	7.5	methanol	33.1	35.96	88.77	68.75	36.67	88.98

After screening and determining the important parameters, optimization was carried out with reduced runs using most important parameters. The data is displayed in Table 7. The tool employed for optimization has an advantage to determine the interaction between the independent

quantitative variables, namely sample mass, speed, and time of centrifuge. It then models the system mathematically. This saved time and cost by reducing the number of trials and experiments that could be done using the traditional way of dealing with one parameter at a time while keeping the rest of the parameters constant.

Table 7: Experimental design for the optimization model

Exp No.	Run order	Sample mass	Centrifuge time	Centrifuge speed	DMP	DEP	4-NP	DDT	DDE	DDD
1	4	0.5	5	3400	41.21	83.26	64.01	20.14	71.83	66.96
2	6	3	5	3400	49.82	54.11	41.17	81.04	58.75	74.12
3	12	0.5	20	3400	16.69	21.22	98.14	66.12	64.42	41.22
4	8	3	20	3400	24.85	71.23	47.65	82.82	72.46	77.62
5	3	0.5	5	4000	58.15	52.51	72.95	66.61	46.85	94.63
6	11	3	5	4000	28.69	74.71	85.96	72.77	85.12	72.01
7	15	0.5	20	4000	41.12	34.32	114.22	64.99	74.12	49.21
8	17	3	20	4000	35.19	86.66	79.66	36.66	100.07	47.86
9	16	0.1	13	3700	55.45	82.12	76.98	12.9	66.21	72.18
10	9	3.4	13	3700	58.46	101.32	71.12	35.96	85.87	55.66
11	2	1.75	2	3700	35.65	30.21	81.02	45.86	61.42	88.58
12	14	1.75	23	3700	12.17	32.14	31.82	64.75	61.21	63.33
13	1	1.75	12.5	3300	19.98	22.12	87.82	86.21	42.78	57.88
14	10	1.75	12.5	4100	32.88	52.41	71.14	54.27	65.42	72.16
15	5	1.75	12.5	3700	34.65	37.82	64.31	51.84	38.75	72.42
16	13	1.75	12.5	3700	33.42	42.17	65.22	55.12	39.12	69.17
17	7	1.75	12.5	3700	39.55	50.44	71.26	52.08	42.69	73.26

3.4.2 Determination of the quantities of organic EDCs in real samples

The QuEChERS extraction method was done using the modified procedure reported by Rawn et al. (2010). Homogenized samples with no EDCss detected on previous occasions were used for recovery studies and for the preparation of matrix-matched standards for calibration. Accurately weighed 0.7 g of homogenised sample was put in a polypropylene centrifuge tube and was spiked with 200 µL and 500 µL of 1000 µg/L of a standard mixture of 5 EDCs. The spiked samples were allowed to equilibrate for 30 min. Then 6 ml of 99.8% methanol was added and the sample was vortexed for 1 min. This was followed by salting-out step with additions 0.5 g sodium chloride and 1 g of anhydrous magnesium sulphate into the tube and the mixture was vortexed for 1 min and then centrifuged for 5 min at 4000 rpm. After centrifuge, supernatant was transferred into another polypropylene centrifuge tube to clean-up with 100 mg anhydrous magnesium sulphate

and 75 mg primary/secondary amine (PSA). The solution was vortexed for 30 seconds and then centrifuged for 5 min at 4000 rpm and filtered using a 0.22 mm PTFE into 2 mL vials and injected in the GC-ECD and/or GC x GC/TOFMS for analysis. All the samples were prepared in triplicate. The GC x GC/TOFMS (Figure 6) was used to confirm the identification of compounds in samples. This was carried out based on two conditions:

Figure 6: Gas Chromatography×Gas Chromatography/Time of Flight Mass Spectrometer. Photo captured from the University of Witwatersrand Analytical Laboratory, Johannsburg, South Africa

(i) GC-ECD conditions

The GC 7890A (Agilent Technologies, DE, USA) equipped with an Electron Capture Detector (ECD) with a wall-coated open tubular (WCOT) fused silica capillary column (30 x 0.25 mm ID, 0.25 μm film thickness) was used to optimize the QuECHERS method. The GC-ECD conditions were selected to get the best separation. The GC conditions and the detector response were adjusted so as to match the relative retention times and response. The conditions used for the analysis were: capillary column coated with ZB-5 (30 m*0.25 mm, 0.25 μm film thickness). Nitrogen (99.999%) was used as carrier gas flowing at 1.2 mL/ min. The oven temperature was programmed from 60 °C (5 min) to 150 °C at a rate of 10 °C /min (1 min), was further increased to 200 °C at a rate of

30 °C/min (3 min) then lastly to 300 °C at a rate of 15 °C/ min for 10 min. The temperature of the injector in split less mode (volume injected 1 mL) was held at 300 °C and electron-capture detector temperature was 250 °C. Volume injected was 10 µL. Figure 15 shows the chromatogram of EDCs as obtained from GC-ECD.

(ii) GC×GC/TOFMS conditions

A LECO GC-MS with a capacity for a GC x GC equipped with a TOF/MS detector was used to identify and quantify selected EDCs in samples. The software used is Pegasus ChromaTOF. The conditions of the mass spectrometer were as follows: transfer line temperature 320 °C; ion source temperature 250 °C; and multiplier voltage 1450 V. A programmed temperature vaporization injector operating in solvent-split mode was employed. The volume injected was 10 µL, split flow 50 mL/min and injection time: 0.50 min, injection flow: 100 mL/min. The oven temperature programme was as follows: initial temperature of 50 °C increased to 150 °C at 10 °C/min; followed by an increase to 300 °C at a rate of 5 °C/min. Helium was used as carrier gas flow rate of 1 mL/min. Ion trap mass detection was operated in full scan mode from 50 to 500 amu. All the samples were prepared in triplicate.

3.4.3 Quality assurance

Quality assurance in the analysis of EDCs involved taking considerable care of the following*:*

(i) Reagents and chemicals

The target organic EDCs standards used namely Diethyl phthalate (CAS No 84-66-2), Dimethyl phthalate (CAS No 131-11-3), 4-Nonyl phenol (CAS No 104-40-5), 4,4'-Dichlorodiphenyldichloroethylene (DDE) (CAS No 72-55-9) and 4,4'-Dichlorodiphenyldichloroethane (DDD) (CAS No 72-54-8) were purchased from Merck, Johannesburg, South Africa. All the standard EDCs were over 95% pure. Both stock and standard reagents were made using methanol. Acetonitrile and acetone were also obtained from Merck, Johannesburg, South Africa. Analytical grade $MgSO_4$ was purchased from Merck, Johannesburg, South Africa. For clean-up, primary and secondary amine (PSA) was acquired from Merck, Johannesburg, South Africa. Nitrogen gas (99.999%) was used for blowing and evaporation of the

solvent to the needed volume of 2 mL. 55% w/v nitric acid, HNO_3 and 30% w/v hydrogen peroxide, H_2O_2, supra pure grade were purchased from Merck (Johannesburg, South Africa).

(ii) Preparation of the stock solution

Individual EDCs stock solutions were prepared from neat certified materials in methanol. Solutions of 1000 mg/L of each compound were prepared in 25 mL volumetric flasks by dissolving 25 mg of each standard compound in their respective volumetric flasks and filling it to the mark with methanol. The stock solutions were stored at -20° C. The working standard solution of the five compounds was prepared by withdrawing 100 μL of each prepared stock solution into a 10 mL volumetric flask and diluting to the mark with methanol. The final concentration of each endocrine disrupting compound in the 10 mL flask is 10 mg/L. It is from this final concentration that a 1 mg/L standards solution was made and other standards for calibration curve. The prepared mixed solutions were then stored at 4℃ until analysis.

(iii) Preparation of calibration curves

From the 10 mg/L standards solution prepared earlier, a standard solution of EDCs of different concentration ranging from 0.2 mg/L to 1.0 mg/L was prepared and used for determination of calibration curve. The calibration curves were linearly fitted from GC-ECD/GC-MS.

3.4.4 Statistical analysis and method validation for EDCs

Both descriptive and inferential statistical analysis of data were performed using Microsoft Excel 2016 Software with a level of significance maintained at 95% using single factor ANOVA with post-hoc analysis using Bonferroni correction. To validate the method, percentage (%) recoveries together with limit of detection (LOD), limit of quantification (LOQ) and linearity were also determined. The blank correction problem was taken into consideration to address the problem of aerial contamination of reagents and equipment by the ubiquitous phthalates. The calculation of LOD and LOQ were as follows:

$$LOD = \frac{3.3\sigma}{S} \quad (1)$$

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

Where σ is the standard deviation of triplicate measurements and S is the slope of the calibration curve.

The % recoveries were calculated as follows:

$$\%\ Recovery = \frac{C_O - C_i}{C_O} \times 100 \qquad (3)$$

Where C_O is concentration of analyte in spiked sample and C_i is the concentration of analyte in unspiked sample.

3.4.5 Determining the quantities of heavy metals

Quantities of heavy metals were obtained by analyte extraction and analysis. This involved a mass of 0.15 g of pulverized dried samples weighed using an analytical balance (Precisa 180A, Switzerland) and placed into acid washed digestion tubes (PTFE-TFM liners) onto which 10 mL nitric acid, HNO_3 and 2 mL hydrogen peroxide, H_2O_2 were added before being placed into the Anton Paar Microwave Go digester. The digestion programme included a ramp time of 35 minutes at 180 °C held for 30 minutes with a temperature limit of 190 °C (Ramalepe 2022). The tubes were sealed and placed in a motor, which was then subjected to Anton Paar Microwave Go (Figure 7) digestion. Extraction occurred for about 30 minutes. After digestion, the digested solution was transferred to 50 mL PTFE tubes into which distilled water was added and diluted to 50 mL mark. Analysis of metal concentrations was done using ICP-OES (Spectro, Kleve, Germany) (Figure 8).

Figure 7: Anton Paar Microwave Go Digester. Photo captured from the University of Witwatersrand Analytical Laboratory, Johannsburg, South Africa

Figure 8: Inductively Coupled Plasma-Optical Emission Spectrometer. Photo captured from the University of Witwatersrand Analytical Laboratory, Johannsburg, South Africa

For quality assurance and quality control for the reliability of the results, proper care was taken in handling and cleaning of glassware. Di-ionized water was used for all rinsing and dilutions throughout the study and the reagents were of analytical grade reagents. Blank determinations were used to correct the instrument readings. Standards were prepared for each metal from their stock solution for calibration of the instrument. Precision and accuracy of analysis were checked through repeated analysis. Verification of applied analytical method was done through spiking all matrices with 100 µL of 1000 ppm stock solution containing all metals under analysis. For the analysis of trace elements at mg/kg levels, recovery of 70-120% is considered acceptable (Fung et al. 2017). All metals were recovered within that range indicating good analytical performance.

3.4.6 Statistical analysis of heavy metals

Both descriptive and inferential statistical analysis of data were performed using Microsoft Excel 2016 Software with a level of significance maintained at 95% using single factor ANOVA with post-hoc analysis using Bonferroni correction.

3.4.7 Health risk assessment of organic EDCs

The non-cancer risk to consumers was estimated according to the methods recommended by Wang et al. (2015). The estimated daily intake (EDI) was estimated using the following formula:

$$EDI = C \times \frac{IR}{Bw} \quad (4)$$

where C is concentration of particular food, B_w is body weights of 70 kg for adults (Chungu et al. 2019) and IR is rate of food consumption for fish and vegetables in Zambia is 0.06 kg/person/day (Halimatou et al. 2014). The hazard quotient was calculated as:

$$HQ = \frac{EDI}{RfD} \quad (5)$$

where RfD is defined as the oral reference dose of contaminants; 10 mg/kg/day and 0.8 mg/kg/day for DMP and DEP, respectively (Ai et al. 2023).

The hazard index is the summation of target hazard quotients of different chemicals. The hazard index larger than 1 indicates health risk associated with the EDC detected in the food sample was calculated as shown in the following equation:

$$HI = \sum \mathrm{HQ} = \mathrm{HQ\ DMP} + \mathrm{HQ\ DEP}. \quad (6)$$

Where, ∑HQ is the sum of Hazard Quotients of pollutants. HQ DMP and HQ DEP are the hazard quotients for dimethyl phtalate and diethyl phtalate, respectively. If ∑HQ < 1, the population is not at risk. If ∑HQ ≥ 1, the population is at risk (Kortei et al. 2020).

3.4.8 Health risk assessment for heavy metals

The risk to human health because of consuming the foodstuffs investigated was evaluated by calculating the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and carcinogenic risk for the carcinogenic metals. These were undertaken as follows:

(i) Estimated daily intake (EDI) for heavy metals

The EDI was calculated using equation 4 above.

(ii) The target hazard quotient (THQ) for heay metals

The THQ is defined as the ratio between exposure to a potential hazard element and its reference dose, and it approximates the non-cancer risk owing to exposure (Kortei et al. 2020). It was calculated using the following equation:

$$THQ = \frac{EDI}{RfD} \quad (7)$$

where RfD is the oral reference dose in mg/kg per day based on the safe maximum level for oral ingestion of the element by an adult. The RfDs are as follows; aluminium (1.0); copper (0.04); iron (0.7); manganese (0.14); cadmium (0.001) and zinc (0.3) (mg/ kg/day), respectively (USEPA 2011). For an element, a THQ < 1 signifies low risk of antagonistic effects owing to exposure to that element whilst a THQ > 1 proposes possible risks to health due to exposure to that element.

(iii) The hazard index (HI) for heavy metals

The hazard index is the sum of the target hazard quotients as described in the following equation:

$$HI = \sum \mathrm{THQ} = \mathrm{THQ\,Fe} + \mathrm{THQ\,Al} + \mathrm{THQ\,Mn} + \mathrm{THQ\,Cd} + \mathrm{THQ\,Cu} + \mathrm{THQ\,Zn}. \quad (8).$$

Where, ∑THQ is the sum of Target Hazard Quotients of metals. THQ Al, THQ Fe, THQ Cd, THQ Mn, THQ Cu, and THQ Zn are the target hazard quotients for aluminium, iron, cadmium, copper, manganese and zinc, respectively. The assumption is that magnitude of adverse effect directly related to the sum of multiple metal exposures. If the sum of the hazard quotients is < 1, the population is at risk. If ∑THQ is ≥ 1, the population experiences health issues.

(iv) Carcinogenic risk (CR)

The CR was calculated in relation to the USEPA Region III Risk-Based Concentration Table (USEPA 2011). The CR is an estimation of the probability of an individual to develop cancer due to exposure to the potential carcinogen over a lifetime.

$$\mathrm{CR} = \mathrm{EDI} \times \mathrm{CPSo} \quad (9)$$

where CPSo is the oral slope factor of a particular carcinogen in mg/kg per day, which is 6.3 for cadmium (Kortei et al. 2020; Fanfu et al., 2015). Values above 10^{-4} indicate higher probabilities of CR (Javed and Usmani 2016). If multiple carcinogenic elements are involved, the sum of carcinogenic risks is obtained (assuming additive effects). Risks in the range of 1.0×10^{-6} to 1.0×10^{-4} are acceptable (Fanfu et al. 2015).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

This chapter presents the results obtained from analysis of foodstuffs and discusses the results by comparing the results from the three towns. It presents the statistical analysis of the results obtained and the health risk assessment to the local consumers. The results and discussion are presented in two parts, starting with analysis of organic EDCs followed by analysis of metals.

4.1 Analysis of organic EDCs

Analysis of organic EDCs involved both qualitative and quantitative analysis as discussed in the following sections:

4.1.1 Quality assurance

The chromatogram of target EDCs as obtained from GC-ECD is shown in Figure 9. The peaks and their retention times were used to identify the peaks for the target EDCs.

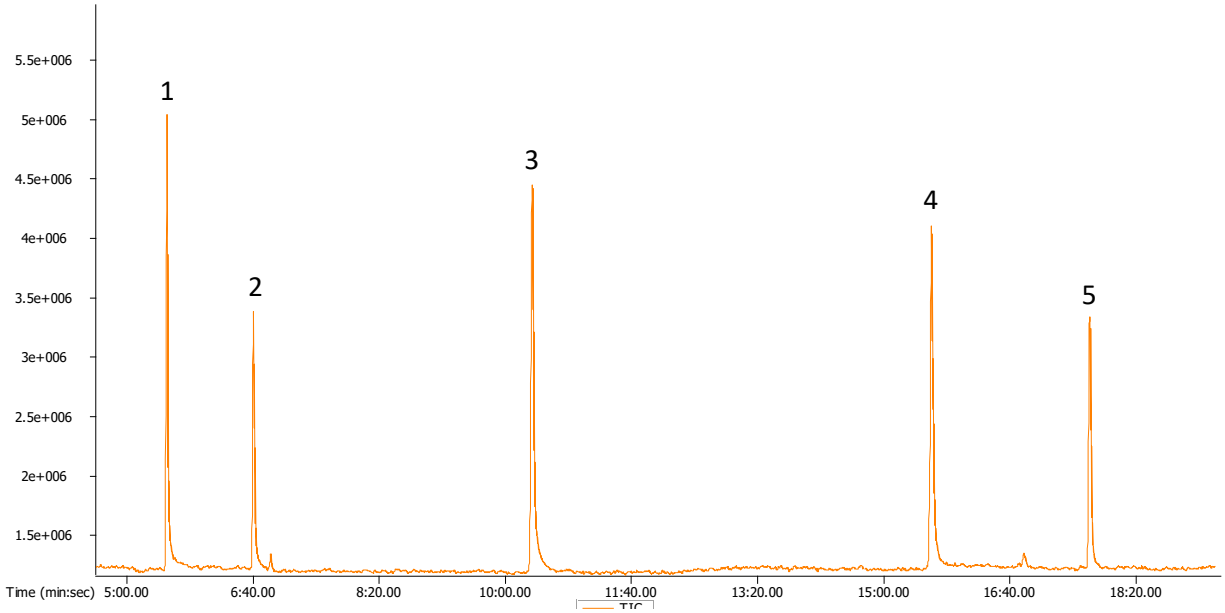


Figure 9: GC-ECD Chromatogram of EDCs standards. The target compounds are in the following order: (1) Dimethylphthalate, (2) Diethylphthalate, (3) 4-n-Nonylphenol, (4) 4,4'-Dichlorodiphenyldichloroethylene (DDE) and (5) 4,4'-Dichlorodiphenyldichloroethane (DDD).

The sensitivity of the method is expressed by limit of detection (LOD), limit of quantification (LOQ) and linearity (R^2) as indicated in Table 8. The LOD and LOQ of the method were assessed based on the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC- ECD. The precision values for the method were expressed as percentage relative standard deviation (RSD, n=3) and linearity (R^2). Blank analyses were done for every batch of six samples analyzed.

Table 8: Limit of detection (LOD in µg/kg), limit of Quantification (LOQ in µg/kg), RSD (%), linearity (R^2) and retension times (TR in mm:sec.msec)

Analyte	LOD	LOQ	RSD	R^2	TR
Dimethyl phthalate	1.61	16.1	2.26	0.9918	05:30.4
Diethyl phthalate	1.18	11.8	1.37	0.9967	06:35.0
4-n-Nonyl phenol	2.89	28.9	2.25	0.9944	10.43.6
4,4'-DDE	1.12	21.2	2.95	0.9939	15.23.7
4,4'-DDD	1.77	17.7	5.63	0.9372	16.38.3

Samples that were fortified with 200 µL and 500 µL of 1000 µg/L concentration of a mixed standard solution containing the EDCs are shown in Table 8. The standard deviation and percent recoveries were calculated in triplicate. For the analysis of EDCs, accuracy and recovery of 70-120% is considered acceptable (Nuapia et al. 2016; Gonzalvez et al. 2008). The procedure can be applied for assessment of the selected EDCs in food samples under study. The recovery percentages in spiked samples ranged from 74.23 to 86.02% for tomato; 76.47 to 91.09% for fish; 72.06 to 74.26% for cassava leaves; 81.36 to 86.16% for spinach; 84.20 to 91.27% for cabbage; 79.63 to 82.20% for rape, and 73.75 to 84.38% for Chinese cabbage. Recoveries for 4-n-nonylphenol, DDE and DDD were not included because they were not detected in original samples.

Table 9: Analytical recoveries (%) ±SD of endocrine disruptors (EDCs) in food samples at 200 µL and 500 µL fortification with 1000 µg/L standard mixture of the EDCs.

	Dimethyl phthalate		Diethyl phthalate	
Sample	200 µL	500 µL	200 µL	500 µL
Tomato	75.23±0.26	86.02±0.19	74.23±0.20	76.02±0.29
Fish	76.47±0.18	76.89±0.21	86.27±0.18	91.09±0.11
Cassava leaves	72.06±0.06	74.26±0.43	72.86±0.26	73.46±0.13
Spinach	84.29±0.22	81.36±0.17	85.37±0.29	86.16±0.14
Cabbage	86.28±0.46	91.27±0.33	84.20±0.61	91.27±0.13
Rape	79.87±0.12	82.20±0.08	79.63±0.12	80.20±0.28
Chinese cabbage	73.75±0.28	75.09±0.31	75.09±0.09	84.38±0.27

4.1.2 Identification of phthalates, 4-nonylphenols and DDT metabolites

Identification of phthalates was achieved by GC-MS. The fragmentation patterns of identified phthalates are shown in Figure 10.

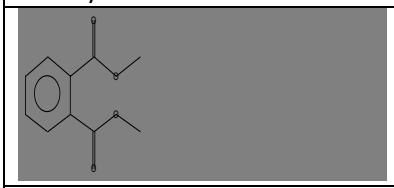
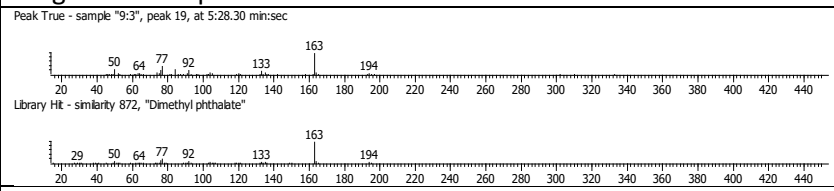
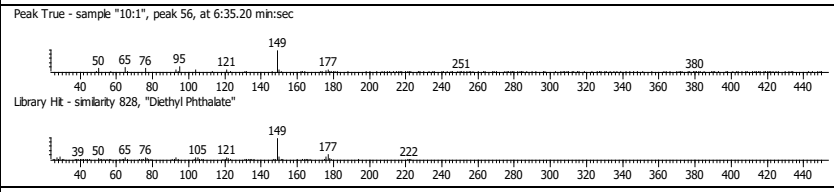
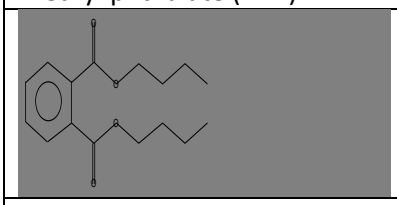
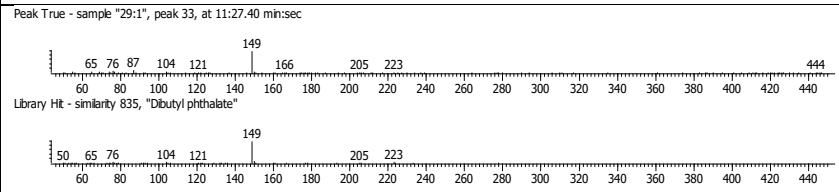
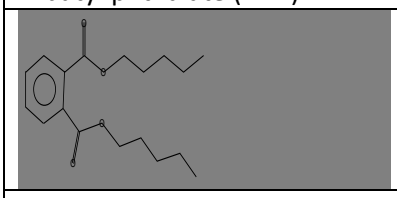
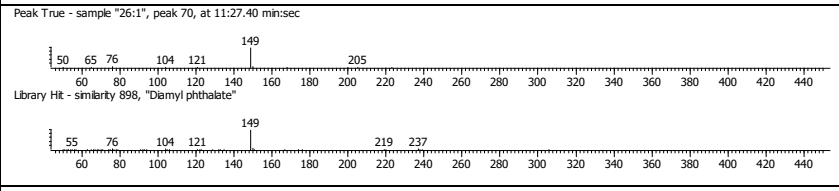
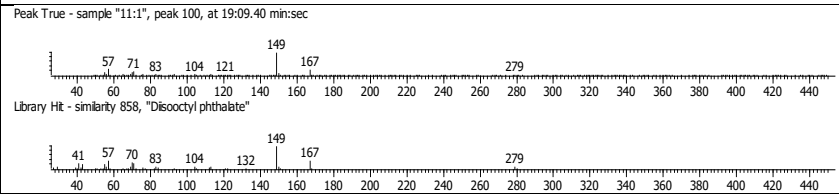
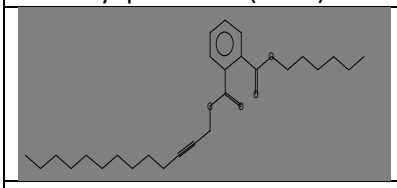
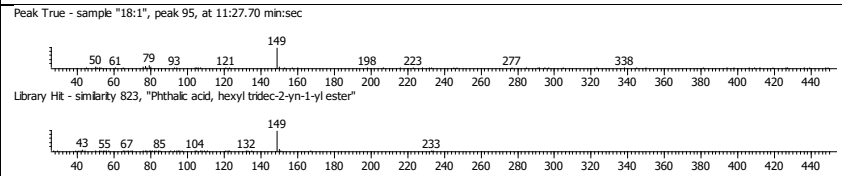
Name/structure	Fragmentation pattern
	
Dimethyl phthalate (DMP)	
	
Diethyl phthalate (DEP)	
	
Dibutyl phthalate (DBP)	
	
Diamyl phthalate (DAP)	
	
Diisoctyl phthalate (DIOP)	
	
Hexyltridec-2-yn-1-ylphthalate (HTDP)	

Figure 10: Structures and fragmentation patterns of the identified phthalates

The distribution of the identified phthalates in food samples is shown in Table 10. Dimethylphthalate (DMP) was only detected in tomato and fish, and rape and fish for Kitwe City and Kabwe Town, respectively. For Lusaka City, DMP was detected in tomato, fish and cassava

leaves. Diethylphthalate (DEP) was not detected in cabbage, cassava leaves and Chinese cabbage for Kitwe City. For Kabwe Town, DEP was detected in all the samples except fish. Only Kapenta, rape and cassava leaves did not have DEP detected in them among samples from Lusaka City. Dibutylphthalate (DBP) was only detected in two samples from Kitwe City, cassava leaves and Chinese cabbage. Three samples from Kabwe, fish, Kapenta and cabbage, did not test positive for DBP while only three samples from Lusaka, fish, spinach and cabbage had DBP. Diamylphthalate (DAP) was identified in only four samples from Kitwe; tomato, cabbage, cassava leaves and Chinese cabbage. Only one sample, cassava leaves, from Kabwe had DAP in it as well as one sample, Chinese cabbage, from Lusaka. Spinach, rape, cabbage and cassava leaves were the only samples from Kitwe in which di-(2-ethylhexyl)phthalate (DEHP) was identified. For samples from Kabwe, DEHP was not detected in tomato, fish and Kapenta. DEHP was identified in only two samples, Kapenta and spinach, from Lusaka. Diisooctylphthalate (DIOP) was only identified in three samples, spinach, cassava leaves and Chinese cabbage, among samples from Kitwe. No sample from Kabwe was positive for DIOP and only in one sample, cabbage, was DIOP detected among the samples from Lusaka. Hexyl tridec-2-yn-1-yl phthalate (HTDP) was identified in fish, rape and cassava leaves for Kitwe samples. Among samples from Kabwe, HTDP was only identified in Kapenta, rape and cabbage. For samples from Lusaka, only cabbage gave a positive result for HTDP. DEHP was the most frequently detected phthalate followed by DBP, which was followed by HTDP, then DAP and DIOP was least abundant. 4-Nonylphenols and DDT metabolites were not detected in all the samples by mass-spectral library screening. Further work is required to quantify and estimate the health risk associated with exposure to these phthalates through consumption of food.

Table 10: Phthalates identified by GC/MS in the samples under study
A mark shows presence of specific phthalate; Nd=not detected, DMP=Dimethyl phthalate, DEP=Diethyl phthalate, DBP=Dibutyl phthalate; DAP=Diamyl phthalate; DEHP=Di-(2-ethylhexyl) phthalate; DIOP=Diisooctyl phthalate, HTDP=Hexyl tridec-2-yn-1-yl phthalate, NP=Nonylphenol, DDE=Dichlorodiphenyldichloroethylene, DDD=Dichlorodiphenyldichloroethane

City/Town	Sample	DMP	DEP	DBP	DAP	DEHP	DIOP	HTDP	4-n-NP	DDE	DDD
Kitwe	Tomato	√	√	Nd	√	Nd	Nd	Nd	Nd	Nd	Nd
	Fish	√	√	Nd	Nd	Nd	Nd	√	Nd	Nd	Nd
	Kapenta	Nd	√	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Spinach	Nd	√	Nd	Nd	√	√	Nd	Nd	Nd	Nd
	Rape	Nd	√	Nd	Nd	√	Nd	√	Nd	Nd	Nd
	Cabbage	Nd	Nd	Nd	√	√	Nd	Nd	Nd	Nd	Nd
	Cassava leaves	Nd	Nd	√	√	√	√	√	Nd	Nd	Nd
	Chinese cabbage	Nd	Nd	√	√	Nd	√	Nd	Nd	Nd	Nd
Kabwe	Tomato	√	√	√	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Fish	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Kapenta	√	√	Nd	Nd	Nd	Nd	√	Nd	Nd	Nd
	Spinach	√	√	√	Nd	√	Nd	Nd	Nd	Nd	Nd
	Rape	Nd	√	√	Nd	√	Nd	√	Nd	Nd	Nd
	Cabbage	√	√	Nd	Nd	√	Nd	√	Nd	Nd	Nd
	Cassava leaves	√	√	√	√	√	Nd	Nd	Nd	Nd	Nd
	Chinese cabbage	√	√	√	Nd	√	Nd	Nd	Nd	Nd	Nd
Lusaka	Tomato	√	√	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Fish	√	√	√	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Kapenta	Nd	Nd	Nd	Nd	√	Nd	Nd	Nd	Nd	Nd
	Spinach	Nd	√	√	Nd	√	Nd	Nd	Nd	Nd	Nd
	Rape	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Cabbage	Nd	√	√	Nd	Nd	√	√	Nd	Nd	Nd
	Cassava leaves	√	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	Chinese cabbage	Nd	√	Nd	√	Nd	Nd	Nd	Nd	Nd	Nd

4.1.3 Quantification of selected EDCs in food samples

This section presents the concentrations obtained for selected organic EDCs in the studied foodsuffs, a comparison of the obtained results with similar studies, health risk assessment of organic EDCs and limitations of the study and future outlook.

(i) Concentration of EDCs in the samples from two cities and one town

The mean concentrations of the quantified EDCs are summarized in Table 11. 4-n-nonyl phenol, 4,4'-DDE and 4,4'-DDD were not detected in all the samples. Therefore, these three EDCs were omitted on the results table and in the subsequent discussion.

Table 11: Mean concentrations of EDCs (μg/kg) in foods per dry weight
Nd=not detected, DMP=Dimethyl phthalate, DEP=Diethyl phthalate

City/Town	Sample	DMP	DEP
Kitwe	Tomato	91.05±1.45	63.27±8.92
	Fish	101.76±1.99	49.91±0.51
	Kapenta	Nd	21.42±17.98
	Spinach	Nd	22.36±4.36
	Rape	Nd	80.69±3.85
	Cabbage	Nd	Nd
	Cassava leaves	Nd	Nd
	Chinese cabbage	Nd	Nd
Kabwe	Tomato	77.14±0.03	82.93±5.54
	Fish	Nd	Nd
	Kapenta	92.85±0.24	135.77±9.60
	Spinach	105.34±0.36	161.67±0.26
	Rape	Nd	143.14±0.77
	Cabbage	123.82±0.38	131.26±2.90
	Cassava leaves	90.16±1.10	91.17±0.21
	Chinese cabbage	92.38±1.42	63.93±0.33
Lusaka	Tomato	85.65±2.08	25.08±3.07
	Fish	98.55±0.40	23.99±9.98
	Kapenta	Nd	Nd
	Spinach	Nd	23.22±17.22
	Rape	Nd	Nd
	Cabbage	Nd	25.66±0.19
	Cassava leaves	95.65±7.34	Nd
	Chinese cabbage	Nd	46.01±2.71

For Kitwe Open Markets, DMP was only detected in tomato and fish with concentrations of 91.05 µg/kg and 101.76 µg/kg respectively. The mean concentrations of DEP ranged from 21.46 µg/kg in Kapenta to 80.69 µg/kg in rape. The sequence of DEP mean concentrations was as follows: Kapenta<spinach<tilapia<tomato<rape. The concentration of 21.46 µg/kg in kapenta was the least concentration recorded for DEP in all the samples from all the open markets of Kabwe, Kitwe and Lusaka.

For Kabwe Open Market, the mean concentrations of DMP ranged from 77.14 µg/kg in tomato to 123.82 µg/kg in cabbage. Only two sample types, fish and rape, had no DMP detected. This was contrary to the results recorded for Kitwe City Open Market where only two samples had DMP detected in them. The sequence for DMP concentrations is as follows: tomato<cassava leaves<Chinese cabbage<Kapenta<spinach<cabbage. The lowest concentration of DMP was found in tomato samples just like was the case with samples from Kitwe Open Market. However, this concentration of DMP was the lowest in samples from all all the open markets of Kabwe, Kitwe and Lusaka. The highest concentration of DMP was also recorded for Kabwe Town Open Market when compared to other two cities. There was no DEP detected in the fish samples. However, DEP was detected in the other samples with mean concentrations between 63.93 µg/kg in Chinese cabbage to 161.67 µg/kg in spinach. The mean concentration sequence was given as Chinese cabbage<tomato<cassava leaves<cabbage<Kapenta<rape<spinach. The concentrations here recorded were higher than those recorded for both Kitwe City Open Market and Lusaka City Open Market.

As Table 11 shows, DMP was only detected in three samples from Lusaka Open Markets; tomato with mean concentration of 85.65 µg/kg, cassava leaves with mean concentration of 95.65 µg/kg and fish with mean concentration of 98.55 µg/kg. It was interesting to note that the lowest concentration of DMP was recorded in tomato samples, which was consistent with the results from the other two open markets of Kitwe City and Kabwe Town. The lowest concentration (23.2 µg/kg) as well as the highest concentration (98.5 µg/kg) obtained were lower than those recorded for samples from Kitwe Open Market. The mean concentrations of DEP ranged from 23.22 µg/kg in spinach to 46.01 µg/kg in Chinese cabbage. The concentration sequence was as follows: spinach<fish<tomato<cabbage<Chinese cabbage. These results are much comparable to those

recorded for Kitwe City Open Market except for the highest concentrations of 46.01 μg/kg, which was much lower than the concentration of 80.69 μg/kg.

The most probable sources of these phthalates include irrigation with wastewater and use of contaminated soils for vegetables, industrial discharges into the aquatic environment and food contact with phthalates in the air since foodstuffs are sold on the open air in open markets. Sewage aquaculture is one potential source of phthalates with wastewaters being used for culturing fishes (Barse et al. 2007). Application of pesticides may have contributed to the presence of phthalates, especially DEP, in vegetables. Phthalate leachate from garden hoses is another source of contamination (Wang et al. 2019).

For statistical analysis of data, single factor ANOVA was used with post-hoc analysis in Microsoft Excel 2016 version. For post-hoc analysis, the Bonferroni correction was used. For DMP mean concentrations, there was not a substantial variance for two cities and one town, $P>0.05$. However, one-way ANOVA for the mean concentrations of DEP showed a significant difference, $P<0.05$. The post-hoc analysis indicated insignificant difference in the levels of DEP for samples from Kitwe City Open Market and Lusaka City Open Market, $P>0.0167$. The variances in the levels of DEP were significant in samples from Kitwe Open Market and Kabwe Open Market and samples from Kabwe Open Market and Lusaka Open Market, $P<0.0167$.

(ii) A comparison with results from similar studies: a discussion

The findings from similar studies of EDCs of interest in this study are presented (Table 12). My study reports no detection of DDT metabolites. However, 4,4'-DDE and 4,4'-DDD were detected and quantified by other studies in cabbage from Pretoria, South Africa; Kinshasa, Democratic Republic of Congo (Nuapia et al. 2016) and Cape Town, South Africa (Olantunji 2019). The two metabolites were also identified in fish from Pretoria, South Africa; Kinshasa, Democratic Republic of Congo, Eastern Lake Tanganyika, Tanzania and River Po, Italy (Nuapia et al. 2016; Mahugija et al. 2018; Viganó et al. 2015) and spinach from Cape Town, South Africa (Olantunji 2019). Just like in my study, 4-n-Nonylphenol was not detected in the study conducted by Lu et al. (2013) from Florida, United States of America. In studies conducted by She et al. (2012) from local Supermarkets, China; Viganó et al. (2015) from River Po, Italy and Chai et al. (2012) from Pearl River Delta, South China, 4-n-Nonylphenol was detected and quantified in cabbage, fish,

Chinese cabbage and spinach. Kannan et al. (2003) reported a concentration of 4-n-Nonylphenol of less than 3.3 μg/kg in fish from Kalamazoo River, Michigan, United States of America.

Table 12: Mean concentrations of Dimethyl phthalate (DMP), Diethyl phthalate (DEP), 4-n-Nonylphenol (4-n-NP), Dichlorodiphenyldichloroethylene (DDE), Dichlorodiphenyldichloroethane (DDD) (μg/kg) in foods as reported by other studies. Nd=not deteced. Hyphen means not investigated. Results were just reported as mean concentrations, without ±SD, in order to suit all results as most of the authors just reported mean concentrations.

Country/sample	Author	DMP	DEP	4-n-NP	4,4'-DDE	4,4'-DDD
Tomato						
China	Chen et al. 2017	210	315	-	-	-
Florida, USA	Lu et al. 2013	-	-	nd	-	-
Zambia	this study (Kabwe)	77.14	82.93	nd	nd	Nd
Zambia	this study (Kitwe)	91.05	63.27	nd	nd	Nd
Zambia	this study (Lusaka)	85.65	25.08	nd	nd	Nd
Cabbage						
China	Yan et al. 2020	140	60	-	-	-
China	Jianteng et al. 2016	3.36	1.18	-	-	-
China	She et al. 2012	-	-	21.59	-	-
South Africa	Nuapia et al. 2016	-	-	-	106.65	95.67
South Africa	Olatunji 2019	-	-	-	11.6	12.5
D.R. Congo	Nuapia et al. 2016	-	-	-	81.05	61.05
Zambia	this study (Kabwe)	123.82	131.26	nd	nd	Nd
Zambia	this study (Kitwe)	nd	nd	nd	nd	Nd
Zambia	this study (Lusaka)	nd	25.66	nd	nd	Nd
Fish						
South Africa	Nuapia et al. 2016	-	-	-	125.78	105.74
D.R. Congo	Nuapia et al. 2016	-	-	-	90.09	73.52
China	Cheng et al. 2013	1	nd	-	-	-
Pakistan	Munshi et al. 2013	100	123	-	-	-
Korea	Lee et al. 2019	3.3	4.9	-	-	-
Tanzania	Mahugija et al. 2018	-	-	-	100.1	35.42
Michigan, USA	Kannan et al. 2003	-	-	<3.3	-	-
Italy	Luigi et al. 2015	-	-	3.6-26.8	17.3-2902	3.6-98.2
Zambia	this study (Kabwe)	77.14	82.93	nd	nd	Nd
Zambia	this study (Kitwe)	101.76	49.91	nd	nd	Nd
Zambia	this study (Lusaka)	98.55	23.99	nd	nd	Nd
Chinese cabbage						
China	Wang et al. 2015	14	52	-	-	-
China	Chai et al. 2012	-	-	5.3	-	-

Zambia	this study (Kabwe)	92.38	63.93	nd	nd	Nd
Zambia	this study (Kitwe)	nd	nd	nd	nd	Nd
Zambia	this study (Lusaka)	nd	46.01	nd	nd	Nd
Spinach						
South Africa	Olatunji 2019	-	-	-	10.5	10.1
China	Chai et al. 2012	-	-	6.41	-	-
Zambia	this study (Kabwe)	105.34	161.67	nd	nd	Nd
Zambia	this study (Kitwe)	nd	22.36	nd	nd	Nd
Zambia	this study (Lusaka)	nd	23.22	nd	nd	Nd

Chen et al. (2017) from 10 cities that include Shenyang, Beijing, Shougaung, Xianyang, Siyang, Haimen, Nanjing, Changshu, Fuzhou and Kunming in China and Yan et al. (2020) from North China Plain reported levels of 210 μg/kg in tomato and 140 μg/kg in cabbage respectively. There results were higher than what I report in this study. A mean concentration of 100 μg/kg was reported in fish from Virginia Beach, Pakistan (Munshi et al. 2013) which is comparable to the findings in this study. Other studies reported lower concentrations of DMP in fish from Hong Kong Market, China (Cheng et al. 2013), Asan Lake of Korea (Lee et al. 2019) and Chinese cabbage from Nanjing City, China (Wang et al. 2015).

The mean concentrations of DEP reported by Chen et al. (2017) in tomato from the 10 cities in China as aforementioned and Munshi et al. (2013) in fish from Virginia Beach, Pakistan were higher than what I report in this study. The concentration of 60 μg/kg in cabbage from the North China Plain as reported by Yan et al. (2020) is lower than what I have reported for cabbage samples from Kabwe Town but higher than in samples from Lusaka City. Wang et al. (2015) reported a concentration of 52 μg/kg of DEP in Chinese cabbage from Nanjing City, China, which is lower than my result for Chinese cabbage samples from Kabwe Town but higher than in samples from Lusaka City. Other similar studies from Eastern, China (Jianteng et al. 2018) Asan Lake of Korea (Lee et al. 2019) reported concentrations of DEP, which are lower than my findings. Cheng et al. (2013) reported no detection of DEP in fish samples from Hong Kong Market, China. Studies that report the EDCs of interest for this study in rape, cassava leaves and kapenta are limited.

(iii) **Health risk assessment**

The EDI of all the foodstuffs did not exceed the tolerable intakes as proposed by Wang et al. (2015). The hazard quotients and the hazard indices of all the foods in all the two cities and one

town were below the threshold (<1) (Table 13). These results indicate that, for DMP and DEP contamination, there is no non-carcinogenic risk associated with consumption of the foods under study by the local consumers. The total phthalate risk can best be ascertained if all the phthalates are analyzed, which phthalates were identified in the foodstuffs (Figure 10).

Table 13: Mean concentrations of EDCs (µg/kg), estimated daily intake (EDI) (µg/kg/day), hazard quotient (HQ) and hazard index (HI) for non-carcigenic risk for adults
Nd=not detected

		Dimethyl phthalate			Diethyl phthalate			HI
Town	Sample	Conc.	EDI	HQ	Conc.	EDI	HQ	ΣHQ
Kitwe	Tomato	91.05	0.078	0.0078	63.27	0.054	0.068	0.076
	Fish	101.76	0.087	0.0087	49.91	0.043	0.053	0.062
	Kapenta	Nd	-	-	21.42	0.018	0.023	0.023
	Spinach	Nd	-	-	22.36	0.019	0.024	0.024
	Rape	Nd	-	-	80.69	0.069	0.086	0.086
	Cabbage	Nd	-	-	Nd	-	-	-
	Cassava leaves	Nd	-	-	Nd	-	-	-
	Chinese cabbage	Nd	-	-	Nd	-	-	-
	Sum of hazards for all foods			**0.0165**			**0.254**	**0.271**
Kabwe	Tomato	77.14	0.066	0.0066	82.93	0.071	0.089	0.095
	Fish	Nd	-	-	Nd	-	-	-
	Kapenta	92.85	0.079	0.0079	135.77	0.12	0.15	0.15
	Spinach	105.34	0.091	0.0091	161.67	0.14	0.17	0.18
	Rape	Nd	-	-	143.14	0.12	0.15	0.15
	Cabbage	123.82	0.11	0.011	131.26	0.11	0.14	0.15
	Cassava leaves	90.16	0.077	0.0077	91.17	0.078	0.098	0.11
	Chinese cabbage	92.38	0.079	0.0079	63.93	0.055	0.068	0.076
	Sum of hazards for all foods			**0.0502**			**0.865**	**0.911**
Lusaka	Tomato	85.65	0.073	0.0073	25.08	0.021	0.027	0.034
	Fish	98.55	0.084	0.0084	23.99	0.021	0.026	0.034
	Kapenta	Nd	-	-	Nd	-	-	-
	Spinach	Nd	-	-	23.22	0.019	0.025	0.025
	Rape	Nd	-	-	Nd	-	-	-
	Cabbage	Nd	-	-	25.66	0.022	0.027	0.027
	Cassava leaves	95.65	0.082	0.0082	Nd	-	-	0.0082
	Chinese cabbage	Nd	-	-	46.01	0.039	0.049	0.049
	Sum of hazards for all foods			**0.0239**			**0.154**	**0.1772**

(iv) Limitations of the study and future outlook

Information for the exposure of humans to EDCs through ingestion of food and environmental matrices in Zambia is uncommon. In the current study, only few representative foodstuffs (vegetables and fish) were used for approximation of exposure to humans. In addition, only two phthalates were considered for exposure assessment. However, straight exposure from consummables (e.g. drugs and cosmetics, toys and cleaning materials) were unaccounted for possibly leading to underestimation of total daily intake of EDCs. Moreover, the present study only focused on two cities and one town of Zambia, and the approximations might be differing to a large degree per dissimilar geographical location. Nonetheless, this study presents a case, stressing a scenario of EDCs exposure in the one town and two cities, Kabwe, Kitwe and Lusaka, of Zambia. Therefore, high-quality evaluations is needed for approximating present state of human exposure to a number of EDCs. Furthermore, a holistic risk assessment that includes age and gender differences need to be measured.

4.2 Analysis of heavy metals

This section describes the results obtained for analysis of heavy metals. This includes quality control and assurance, mean concentrations of metals in foodstuffs, comparison with results from similar studies and a health risk assessment.

4.2.1 Quality control and assurance

To validate the method, the linearity (R^2), limit of detection (LOD) and limit of quantification (LOQ) were calculated for each analyte (Table 14). For all the metal analytes, the R^2 was found to be above 0.99. All the LOQ values were much lower than the recorded mean concentrations of each heavy metal (Miyanza et al. 2023).

Table 14: Linearity (R^2), limit of detection (LOD) and limit of quantification (LOQ) in ppm
Al=Aliminium; Cd=Cadmium; Cu=Copper; Fe=Iron; Mn=Manganese; and Zn=Zinc

Parameter	Al	Cd	Cu	Fe	Mn	Zn
R^2	0.9998	0.9999	0.9995	0.9998	0.9999	1.00
LOD	0.176	0.125	0.295	0.166	0.151	0.059
LOQ	0.532	0.377	0.894	0.504	0.458	0.179

4.2.2 Mean concentrations of heavy metals

The aluminium, cadmium, copper, iron, manganese and zinc levels for the foodstuffs analysed are summarized (Table 15) and all the values are reported as dry weight. For Kitwe, the aluminium content ranged from 236 mg/kg in fish to a high of 3472.3 mg/kg in Chinese cabbage, the cadmium content ranged from 3.0 mg/ kg in cassava leaves to 4.7 mg/kg in fish. Rape samples had the lowest mean content of copper at 81.3 mg/ kg with Kapenta samples having the highest content of copper at 294.7 mg/kg, the iron content ranged from 383 mg/kg in cabbage to as high as 1757.7 mg/kg in spinach. Manganese lowest mean content was recorded at 64.7 mg/kg in fish having its highest content of 883.7 mg/kg in cassava leaves, and the zinc content ranged from 119.7 mg/kg in tomato to 826 mg/kg in fish (Miyanza et al. 2023).

The Lusaka samples had mean contents as follows; the aluminium content ranged from 163.3 mg/kg found in fish to a high of 1698.7 mg/kg in Chinese cabbage, the cadmium content ranged from 2.0 mg/ kg in cassava leaves to 5.3 mg/kg in Kapenta. The lowest mean content of copper at 73.3 mg/ kg was found in cabbage with Kapenta samples having the highest content of copper at 147.3 mg/kg, the iron content of 453 mg/kg was found in cabbage and a content as high as 1508.3 mg/kg was found in cassava leaves. The manganese lowest mean content was obtained at 18.7 mg/kg in fish having its highest content of 512 mg/kg in tomato, and the zinc mean content ranged from 83.3 mg/kg in cabbage to 387 mg/kg in Kapenta (Miyanza et al. 2023).

For Kabwe, the aluminium content ranged from 120.3 mg/kg found in Chinese cabbage to 662.7 mg/kg in rape, the cadmium content ranged from 1.9 mg/ kg in spinach to 3.9 mg/kg in rape. Cabbage samples had the lowest mean content of copper at 99.9 mg/ kg with cassava leaves samples having the highest content of copper at 108.5 mg/kg, the iron content ranged from 124.5 mg/kg in spinach to 618.8 mg/kg in cassava leaves. Manganese lowest mean content was recorded at 13.6 mg/kg in tomato having its highest content of 86.2 mg/kg in spinach, and the zinc content ranged from 10.6 mg/kg in spinach to 261.4 mg/kg in rape (Miyanza et al. 2023).

The aluminium lowest content was recorded in fish and highest content in Chinese cabbage for Kitwe and Lusaka cities. However, samples from Lusaka recorded lower concentration of aluminium. The cadmium content was both low in cassava leaves for Lusaka and Kitwe, but lowest in spinach for Kabwe samples. However, the highest cadmium content was recorded in Kapenta

from Kitwe. The copper content was lowest in samples from Lusaka but it was highest in Kapenta from Kitwe. The iron content was lowest in spinach from Kabwe and highest in spinach from Kitwe City. The manganese content was both lower in fish for Kitwe and Lusaka cities but the lowest content was recorded in tomato samples from Kabwe Town. The highest manganese content was recorded in cassava leaves from Kitwe City. For zinc, the lowest content was recorded in samples from Kabwe Town (Miyanza et al. 2023).

All the samples exceeded the maximum allowable limits for cadmium as set by the FAO/WHO (2001) (Table 15). Cabbage from Lusaka City was the only sample that did not exceed the maximum allowable limits for copper. For Kabwe, only cabbage and cassava leaves had levels of iron exceeding the allowable limit. For Kitwe, only cabbage had levels of iron within the allowable limits. All the foodstuffs from Lusaka had levels of iron above the allowable limit. The levels of manganese were within the acceptable limits for all the foodstuffs, except for cassava leaves from Kitwe and Lusaka cities. The levels of zinc were within acceptable limits in foodstuffs from Kabwe except for rape. All the foodstuffs from Kitwe exceeded the allowable limit for zinc. Only cabbage and rape had levels of zinc within allowable limits among the foodstuffs from Lusaka (Miyanza et al. 2023).

Comparatively, the concentrations were generally higher in samples from Kitwe than those from Lusaka with Kabwe having the lowest concentrations probably because there is no more mining in the area. It is not surprising that Kitwe had higher levels of heavy metals because it is the mining area. However, it was expected that the levels of heavy metals for samples from Kabwe would be higher than those for Lusaka because of the mining history of Kabwe. However, such conclusions would be satisfactory if the samples were grown on the soils of each study area. In this case, the samples were randomly bought from open markets without foreknowledge of where they were grown (Miyanza et al. 2023).

Table 15: Metal concentrations (mg/kg, n=3) from selected food samples sold on open markets in Zambia Al=Aliminium; Cd=Cadmium; Cu=Copper; Fe=Iron; Mn=Manganese; and Zn=Zinc; MAL=Maximum allowable limits

City/Town	Sample	Al	Cd	Cu	Fe	Mn	Zn
Kitwe	Cabbage	449.7±7.0	3.33±0.33	113.3±3.0	383±6.0	77.3±2.0	180±3.7
	Rape	1245±5.0	3.33±0.33	81.3±1.0	540±5.7	101.3±10	213.7±2.3
	Cassava leaves	714.3±9.0	3.0±0.33	105.3±1.0	707.3±7.3	883.7±6.0	135±2.3
	Chinese cabbage	3472.3±25	3.7±0.67	259.3±3.7	1539.3±12	242.7±1.3	181.7±1.3
	Spinach	2661.3±12	3.30±0.33	226.7±1.7	1757.7±18	146.7±2.0	125±1.7
	Kapenta	998.3±10	4.3±1.0	294.7±6.3	559±13	85.7±2.3	428.7±7.7
	Tomato	1813.3±20	4.0±0.33	84.3±6.0	1233.7±27	197.7±4.7	119.7±3.3
	Fish	236±15	4.7±0.33	82.3±2.3	540±14	64.7±2.0	826±19
Lusaka	Cabbage	281.7±4.7	3.0±0.23	73.3±1.0	453.3±10	78.3±1.7	83.3±2.0
	Rape	742.3±20	3.3±0.33	76±0.67	766.7±3.0	201±2.0	86±1.0
	Cassava leaves	1343.7±8.0	2.0±0.67	130.3±3.0	1508.3±19	510±8.3	198±2.3
	Chinese cabbage	1698.7±17	3.3±0.33	75±1.3	1044.3±21	137.7±2.7	103±3.0
	Spinach	1554.7±26	3.0±0.33	95.3±3.0	1158.7±34	393±12	120.7±3.3
	Kapenta	732.3±8.7	5.3±0.33	147.3±4.0	522.7±8.0	39±0.67	387±8.0
	Tomato	1457.3±18	2.7±0.67	94±1.3	1183.7±2.7	512±3.3	226±1.0
	Fish	163.3±6.7	4.0±0.33	78±0.67	596.7±8.7	18.7±0.33	249.6±5.3
Kabwe	Cabbage	336.9±2.66	2.9±0.33	99.9±1.8	529.3±7.2	61.3±0.32	17.6±0.49
	Rape	662.7±6.01	3.9±0.59	106.2±4.0	245.8±3.4	45.9±0.54	261.4±3.5
	Cassava leaves	574.4±4.7	2.6±0.33	108.5±3.3	618.8±8.7	32.9±0.67	51.2±0.82
	Chinese Cabbage	120.3±0.89	2.6±0.56	107.8±6.2	380.6±3.5	57.6±1.09	83.2±1.5
	Spinach	150.5±1.98	1.9±0.31	102.2±1.9	124.5±2.0	86.2±0.47	10.6±0.56
	Kapenta	244.8±2.22	2.9±0.09	100.5±3.7	242.6±3.1	40.3±0.33	36.6±0.3
	Tomato	143.9±1.71	2.9±0.05	104.5±4.3	357.6±4.9	13.6±0.63	19.3±0.14
	Fish	235.2±3.89	2.6±0.42	101.5±3.0	331.3±6.0	44.6±0.22	20.6±0.46
MAL	FAO/WHO, 2001	-	0.2	73.3	425.5	500	99.4

Statistical analysis, both descriptive and inferential, performed using Microsoft Excel 2016 Software with a level of significance maintained at 95% revealed that there was no significant difference in the concentrations of metals for samples from Kitwe City Open Market and Lusaka City Open Market, $P>0.0167$. However, there was a significant differences in the mean

concentrations of metals in samples from Kitwe Open Market and Kabwe Open Market with Kitwe City recording higher concentrations and samples from Kabwe Open Market and Lusaka Open Market with Lusaka City recording higher concentrations , $P<0.0167$ (Miyanza et al. 2023).

4.2.3 Comparison with results from similar studies: a discussion

The mean concentrations of metals in vegetables from different countries are presented (Table 16). The results show that mean concentrations of aluminium in the studied samples from other countries are lower than those found in this study. The results also show that aluminium is not frequently analysed as compared to other metals analysed in this study. Similar results which range from 1.5 mg/kg to 5.3 mg/kg were recorded for cadmium by Naupia et al. (2018) in Pretoria, South Africa and Kinshasa, Democratic Republic of Congo and Rebeyehu and Bayissa (2020) in Mojo area, Ethiopia. The same results were recorded by Ali and Al-Qahtani (2012) in 4 cities of Saudi Arabia including Riyadh City, Tabouk City, Dammam City and Jazan City; Eneji et al. (2014) in North Central Nigerian rivers, Adekiya et al. (2019) in Osun State, Nigeria and Somda et al. (2019) in Ouagadougou, Burkina Faso. However, Mubofu (2012) reported higher results of 10 mg/kg for cabbage and Chinese cabbage from Karakoo Market in Dar es Salaam, Tanzania and Chopra and Pathak (2015) reported 14.58 mg/kg for spinach in Dehradun, India. This study found the highest content of copper in all samples than reported by other studies (Table 14) except for Somda et al. (2019) who obtained 242.9 mg/kg for tomato in Ouagadougou, Burkina Faso. This result was higher than all the results reported in this study except for the content of Chinese cabbage and Kapenta from Kitwe City (Miyanza et al. 2023).

Table 16: Mean concentrations (mg/kg) of heavy metals from similar studies
Al=Aliminium; Cd=Cadmium; Cu=Copper; Fe=Iron; Mn=Manganese; and Zn=Zinc; nd=not detected

Country/sample	Authors	Al	Cd	Cu	Fe	Mn	Zn
Tomato							
Botswana	Bati et al. 2017	-	0.33	14.7	119.6	19.1	45.5
Nigeria	Oyareme et al. 2020	-	0.82	0.44	4.23	0.53	1.38
Nigeria	Adekiya et al. 2018	-	2.53	20.4	-	-	5.37
Ethiopia	Gebeyehu and Bayissa 2020	-	0.56	16.27	85.1	27.2	24.5
Saudi Arabia	Mohamed et al. 2003	-	0.77	4.47	60.2	7.39	14.4
Saudi Arabia	Ali and Al-Qahtani 2012	-	2.45	7.46	364.6	27.84	22.91
Burkina Faso	Somda et al. 2019	-	2.78	242.9	-	-	146.4
Algeria	Bounar et al. 2020	-	0.45	-	-	-	6.57
South Africa	Gupta et al. 2018	-	-	0.72	14.3	1.01	1.26
India	Rao et al. 2017	-	0.94	0.47	16.38	-	-
Zambia	this study (Kabwe)	143.9	2.9	104.5	357.6	13.6	19.3
Zambia	this study (Kitwe)	1813	4.0	84.3	1233.7	197.7	119.7
Zambia	this study (Lusaka)	1457	2.7	94	1183.7	512	226
Fish							
Zambia	Mbewe et al. 2016	-	0.58	3.78	10.51	1.67	-
South Africa	Naupia et al. 2018	4.63	2.5	11.75	-	11.84	50.69
DR. Congo	Naupia et al. 2018	9.1	0.64	6.53	-	10.51	14.17
Nigeria	Eneji et al. 2014	-	4.76	-	-	72.5	-
Malaysia	Taweel et al. 2013	-	0.04	1.69	-	-	26.13
Zambia	this study (Kabwe)	235.2	2.6	101.5	331.3	44.6	20.6
Zambia	this study (Kitwe)	236	4.7	82.3	540	64.7	826
Zambia	this study (Lusaka)	163.3	4.0	78	596.7	18.7	249.6
Cabbage							
South Africa	Naupia et al. 2018	18.93	3.93	15.7	-	18.31	75.12
DR. Congo	Naupia et al. 2018	52.1	2.93	3.78	-	14.14	27.93
Botswana	Bati et al. 2017	-	0.8	8.06	33.1	38.6	235.4
Ethiopia	Weldegebriel et al. 2012	-	0.18	0.99	-	3.82	3.07
Ethiopia	Gebeyehu and Bayissa 2020	-	1.56	9.42	490.46	302.2	23.53
Ethiopia	Gezahegn et al. 2017	-	-	10.4	100.7	65.4	43.2
Saudi Arabia	Mohamed et al. 2003	-	0.56	0.43	76.9	21.79	14.9
Tanzania	Mubofu 2012	-	10	0.56	-	1.8	4.18
South Africa	Gupta et al. 2018	-	-	0.68	6.15	2.91	5.36
Zambia	this study (Kabwe)	336.9	2.9	99.9	529.3	61.3	17.6
Zambia	this study (Kitwe)	449.7	3.33	113.3	383	77.3	180
Zambia	this study (Lusaka)	281.7	3.0	73.3	453.3	78.3	83.3
Rape							
Botswana	Bati et al. 2017	-	0.53	5.63	94.5	70.2	47.3

Zambia	this study (Kabwe)	662.7	3.9	106.2	245.8	45.9	261.4
Zambia	this study (Kitwe)	1245	3.33	81.3	540	101.3	213.7
Zambia	this study (Lusaka)	742.3	3.3	76	766.7	201	86
Cassava leaves							
Zambia	Kříbek et al. 2019	-	-	85.5	-	-	82.5
Zambia	this study (Kabwe)	574.4	2.6	108.5	618.8	32.9	51.2
Zambia	this study (Kitwe)	714.3	3.0	105.3	707.3	883.7	135
Zambia	this study (Lusaka)	1344	2.0	130.3	1508.3	510	198
Chinese cabbage							
Tanzania	Mubofu, 2012	-	10	0.38	-	3.88	1.19
Zambia	this study (Kabwe)	120.3	2.6	107.8	380.6	57.6	83.2
Zambia	this study (Kitwe)	3472	3.7	259.3	1539.3	242.7	181.7
Zambia	this study (Lusaka)	1699	3.3	75	1044.3	137.7	103
Spinach							
Saudi Arabia	Ali and Al-Qahtani 2012	-	4.13	14.07	94.7	25.13	30.18
Saudi Arabia	Mohamed et al. 2003	-	0.77	2.71	166.4	9.9	9.6
Parkstan	Latif et al. 2018	-	0.35	22.25	968.25	137.3	19.5
Tanzania	Kacholi and Sahu 2018	-	-	0.98	10.6	-	5.76
India	Chopra and Pathak 2015	-	14.58	34.49	-	-	154.21
Bangladesh	Tasrina et al. 2015	-	<0.1	nd	58.09	5.28	8.49
South Africa	Gupta et al. 2018	-	-	1.73	18.7	13.7	1.74
India	Gupta et al. 2020	-	0.86	8.19	-	37.67	31.89
Turkey	Leblebici et al. 2020	-	0.03	5.11	-	38.67	31.3
Zambia	this study (Kabwe)	150.5	1.9	102.2	124.5	86.2	10.6
Zambia	this study (Kitwe)	2661	3.3	226.7	1757.7	146.7	125
Zambia	this study (Lusaka)	1555	3.0	95.3	1158.7	393	120.7

The mean concentrations of iron that range between 124.5 mg/kg and 1757.7 mg/kg for all samples were recorded in this study. Similar concentrations that are close to and are within the aforementioned range were recorded by Bati et al. (2017) in Maun, Botswana and Ali and Al-Qahtani (2012) in the aforementioned cities of Saudi Arabia for tomato samples, Mohamed et al. (2003) in Al-Taif District, Saudi Arabia for spinach and Rebeyehu and Bayissa (2020) in Mojo Area, Ethiopia for cabbage. Latif et al. (2018) reported a mean level of 968.3 mg/kg for spinach in Dera Ghazi Khan District, Pakistan. For manganese, other researchers as indicated (Table 13) reported results that are similar to those obtained in this study (Miyanza et al. 2023).

However, other researchers obtained lower results as compared to the results in this study. Mean concentrations ranging between 0.53 mg/kg and 9.9 mg/kg were reported by Oyareme et al. (2020) in Edo State, Nigeria; Mbewe et al. (2016) in Kafue River, Zambia and Mubofu et al. (2012) in Dar es Salaam, Tanzania. Mohamed et al. (2003) in Al-Taif District, Saudi Arabia; Tasrina et al. (2015) in Pakshi, Bangladesh; Gupta et al. (2018) in Durban, South Africa and Weldegebriel et al. (2012) in Addis Ababa, Ethiopia reported the same for manganese. Most of the results (Table 13) for zinc are above 10.6 mg/kg, which is the lowest result obtained in this study. Somda et al. (2019) in Ouagadougou, Burkina Faso and Bat et al. (2017) in Maun, Botswana reported mean levels of 146.4 mg/kg and 235.4 mg/kg for zinc, respectively, which are higher than most of the results in this study (Miyanza et al. 2023).

4.2.4 Health risk assessment of heavy metals

The risk to human health because of consuming the foodstuffs investigated was evaluated by calculating the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and carcinogenic risk for the carcinogenic metals as described in Chapter 3. The results obtained are presented as follows:

(i) Estimated daily intake

In Table 17, the results for the EDI for the studied metals are presented. All metals have EDI values less than the recommended limits as set by WHO/FAO (2000) except for cadmium. The EDI value for cadmium had the EDI exceeds the tolerable daily intakes in all samples except in tomato, cassava leaves, cabbage from Lusaka and cassava leaves from Kitwe, which gave EDIs lower than the set limits. For Kabwe, the EDIs for cadmium in cabbage and rape samples exceed the recommended limits. The rest of the samples have EDIs lower than the limits (Miyanza et al. 2023).

Table 17: Estimated daily intake (EDI) of the metals under study (mg/Kg)
Al=Aliminium; Cd=Cadmium; Cu=Copper; Fe=Iron; Mn=Manganese; and Zn=Zinc

City/Town	Sample	Al	Cd	Cu	Fe	Mn	Zn
Kitwe	Cabbage	0.38	0.0028	0.097	0.33	0.066	0.15
	Rape	1.1	0.0028	0.069	0.46	0.086	0.18
	Cassava leaves	0.61	0.0025	0.089	0.61	0.75	0.12
	Chinese cabbage	2.9	0.0031	0.22	1.3	0.21	0.15
	Spinach	2.2	0.0028	0.19	1.5	0.12	0.11
	Kapenta	0.85	0.0036	0.25	0.47	0.073	0.36
	Tomato	1.5	0.0034	0.071	1.1	0.16	0.11
	Fish	0.21	0.0041	0.071	0.46	0.055	0.71
Lusaka	Cabbage	0.24	0.0025	0.062	0.38	0.066	0.071
	Rape	0.63	0.0028	0.064	0.65	0.17	0.073
	Cassava leaves	1.1	0.0017	0.11	1.3	0.44	0.16
	Chinese cabbage	1.4	0.0028	0.064	0.89	0.12	0.087
	Spinach	1.3	0.0025	0.081	0.98	0.33	0.11
	Kapenta	0.62	0.0045	0.13	0.44	0.033	0.33
	Tomato	1.2	0.0023	0.081	1.1	0.44	0.19
	Fish	0.14	0.0034	0.066	0.51	0.016	0.21
Kabwe	Cabbage	0.039	0.0027	0.085	0.025	0.052	0.015
	Rape	0.56	0.0038	0.091	0.29	0.039	0.22
	Cassava leaves	0.49	0.0022	0.092	0.52	0.028	0.044
	Chinese Cabbage	0.17	0.0022	0.092	0.32	0.049	0.071
	Spinach	0.18	0.0016	0.087	0.16	0.079	0.0094
	Kapenta	0.038	0.0024	0.085	0.036	0.034	0.031
	Tomato	0.037	0.0024	0.089	0.049	0.011	0.016
	Fish	0.03	0.0022	0.086	0.026	0.038	0.017
WHO/FAO (2000)	**Limits**	**10**	**0.0025**	**1.4**	**14.8**	**2.3**	**11**

(ii) Target hazard quotient and hazard index

The THQs of the metals in the foodstuffs (Figure 11) indicate various outcomes for individual metals in different samples. Cabbage, cassava leaves, Kapenta and fish from Kitwe and cabbage, rape, kapenta and fish from Lusaka had THQ < 1 for aluminium. Cabbage, rape, cassava leaves, Kapenta and fish from Kitwe, and cabbage, rape, Kapenta and fish from Lusaka recorded THQ < 1 for iron. Manganese also had THQ < 1 in cabbage, rape, spinach, Kapenta and fish from Kitwe, and in cabbage, Chinese cabbage, kapenta and fish from Lusaka. Kapenta and fish from Kitwe

gave a THQ < 1 including Kapenta from Lusaka. All the samples from Kabwe recorded THQs < 1 for aluminium, iron, manganese and zinc. Cadmium and copper had THQs > 1 in all the samples from all the two cities and one town. The highest THQs were recorded for cadmium, copper and manganese with values ranging from 3.4 to 6.25 for some samples from Kitwe City. For samples from Lusaka City, the highest THQs were 3.4 and 4.5 for cadmium. The highest THQ for samples from Kabwe was 3.8 for cadmium. These results mean that the hazard indexes (HIs) for all the metals in all the samples were > 1. This entails that there is a probable non-carcinogenic risk through prolonged consumption of the studied foodstuffs for the local population in the three towns. The carcinogenic risk for cadmium was > 10^{-4}, indicating a possibility of the population experiencing cancer because of prolonged consumption of foodstuffs of interest in this study.

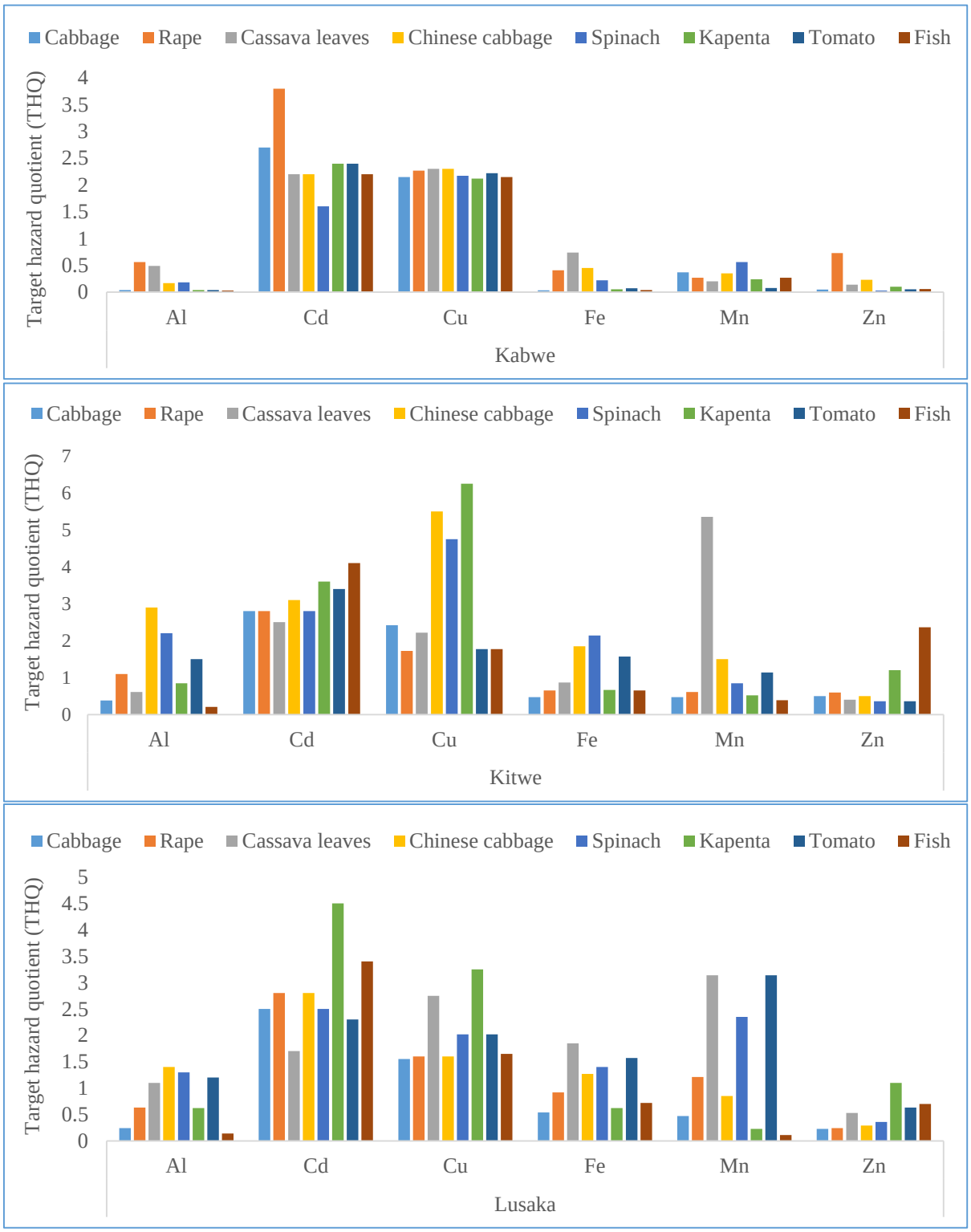


Figure 11: The THQs of metals in each foodstuff obtained from Kabwe, Kitwe and Lusaka in Zambia. Al=Aliminium; Cd=Cadmium; Cu=Copper; Fe=Iron; Mn=Manganese; and Zn=Zinc

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

This chapter presents the conclusions and recommendations derived from the results obtained and discussed in Chapter 4.

5.1 Conclusions

The EDCs 4-n-Nonyl phenol and DDT metabolites were not detected in all the samples in this study. Though DDT is still being used in some areas, these results could indicate its non-use or usage that does not affect the food environment in the study areas. The general population in the study areas is free from the health effects of these EDCs. On the other hand, phthalates were identified in most of the foodstuffs. DMP was identified and quantified in two samples from Kitwe Open Market, three samples from Lusaka Open Market, and was not identified in only one sample from Kabwe Open Market. However, there was no significant difference in the mean concentrations of DMP in all the samples from markets Kabwe, Kitwe and Lusaka, $P>0.05$. The mean concentrations of DEP showed no significant difference for samples from Kitwe and Lusaka Open markets, $P>0.05$. However, a significant difference, $P>0.0167$, in the mean concentrations was found between samples from Kitwe and Kabwe Open markets, and samples from Kabwe and Lusaka Open markets with samples from Kabwe Town having higher concentrations, up to 123.82 μg/kg compared to 101.76 μg/kg and 98.55 μg/kg, in both cases.

For heavy metals, there was no significant difference in the concentrations of metals for samples from Kitwe and Lusaka Open markets, $P > 0.0167$. However, there was a significant difference in the mean concentrations of metals in samples from Kitwe and Kabwe Open markets, and samples from Kabwe and Lusaka Open markets, $P < 0.0167$. Samples from markets in Kitwe recorded the highest average metal concentrations followed by samples from markets in Lusaka as follows:

(i) The mean concentrations of dimethyl phthalate (DMP) ranged from 91.05 to 101.76 μg/kg, 77.14 to 123.82 μg/kg, and 85.65 to 98.55 μg/kg for samples from Kitwe, Kabwe and Lusaka Open markets respectively;

(ii) The mean concentrations of diethyl phthalate (DEP) ranged from 21.46 to 80.69 μg/kg, 63.93 to 161.67 μg/kg and 23.22 to 46.01 μg/kg for samples from Kitwe, Kabwe and Lusaka Open markets respectively; and

(iii) The investigation revealed that the mean level of heavy metals ranged: Cd 3.0±0.33 to Al 3472.3±25 mg/kg for Kitwe; Cd 2.0±0.67 to Al 1698.7±17 mg/kg for Lusaka; Cd 1.9±0.31 to Al 662.7±6.01 mg/kg for Kabwe.

This suggest that the population of Kitwe is at the highest risk followed by the population of Lusaka. The population of Kabwe has the least risk to health.

The health risk analysis of DMP and DEP, HI<1, gives an indication that the consumers are safe from the health effects that result from exposure to these phthalates through consumption of food, which is the main route of exposure.

Although the estimated daily intakes of the heavy metals for all the foodstuffs were within tolerable intakes, the foodstuffs analysed presented undue risk of adverse health effects, whether non-carcinogenic in the case of Target Hazard Quotients (THQ) for aluminium, iron, manganese, and zinc in selected samples. Cadmium and copper presented undue risk due to higher THQs recorded. The Hazard Index (HI) for all metals in all samples from all markets in Kabwe, Kitwe and Lusaka was greater than 1 indicating possible non-carcinogenic risk. The Carcinogenic Risk (CR) for cadmium was higher than 10^{-4} in all samples from all markets in Kabwe, Kitwe and Lusaka. This indicates possible carcinogenic risk from prolonged consumption of studied foods.

5.2 Recommendations

The following are recommended from this study:

(i) Academic and research institutions and the Ministry of Technology and Science should undertake further studies in order to make wholesome conclusions with regards to health risk;

(ii) Food quality assurance and control experts and National Food and Nutrition Commission should routinely check the quality of foodstuffs consumed by the Zambian nationals because some findings in this study show a significant health risk to the consumers; and

(iii) The National Food and Nutrition Commission should update the food balance sheet to reflect the real consumption accounting for both social-economic and cultural differences. The food balance sheet should also consider all varieties of food, unlike the current one that has general terms for many food categories.

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