PERSISTENT ORGANIC POLLUTANTS IN WATER, SEDIMENTS AND FISH FROM LAKE VICTORIA AND IMPLICATIONS TO HUMAN HEALTH RISKS

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN FOOD SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

2019

EXTENDED ABSTRACT

Lake Victoria is an important freshwater resource in Tanzania and its neighbouring countries namely Uganda, Kenya, Rwanda, Burundi and Democratic Republic of Congo (DRC). Millions of people dotted around the lake depend on it as a major source of household and industrial water supply, irrigation, transport and provides for fishing as a main source of animal derived protein as well as income. In Tanzania, the lake provides employment opportunities to more than four million people engaged directly or indirectly with fishing activities. The frequently fished and consumed fish species that are of commercial interest include; Nile perch (*Lates niloticus*), Nile tilapia (*Oreochromis niloticus*) and the freshwater sardines (*Rastrioneobola argentea*) commonly known as *dagaa* in Tanzania. The fish are further processed to feed the domestic, regional and international markets. Current estimates from the lake put the annual contribution of the fisheries sector to the Tanzanian Gross Domestic Product (GDP) up to 2.5%.

There have been unsubstantiated claims that despite using agrochemicals such as pesticides in agriculture, some fisher folks have been using such chemicals for fishing and preservation of fish products during storage. The same organochlorine pesticides are still in use in agricultural activities within the lake basin and there is a possibility that they accumulate in water, sediments and fish. Furthermore, there have been incinerations of medical wastes, discharge of electronic wastes and untreated wastewaters from industrial establishments in water bodies that tend to increase the loading of polychlorinated biphenyls (PCBs) in water, sediments and fish. It is likely that human beings will get exposed to these chemicals through drinking water and eating fish from the lake.

The present study overall objective was therefore to assess fish consumers' eating habits and the prevalence, levels and human health risks of indicator PCBs and OCPs in water, sediments, fish and fish products from Lake Victoria in Tanzania.

The specific objectives of the study were:

i. To establish levels of indicator PCBs and OCPs in water, sediments and fish from Lake Victoria.
ii. To determine the prevalence and quantify residual levels of indicator PCBs and OCPs in processed fish products from Lake Victoria.
iii. To assess fish consumers' eating habits.
iv. To evaluate the probable human health risks associated with consumption of persistent organic pollutants (POPs) contaminated fish and fish products from Lake Victoria.

Water and sediment samples were collected from twelve nationally designated sampling points (considered as important hotspots). Thirty six samples of both water and sediments (a total of 72 samples) were collected for analysis. A total of 162 fresh fish samples were collected from nine selected landing sites and 480 samples of processed fish products from Kirumba Fish Market for analysis of POPs. Extraction of the samples was done using a QuEChERS method and POPs analysis using a gas chromatography equipped with electron capture detectors (GC-ECDs) and a gas chromatography equipped with mass spectrometry (GC-MS).

A questionnaire was also administered to 122 fish consumers to assess the fish consumption habits and patterns. The main focus of administering the questionnaire was to identify the types of fish species consumed, frequencies of fish consumption, quantities

of fish consumed and factors affecting fish consumption in the population around the lake.

The results showed that some organochlorine compounds were present in water, sediments, fish and fish products from Lake Victoria in Tanzania. This is likely due to human anthropogenic activities being undertaken along the lake, long range atmospheric transport and environmental persistence of the compounds. For water samples, the ∑PCBs ranged between 0.95 and 2.24 µg/L while for sediments the range was between <LOD and 10.28 µg/kg- dry weight (dw) basis being dominated by CB 138 and CB 153 because of their structures and high degree of chlorination. For OCPs; Aldrin and Dieldrin were the highest (6.13 ±0.90 and 6.82±0.53 µg/kg- dw, respectively) and p, p'- DDT was the lowest (0.82±0.12 µg/kg- dw) in sediments whereas for water samples the highest concentration was γ- HCH (1.60±0.32 µg/L) and the lowest was α- endosulfan (0.15±0.01 µg/L).

There were high levels of organochlorine compounds in sediments than the water samples suggesting that sediment serves as a sink for organochlorine pollutants and therefore act as a source of these compounds in case of environmental changes. However, the levels of POPs measured in water and sediments in this study were far below the Maximum Residual Limit (MRL) set by FAO/WHO and the European Union (EU) except Aldrin and Dieldrin through which based on the Threshold Effect Concentration (TEC) for freshwater ecosystems, Aldrin and Dieldrin are the only OCPs that seem to be a threat to Lake Victoria environment.

For the fresh fish species which were considered in the current study; *L. niloticus* and *O. niloticus*, the results revealed that fish species sampled were undersize, reflecting overfishing and abuse of bylaws.

The abuse may be going beyond overfishing and touching other environmental protection bylaws. The results showed further that four organochlorine pesticides (β-HCH, HCB, Aldrin and Dieldrin) were identified in fresh muscles of *L. niloticus* and *O. niloticus* at measurable quantities at concentration between <0.24 and 1.19 µg/kg. There were slightly higher levels of the identified OCPs in *L. niloticus* than in *O. niloticus* muscles due to differences in trophic levels and feeding habits. However, the levels of OCPs measured were below the MRL of 200 µg/kg set for fish by FAO/WHO and other competent authorities suggesting that the fresh fish muscles were safe for human consumption.

The seven indicator PCBs considered in this study were not detected in all fresh fish species investigated. Low levels of the detected residues and non detection of many POPs which were considered indicates that currently POPs pollution in Lake Victoria has not reached alarming concentrations but requires a strict continuous monitoring to minimize contaminants loading.

Nine OCPs were detected at different measurable concentrations in different processed fish products available in the markets from Lake Victoria. The highest amount was p, p'-DDE (3.73±0.31 µg/kg) in deep fried *O. niloticus* while the lowest level was α- HCH (0.14±0.06 µg/kg) in smoked *O. niloticus*. The OCPs were more prevalent in smoked products compared to other processed fish products. This is attributed to a reduced co-distillation of the compounds with water vapour during smoking. However, there was high prevalence of some OCPs in deep fried products compared to other fish products.

This is probably due to illegal use of some of these chemicals for fish preservation during storage. In this study, the levels of OCPs in processed fish products were below MRL set by different international statutory bodies for fish and fish products implying that the fish products are safe for human consumption with reference to OCPs concentrations.

For the seven indicator PCBs which were considered in this study, six of them were detected at different levels in processed fish products. The total PCBs loading ranged from 16.05±3.04 µg/kg (deep fried products) to 31.55±16.66 µg/kg (trims). There was a significant difference ($p<0.05$) in PCBs loading for deep fried products (with significantly lower levels) compared to other products which were investigated in this study. This observation is mainly because at high temperatures, cooking oil serves as an extracting solvent and therefore the PCBs are transferred the cooking oil. The PCBs loading was dominated by CB 138, CB 153 and CB 180. The same PCBs were more prevalent (between 20% and 80%) in all fish products than other congeners. This is mainly due to their structures, chemical stability and high degrees of chlorination. However, the mean levels of ∑PCBs in this study were below the MRL of 75 µg/kg set for fish and other fish products by the European Commission indicating that the products are safe for human consumption with respect to indicator PCBs.

Lifetime cancer risks for both OCPs and PCBs in adults and children were low while the non cancer risks associated with consumption of contaminated fish and fish products were insignificant as the hazard indices (HI) were very low (<1). The risk is considered significant if HI is greater than 1.

Results on fish consumer's preferences, quantities and types of fish consumed and factors affecting fish eating habits in this study showed that *L. niloticus, O. niloticus* and *R. argentea* are widely consumed fish species. The average per capita fish consumption per

day was quantified to be 0.37 kg (~135 kg/year). This level is far greater than the national, worldwide and the per capita fish consumption of a great fish consumer reported in literatures. The main reasons for this high fish consumption in the study area were found to be easy accessibility and availability of different fish species.

The major factors affecting fish consumption habits were reported to be price (affordability), convenience, accessibility, availability, nutritional and healthy concerns.

The study revealed a slightly higher proportion of male fish consumers (54.1%) than female fish consumers (45.9%) implying that males usually eat outside their homes better meals than home meals. The study revealed further a direct relationship between fish consumption and age of consumers due to knowledge and health reasons and an inverse relationship between fish consumption and family size due to economic reasons. In order to determine the actual human health risks associated with consumption of fish products from Lake Victoria for consumers dotted around the lake, it is therefore necessary to employ the actual per capita fish consumption of 0.37 kg established herein for risk computation rather than the hypothetical per capita fish consumption rate of 0.02 kg reported in literature.

Data generated from this study will therefore help decision makers to devise more appropriate and cost effective risk mitigation measures that will minimize the build-up of these organochlorine compounds in the aquatic ecosystems to safeguard consumer's health and the environment. The results will also help to create awareness of the actual adverse effects associated with the established organochlorine compounds among stakeholders in the fisheries sector.

DECLARATION

I, Alex Ngungulu Wenaty , do here by declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

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ACKNOWLEDGEMENTS

I thank God the Almighty for his guidance for the whole period of my study. My profound appreciation goes to my supervisors; Professor B. E. Chove, Professor R. H. Mdegela and Dr. F. P. Mabiki for their guidance, patience, constructive criticisms and moral support throughout this study period.

My sincere thanks go to my sponsor, the Danish International Development Agency (DANIDA) through the "Innovations and Markets for Lake Victoria Fisheries (IMLAF) Project" (DFC file No. 14 – P01 – TAN) for financial support to complete this work. I am grateful to the Sokoine University of Agriculture for granting me a study leave. Mr. Arvid Fromberg of the Technical University of Denmark (DTU) is greatly acknowledged for his technical assistance during gas chromatographic (GC) analysis of the samples in Denmark. Professor Anders Dalsgaard of the University of Copenhagen in Denmark deserves many thanks for his technical and constructive guidance during my stay in Denmark. Special thanks go to Mr. Malulu and other Tanzania Pesticides Research Institute (TPRI) staff for their technical and moral support during analysis of water and sediment samples.

Moreover, I am grateful to the National Fish Quality Control Laboratory staff, Chemical Laboratory Section in Mwanza particularly; Mr. Stephen Lukanga (Zonal Officer Incharge), Mr. John Killian (Head of Chemical Laboratory), Mr. Michael Mhina, Mr. Donny Thomas, Ms. Anna Uswege, Mr. Hassani Mengi and Ms. Elinziwanza Mshiu for allowing me to work in their laboratory during samples extraction and clean up and for their technical assistance during samples collection and processing. I am also very

grateful to Dr. Ofred Mhongole for his support with field data collection and providing the help I needed.

I also thank Mr. Zephania Sanga; the IMLAF Project driver who worked tirelessly to make this work done. My sincere gratitude goes to my fellow PhD students Mr. Davis Chaula, Mr. Zebedayo Baniga and Ms. Josephine Mkunda for their moral support during the study period and my fellow staff at the Department of Food Technology, Nutrition and Consumer Sciences; Prof. Henry S. Laswai for mentoring me tirelessly during the study period.

Furthermore my special thanks go to fishing community, fisheries officers, fish processors, sellers and consumers in Mwanza, Mara, Kagera and Geita for providing me with valuable information and samples during data collection.

I am thankful to my parents, Wenaty Ngungulu and late Elizabeth Ngovano for their never ending support and directing me on the right path. I could have never reached this far if it was not for your support, guidance and love. My siblings Ms. Pierina, Mr. Akwilino, Mr. Ponsiano, Ms. Jocomina and Ms. Zuhura Ngungulu; thank you so much.

Finally, my very special thanks go to my lovely wife, Yasinta A. Kongela and my children Elizabeth, Lilian and Kelvin A. Ngungulu. I thank you all for understanding, being patient and loving me throughout this journey.

DEDICATION

I dedicate this work to my brother Ponsiano Wenaty Ngungulu who raised me during the early stages of my academic arena and my family, Yasinta A. Kongela, Elizabeth, Lilian and Kelvin A. Ngungulu who prayed for me throughout my study period.

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1. Wenaty A., Mabiki F., Chove B., and Mdegela R. (2019). Assessment of organochlorine compounds contamination on the Lake Victoria water and sediments: a case study in Tanzania. *Accepted by the African Journal of Aquatic Sciences (Article in press)*.
2. Wenaty A., Arvid F., Mabiki F., Chove B., Dalsgaard A., and Mdegela R. (2019). Persistent organochlorine compounds levels in selected fish species from Lake Victoria and implications to human health risks**.** *Agriculture, Forestry and Fisheries* 8(1):1 – 9.
3. Wenaty A., Arvid F., Mabiki F., Chove B., Dalsgaard A., and Mdegela R. (2019). Assessment of Health Risks Associated with Organochlorine Pesticides Levels in Processed Fish Products from Lake Victoria. *African Journal of Food Science* 13(5): 101 – 110.
4. Wenaty A., Mabiki F., Chove B., Dalsgaard A., and Mdegela R. (2019). Occurrence, quantities and probable human health risks of indicatory polychlorinated biphenyls in processed *L. niloticus* products from Lake Victoria in Tanzania. *Accepted by the African Journal of Environmental Science and Technology (Article in press).*
5. Wenaty A., Mabiki F., Chove B., and Mdegela R. (2018). Fish Consumers Preferences, Quantities of fish consumed and Factors Affecting Fish Eating Habits: A Case of Lake Victoria in Tanzania. *International Journal of Fisheries and Aquatic Studies* 6(6): 247 – 252.

DECLARATION

I, Alex Wenaty**,** do hereby declare to the Senate of Sokoine University of Agriculture that the listed papers above that make this thesis summarize my independent efforts, it is my original work and will not be part of another thesis in the “published Papers” format in any other University.

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AT	Averaged Time
ATSDR	Agency for Toxic Substances and Disease Registry
BW	Body Weight
C	Concentration of the toxicant
CB	Chlorinated Biphenyl
CDI	Chronic Daily Intake
CR	Ingestion/Consumption Rate
C_R	Life time Cancer Risk
DDD	Dichloro- diphenyl- dichloroethane
DDE	Dichloro- diphenyl- dichloro- ethylene
DDT	Dichloro- diphenyl- trichloroethane
DFC	DANIDA Fellowship Centre
DTU	Denmark Technical University
DW	Dry weight basis
EC	European Commission
ECDs	Electron Capture Detectors
ED	Exposure Duration
EF	Exposure Frequency
EMRL	Extraneous Maximum Residue Limit
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GC	Gas Chromatography
GDP	Gross Domestic Product

GPS	Geographical Positioning System
HCB	Hexachlorobenzene
HCHs	Hexachlorocyclohexanes
HI	Hazard Index
HQ	Hazard Quotient
IMLAF	Innovations and Markets for Lake Victoria Fisheries Project
LLE	Liquid – Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LVBC	Lake Victoria Basin Commission
LVFO	Lake Victoria Fisheries Organization
MRL	Maximum Residue Limit
MS	Mass Spectrometry
NA	Not Available
ND	Not Detected/Determined
NFQCL	National Fish Quality Control Laboratory
OCPs	Organochlorine Pesticides
PCBs:	Polychlorinated Biphenyls
POPs	Persistent Organic Pollutants
PSA	Primary Secondary Amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
RfD	Reference Dose
SAS	Statistical Analysis Software
SC	Stockholm Convention
SD	Standard Deviation

SF	Cancer Slope Factor
SPSS	Statistical Package for Service Solutions
TEC	Threshold Effect Concentration
TPRI	Tanzania Pesticides Research Institute
URT	United Republic of Tanzania
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WW	Wet Weight basis
ZOI	Zonal Officer In charge
α- HCH	Alfa Hexachlorocyclohexane
β- HCH	Beta Hexachlorocyclohexane
γ- HCH	Gamma Hexachlorocyclohexane

CHAPTER ONE

1.0 Introduction

1.1 Background Information

Persistent organic pollutants (POPs) are substances that possess a combination of physical and chemical properties such as long range atmospheric transport (LRAT) (Li *et al*., 2007), accumulation in fatty tissues of living organisms (Cok *et al*., 2007; Bordajandi *et al*., 2008; Polder *et al*., 2014) and difficult in degradation in the environment (Field and Sierra- Alvarez, 2008) due to their stability under different environmental conditions. They are also characterized by their low water solubility (Deribe *et al*., 2011), lipophilicity (Sharma *et al*., 2009) and their tendency to biomagnify at higher trophic levels (Fiedler, 2007).

POPs are mainly man-made organic compounds which have been very useful for agricultural and industrial purposes and for vector control because of their unique physicochemical properties (Polder *et al*., 2014). They can accumulate in the food chain and remain in fat rich tissues of living organisms where they are likely to increase in concentration with time. Their impacts of accumulation on lifespan of an individual remain unclear but researches have suggested that they function as endocrine disruptors (Polder *et al*., 2014; Lee *et al*., 2016).

The increased concerns about the human health risks of endocrine disruptors such as POPs have resulted in legal restrictions of their use in most developed and developing nations (Kim and Lee, 2010). However, the harmful effects of low- dose endocrine disruptor exposure on health remain a big problem that requires special attention (Ha *et al*., 2009; Lee *et al*., 2011; Lee *et al*., 2014; Lee *et al*., 2016).

Studies have indicated that children are more likely to metabolize endocrine disruptors and are therefore more susceptible to their harmful impacts compared to adults (Bearer, 1995; Lee *et al*., 2016).

Tanzania ratified the Stockholm Convention (SC) in early 2004 and some of the POPs listed in the Stockholm Convention 2001 for initial elimination and reduction in usage because of their adverse effects to environmental media and living organisms. They include organochlorine pesticides (OCPs) such as Aldrin, Dieldrin, Endrin, Heptachlor, Chlordane, Dichlorodiphenyltrichloroethane (DDT), Hexachlorobenzene (HCB), Lindane (γ- hexachlorocyclohexanes (HCH), β- HCH, α- HCH and Polychlorinated Biphenyls (PCBs) (Polder *et al*., 2014).

The main sources of the listed POPs are agriculture, waste incineration, industrial chemical processes, unregulated disposal of textiles, building materials, obsolete electronic wastes (e- wastes) and burning of wastes and vegetation (Tuppurainen *et al*., 1998; Xu *et al*., 2009; Moon *et al*., 2012; Antunes *et al*., 2012; Liu *et al*., 2013; Ssebugere, 2015). In some cases the sources of POPs in environmental compartments and food items have been reported to be long range atmospheric transport as a result of their volatile nature and environmental persistence.

When released into aquatic ecosystems, the compounds tend to accumulate in bottom sediments for long period of time and are subject to partitioning between phases, degradation and transport processes (Evenset *et al*., 2007; Ssebugere, 2015). The solubility of many POPs in water is dictated by chlorine content, thus congeners with low chlorine content tend to be more volatile and are somewhat soluble in water.

Many congeners adsorb to organic materials in sediments and soils, adsorption tend to increase with chlorine content of the congener and with the organic content of the other material (ATSDR, 1995). Relative to water column, surface sediments are a more appropriate environmental media that can be related to the concentration of POPs in the aquatic organisms (Nguyen *et al*., 2005).

These contaminants have the potential to bio- accumulate across the food chain, building up in top predatory species via consumption of contaminated biota (Bjermo *et al*., 2013; Frouin *et al*., 2013). Therefore, it is anticipated that fish at the top of the aquatic food web are more likely to be affected by exposure to such persistent organic pollutants (Bervoets and Blust, 2003; Wang *et al*., 2011). There are several organochlorine compounds which occur naturally in the environment and some being anthropogenic but the most important POPs for public health and environmental concerns are organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs).

1.1.1 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are synthetic organic compounds characterized by their lipophilicity (Cok *et al*., 2007; Bordajandi *et al*., 2008; Bjermo *et al*., 2013), persistence in the environment (due to longer half-lives), toxicity, long range atmospheric transport (LRAT) (Liu *et al*., 2007), accumulation in biota and increase in concentration with time at higher trophic level (Polder *et al*., 2014; Oluoch-Otiego *et al*., 2016). PCBs are synthetic chlorinated hydrocarbon compounds that consist of two benzene rings linked together by a single carbon – carbon bond with from 1 to all 10 of the hydrogen atoms replaced with chlorine atoms (Fig. 1).

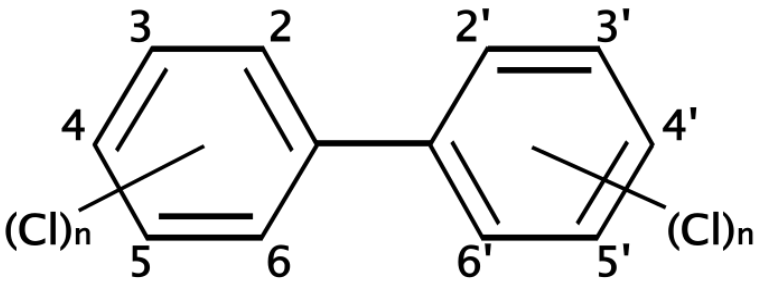

Figure 1.1: General chemical structure and numbering system for PCBs
(Source: http://environmentalchemistry.com/yogi/chemistry/pcb.html)

They are a group of 209 different chemicals which share a common structure but vary in a number of attached chlorine atoms and have been mainly used in plasticizers, surface coatings, inks manufacturing, adhesives, flame retardants, pesticide extenders, paints and microencapsulation of dyes for carbonless duplicating papers (ATSDR, 1995). PCBs resist both acids and alkalis and are relatively heat- stable, thus they have been used in dielectric fluids in transformers and capacitors (ATSDR, 1995; Ssebugere *et al*., 2014a).

As commercial formulations, PCBs were previously employed as dielectric fluids in power transformers and capacitors, insulating materials, coolants, plasticizers in rubber and plastic products as well as hydraulic fluid (Meijer *et al*., 2003; Johnson *et al*., 2006). Accidental leaks and fires in electrical equipment, historical disposal in dumps, accidents in transportation of equipment containing PCBs and leakage from hazardous waste sites are the possible means through which PCBs may be introduced in the environment (Yang *et al*., 2009; Oluoch- Otiego *et al*., 2016). According to Stockholm Convention, PCBs are considered as persistent organic pollutants (POPs), thus depending on their concentrations, the type of PCB congeners and extent of exposure (Zhang *et al*., 2014), the PCBs might seriously threaten the environment, animal as well as human health (Berg *et al*., 2013; Pizarro- Aranguiz *et al*., 2015; Oluoch- Otiego *et al*., 2016).

Despite the restricted production and use of PCBs many years ago, their presence in environmental media is still detectable. This is because of their high biostability, hydrophobicity and resistance to both chemical and biological degradation (Ribas-Fito *et al.*, 2001). Similarly, since it is hard to control their use in the industry and agriculture, PCBs are now known as ubiquitous pollutants in the environmental compartments worldwide (Meijer *et al.*, 2003; Xing *et al.*, 2005; Yang *et al.*, 2008; Oluoch- Otiego *et al.*, 2016). Studies have revealed further that, once they enter into the aquatic ecosystems, small quantities of PCBs may be re-dissolved at the interface between water and sediments or incorporated into sediments (Karvonen *et al.*, 2013; Huang *et al.*, 2015). Sediment- dwelling organisms may take them up to surface water (McLeod *et al.*, 2008) or the compounds may build up in aquatic organisms like fish thereby accumulating to much higher levels compared to those present in water and sediments (Sures *et al.*, 1999).

Depending on concentration of PCBs in the environment and the type of species involved, bioaccumulation of PCBs in water biota tends to increase over time (Fu and Wu, 2006). Based on this fact, fish that are close to the top of the aquatic food web have a relatively long life span and are therefore expected to concentrate high quantities of PCBs (Brazova *et al.*, 2012a). Thus, the amounts of PCBs accumulated in fish species is a good indicator of environmental pollution and may be used as a suitable tool to assess the degree of PCB pollution in environmental compartments (Fang *et al.*, 2009; Hu *et al.*, 2009; Brazova *et al.*, 2012b). The PCBs are also regarded as endocrine disruptors (EDs) (Bell, 2014; Gueke *et al.*, 2014) as they alter the normal functioning of the endocrine system of the exposed individuals as well as their offsprings. The PCBs are also difficult to degrade in the environment (Field & Sierra- Alvarez, 2008; Frouin *et al.*, 2013).

Most PCB mixtures are reported as potential human carcinogens being responsible for breast, liver and testicular cancers. They have also got negative reproductive effects such as low birth weights, small head circumferences, miscarriages, poor sperm quality and low sperm counts (FAO/WHO, 2008; Bell, 2014). PCBs are compounds which bio accumulate in animals due to their stability and are also toxic depending on the amount of chlorine they contain (WHO, 1992).

Higher levels of PCBs and related compounds in human are associated with various health effects such as lowering intelligence quotient, disorder of thyroid gland, higher rate of endometriosis in women, declining thyroid hormone levels, higher rate of diabetes in pregnant women, lowering age at menarche, and altering play behaviour in children at school age (Masuda, 2003). The World Health Organization of the United Nations reports that extended high level exposure to PCBs is associated with several adverse effects such as reproductive dysfunction, severe chloracne, hyperpigmentation, eye discharges, headaches, vomiting, fever, visual disturbances and respiratory problems. It further reports that females exposed to PCBs tend to have disorders of the reproductive organs and also an increased risk of miscarriages and stillbirth. Moreover, children born to women exposed to PCBs tend to exhibit neurobehavioural deficits and reduced growth (FAO/WHO, 2008).

The contamination pathways in the environment and the food chain are non – point sources through runoff, atmospheric deposition and leaching due to agricultural and industrial activities and vector control practices (Kasozi *et al.*, 2006). The major sources of human exposure to significant quantities of POPs are associated with residues in food, drinking water, air and skin contact. This study focused on only indicatory PCBs (CB 28, CB 52, CB 101, CB 118, CB 138, CB 153 and CB 180) because they are known to be

more persistent and bioaccumulative in the food chain and the environment compared to other congeners. They are therefore assumed to be a suitable representative for all PCBs (Afful *et al*., 2013; Polder *et al*., 2014).

1.1.2 Organochlorine pesticides

Organochlorine pesticides (OCPs) such as DDTs, HCHs, HCB, Aldrin, Dieldrin and Endosulfans are products used for plants protection, fighting against pests and preservation of foodstuffs during storage and the residues derived from their use may be found in food products (Biego *et al*., 2010). They have also been used extensively in mosquito, termites and tsetse fly control programs (Farshid *et al*., 2012). Such compounds are highly lipophilic (Farshid *et al*., 2012; Polder *et al*., 2014), persist in the environment for longer times (El- Mekkawi *et al*., 2009; Ssebugere *et al*., 2014b), readily transported over longer distances and/or tend to bio accumulate through the food chain (Guzzella *et al*., 2005; Henry and Kishimba, 2006; Biego *et al*., 2010). Furthermore, the persistent OCPs accumulate in fat rich tissues of living organisms and are commonly known to cause carcinogenic, mutagenic and teratogenic effects. They as well affect the nervous, immune, reproductive, renal and hepatic systems (Biego *et al*., 2010; Polder *et al*., 2014).

Pesticides are chemical substances with harmful effects on human beings, animals and the environment (Wilson and Tisdell, 2001; Belmonte *et al*., 2005). For a very long period of time a large number of these substances have been used in agricultural activities, control of vectors of human, animal and crop diseases and general agricultural pests control strategies (Ecobichon, 2001). The evidence shows that the use of pesticides in agriculture contributes to the massive worldwide increase of food production (Mdegela *et al*., 2013).

Improvement in crop yield which depends much on pesticide applications is sometimes associated with the occurrence and persistence of pesticide residues in the food value chain such as in the fish value chain which in turn results into public health problems and environmental concerns (Ware and Whitacre, 2004).

Organochlorine pesticides which were considered in this study are associated with many harmful effects in human such as acute and persistent injury to the nervous system, lung damage, injury to the reproductive organs, dysfunction of the immune and endocrine systems, birth defects and various types of cancers (Mansour, 2004).

1.2 Economic Importance of Lake Victoria

Lake Victoria is shared between three East African member countries; Tanzania (51%), Uganda (43%) and Kenya (6%) and it covers a surface area of approximately 68 800 km^2 (LVFO, 2005; URT, 2016). The lake is ranked second largest freshwater resource in the world after Lake Superior in North America. Its catchment area covers 180 959 km^2 of which 44% of this is contributed by Tanzania. The rest of the watershed is shared by Uganda (15.9%), Kenya (21.5%), Rwanda (11.4%) and Burundi (7.2%).

Economically, millions of people dotted around the lake use it as an important source of household and industrial water supply, irrigation water for agricultural production, facilitates boat transport and provides for fishing as a main source of animal derived protein as well as income. The most frequently caught and consumed freshwater fish species of commercial interest are the Nile perch (*L. niloticus*), Nile tilapia (*O. niloticus*) and the freshwater sardine , commonly known as *dagaa* (*R. argentea*) in Tanzania (URT, 2016) (Table 1.1).

Table 1.1: Estimated annual catches (in tons) and foreign value (in million dollars) from 2008 to 2015

	2008		2010		2011		2015	
Fish species	Catch	Value	Catch	Value	Catch	Value	Catch	Value
L. niloticus	80 977	72.3	84 969	119	70 061	131	73 051.53	133
O. niloticus	19 557	11.1	16 971	14.8	19 350	21.7	19 123.85	35
R. argentea	70 001	7.62	58 719	6.46	88 808	18.4	136 905.50	249.2
Others	2 489	1.17	2 270	1.79	3 605	3.2	8 016.61	14.6
Total	173 024	92.2	162 929	142.1	181 824	174.3	237 097.49	431.8

Source (Ssebugere, 2015; URT, 2016)

Table 1.2: Summary of Fisheries Statistics for Lake Victoria in different Regions of Tanzania in 2015

Item	Kagera	Mara	Mwanza	Geita	Total
Total number of landing sites	143	174	243	72	632
Total number of fishermen	21 721	24 220	45 616	7 983	99 540
Total number of fishing vessels	6 539	6 751	12 399	2 530	25 689
Total weight of fish in metric tons	24 194.80	63 862.36	129 694.55	18 535.50	236 287
Total Value of fish in 000's TZS	100 408 586	265 028 794	538 232 382.50	76 922 491	980 592 254

Source (URT, 2016)

Furthermore, Lake Victoria provides employment to approximately four million fishmongers, fishermen and people employed in fish processing plants in Tanzania (URT, 2016). The lake therefore has indirect and direct impacts on poverty reduction efforts put by Tanzania and her neighbouring countries.

The fisheries sector in Tanzania is very important both in terms of providing food and valuable animal proteins and other essential nutrients, income and employment opportunities. In 2013 Lake Victoria accounted for 63% of the main fish landings (URT, 2014). In 2014 Tanzania exported 43,354 tons of fish mainly in the form of Nile perch fillets destined to international markets and dried *dagaa* to regional markets valued at USD.188 million (URT, 2016). Most of the products were from Lake Victoria. According to Eurostat data, Tanzania became the main supplier of Nile perch fillets (mainly from Lake Victoria) to the European Union (EU), exporting 12, 400 tons of the product (47.5% market share), followed by Uganda with 10,800 tons (41%) and Kenya with 2,900 tons (11%). DRC is a very important market within the African region accounting for about 17% of the value of exports (LVFO, 2013; URT, 2016). In 2015 the contribution of the fisheries sector to Gross Domestic Product (GDP) was estimated at 2.5% and the fishery industry accounted for 10% by value of national exports (URT, 2016).

Nile perch fishery is processed into several other products such as fillets and partly frozen for export markets. Other products of Nile perch which are mainly for domestic and regional markets are salted- sundried (commonly known as *Kayabo*), fish trims (commonly referred to as *chips* by the local population), smoked and deep fried products.

The main export destinations for fillets and frozen fish include United States of America, Europe, Hong Kong, Japan, Israel, Singapore, Egypt, United Arab Emirates and South Africa (Ssebugere, 2015; URT, 2016).

Fish folks from all around the lake bring their already processed fish products at Kirumba Fish Market in Mwanza and sell them to buyers who then deliver the products to different markets located within and outside the country, such as Rwanda, Burundi, Kenya, Uganda, Democratic Republic of Congo, Zambia and Malawi where they are used as potential sources of protein to local communities, raw materials (fishmeal) for processing animal feeds (Odongkara *et al*., 2005; URT, 2016) as well as source of income (LVFO, 2013).

The lake's biodiversity in combination with its scenic beauty such as Saanane and Rubondo Islands is one of the important contributors to increased tourist arrivals in Tanzania and its neighbour countries Kenya and Uganda. Its banks are home to many perennial and migratory species of birds and fauna such as hippopotamus and crocodiles (Ssebugere, 2015). Moreover, the lake has great influence on climatic conditions in the basin and provides the cheapest linkage for trade between member states, water for power generation for instance along the River Nile, water for domestic use and for industrial purposes especially in most of the urban areas in Tanzania, Uganda and Kenya.

1.3 Persistent Organic Pollutants Pollution in Lake Victoria

Studies have shown that persistent organic pollutants (POPs) such as indicator PCBs and OCPs entering Lake Victoria are unlikely to be rapidly reduced by dilution or out flow. This is because the lake has only one outlet which is estimated to have a flushing time of 123 years and residence time of 23 years (Kayombo and Jorgensen, 2006).

Since the 1960s, the lake has experienced a serious decline in water quality due to pollution (Odada *et al.,* 2004).

Sources of pollution into Lake Victoria have been reported to include; untreated sewage sludge from major urban centres along the lake shore (Scheren *et al*., 2000), agrochemicals due to their increasing use in the lake basin with large-scale farms of coffee, tea, cotton, rice, maize, sugarcane, tobacco and horticultural produce, industrial polluters like sugar refineries, soft drink and food processing factories, oil and soap mills, leather tanning factories and mining companies and long range atmospheric transport of some POPs (Ssebugere *et al*., 2014a; Polder *et al*., 2014).

Increased inputs to the aquatic environment of these chemicals from anthropogenic activities can result in an increase in the concentrations in water and enhanced bio accumulation in the tissues of aquatic organisms to concentrations that are toxic to the organisms themselves or their consumers (Neff, 2002). Climatic conditions in Africa; Tanzania in particular that favours survival and multiplication of different crop pests and animal vectors inevitably lead to intense pesticide use in agriculture for animal health and increasing crop yield (Linderholm *et al*., 2010).

Pesticides runoff from agricultural land such as sugarcane plantations in Kagera, cotton farms in Mwanza and the neighbouring regions, wastes from industries and mining for instance in Geita and Mara regions, incineration of medical wastes, improper disposal of electronic wastes, inadequate sewage systems, burning of vegetation and leaking of obsolete stockpiles are the main contributors to the POPs pollution of aquatic environment in Africa and Lake Victoria (African Stockpiles, 2000; LVBC, 2008; Polder *et al.*, 2014). The fragile ecosystems in Lake Victoria are also being threatened by

nutrients loading and contaminations from human anthropogenic activities and poor sewage installations resulting into eutrophication and algal blooms (Focard *et al.*, 2008; Nonga *et al*., 2011).

1.4 Persistent Organic Pollutants Residue Studies in Lake Victoria, Africa and the Rest of the World

Tanzania is one of the tropical countries where agricultural production relies on intensive use of agrochemicals such as fertilizers and pesticides. Such tropical conditions in Lake Victoria that favour survival and multiplication of different crop pests and animal vectors inevitably leads to intense pesticide use in agriculture and animal production. Pesticides runoff from farming land, wastes from industries and mining activities, inadequate sewage systems and leaking obsolete stockpiles may contribute to the pollution of aquatic environment in Lake Victoria (LVBC, 2008).

Evidence available shows that during 5-6 months of rainy season large rivers such as Kagera River in Kagera, Mara River in Mara and Mirongo River in Mwanza supply Lake Victoria with fresh water which may transport persistent organic pollutants from possible sources to the lake which in turn accumulate in water, sediments and fish muscles. There are four groups of pesticides currently in use in the Tanzanian side of Lake Victoria; Organophosphorous, Carbamates, Pyrethroids and Organochlorine pesticides such as DDTs, HCHs, Cyclodienes and Endosulfans. While most of the pesticides are used for agricultural activities like in the cotton fields, sugarcane plantations and horticultural production, DDTs have also been used to control mosquito population in areas with frequent malaria outbreaks.

PCBs have not been produced worldwide since 1984. However, studies in Uganda and Kenya, countries that are sharing Lake Victoria with Tanzania have reported significant quantities of PCBs in water, sediments and fish (Ssebugere *et al*., 2014a; Ssebugere *et al*., 2014b; Oluoch-Otiego *et al*., 2016). The compounds are reported to be semi-volatile and this nature has enabled them to travel longer distances in the atmosphere before they are deposited in environmental compartments (Afful *et al*., 2013). It is therefore most likely that fish, sediments and water from the Tanzanian side of Lake Victoria are equally contaminated by PCBs.

In general, studies on residual levels and effects of POPs on the Tanzanian side of Lake Victoria's environment are very limited. In a most recent study to determine the levels of pesticide residues in Nile tilapia (*O. niloticus*) and Nile perch (*L. niloticus*) from Southern Lake Victoria, it was established that most of the fish samples had residual levels above the detection limits but were within the acceptable daily intake (ADI) of 0.006 mg/kg for endosulfan and 0.01 mg/kg for DDT (Henry and Kishimba, 2006).

The study was carried out in 1999 after the ban of exports of Lake Victoria fish to the European Union following the abusive fishing practices (Henry and Kishimba, 2006). This study was carried out on the Tanzanian side of Lake Victoria but was very limited in scope as it focused on only very few oragnochlorine pesticides such as DDTs and Endosulfans and it could not consider the PCBs. In another study, Polder *et al*. (2014) established the levels and patterns of persistent organic pollutants (POPs) in tilapia (*Oreochromis sp*) from four different lakes including Lake Victoria in Tanzania. The scope of this study was also limited as it considered very few samples and few persistent organochlorine compounds. In this study, it was observed that some POPs were

present in fish from different lakes considered, although they were below the MRL recommended by FAO/WHO.

Similarly, another study carried out in the Ugandan side of Lake Victoria to provide baseline information on the current levels of organochlorine pesticides in the aquatic system of Lake Victoria, established that the ratios of DDT to DDE from the samples analyzed suggested previous but not current use of the pesticide implying its high persistence and propensity for accumulation in the environment (Polder *et al*., 2014). Also a study on pesticide residue distribution in sediment and fish samples from the Ugandan side of Lake Victoria suggested a significant use of Lindane and Endosulfan within the Lake region (Waswa and Kiremire, 2004). Ssebugere *et al*. (2014a) and Ssebugere *et al*. (2014b) also researched on persistent organic pollutants levels in sediments and fish from Lake Victoria in Uganda and found measurable levels of POPs in environmental compartments. The main limitation of this study was that it considered only HCHs and PCBs.

Another study by Oluoch- Otiego *et al*. (2016) to determine PCBs in fish (*Lates niloticus, Oreochromis niloticus* and *Rastrineobola argentea*) and their parasites demonstrated moderate to high levels of PCBs in sediments and fish from Lake Victoria, Kenya. Elsewhere around the globe such as Burundi, Ivory Coast, Ghana, Poland, China, India and all over the World, several studies to assess the quality of the aquatic environment and fish in terms of POPs residues have been undertaken (Manirakiza *et al*., 2002; Darko *et al*., 2008; Biego *et al*., 2010; Witczak, 2012; Afful *et al*., 2013). Currently the information on levels, occurrence and risks of persistent organic pollutants in different matrices of Lake Victoria on Tanzanian side is limited, thus needing a study to be conducted.

1.5 The Concept and History of Human Exposure Assessment

Exposure assessment is described as an integral part of the risk assessment and management framework that involves obtaining a realistic estimate of total human exposure to a chemical of concern; mainly expressed in terms of dose per unit weight such as mg/kg (USEPA, 2009). Humans are generally exposed to very complicated mixtures of chemical substances such as the toxic globally distributed lipophilic POPs (Linderholm *et al*., 2010).

The variability of human exposures to chemicals of concern is dependent on sources of the compounds and affected by various factors including geography, social and cultural heritages (Linderholm *et al*., 2010). Human exposures to POPs in developed industrialized nations like European and Asian countries are widely documented while very little is known about the levels and trends of POPs in developing nations like Tanzania.

In order to estimate human exposure to a particular chemical substance, information on actual food consumption such as types of foods consumed, quantities and consumption frequencies is required (Muncke *et al*., 2017). This is because in risk assessment the use of actual food consumption data has been proposed (Odring *et al*., 2014; EFSA, 2016). Exposure assessment is therefore conducted to estimate the magnitude of actual and/or potential human exposure, the frequency and duration of these exposures and the pathways by which human are potentially exposed to chemicals of concern (USEPA, 1997). It is well understood that exposure assessment has been a natural part of human history and civilization (Berglund *et al*., 2001). Different kinds of exposure and various types of foods and environments were encountered by the humans who soon learnt what

could be eaten or not, and what kinds of environments should be avoided to protect the health from toxic compounds (Berglund *et al*., 2001; Mhauka, 2014).

History says that early professionals in the field of exposure assessment were the tasters at the court of the Roman Emperors. These tasters had to consume part of the food to be served to the Emperor in order to reveal if the meal was poisoned or not. If they survived after consuming a given amount of a meal, the meal was obviously perceived not poisoned and that was considered safe for the emperor to eat (Berglund *et al*., 2001). History indicates further that between 1633 and 1714, Bernardino Ramazzini was the first Italian physician to realize and report scientifically that there was an association between occupation, exposure and particular diseases. Ramazzini realized that specific exposures occurring in different occupations may cause the disease. For instance smoke particles and white glowing iron gave the blacksmith sore and inflamed eyes as well as potters became anemic and suffered from exposure to Lead salts used for glazing (Berglund *et al*., 2001; Mhauka, 2014).

It was also reported that human senses were not always sufficient to predict what could be eaten, or what environment could be met without risks of health effects. Food sometimes had proved to contain poisonous substances and in some environments, human could not survive long due to specific prevailing conditions. From harsh experiences, human was required to know that certain types of foods and environments should be avoided if possible for safety reasons (Mhauka, 2014).

1.6 Fish Consumers Eating Habits

It is stated in literature that eating habits refers to why and how people eat, which foods they eat, and with whom they eat, as well as the ways people obtain, store, use, and

discard foods (Rodriguez, 2009). It is further indicated that people eat according to learned behaviours concerning good manners, meal and snack patterns, acceptable foods, food combinations, and portion sizes of foods they eat. The components of a meal taken tend to vary across cultures, but generally include grains, such as rice; meat or a meat substitute, like fish; or beans and accompaniments, such as vegetables. Several food guides offer suggestions on foods to eat, portion sizes, and daily intake (WHO, 2003; Rodriquez, 2009).

Nevertheless, what an individual consumes is largely determined by a number of factors such as personal preferences, habits, family customs, social setting, and other factors including economic factors, availability and accessibility (Loureiro *et al*., 2001; Rodriquez, 2009). In general, the eating habits are commonly formed right from childhood through to the adolescent years (Moreno *et al*., 2007). Wood-Wright (2009) had a study to examine dietary intakes and patterns among United States families and found the similarity between children and their parents' eating habits were weak and noted that factors other than family and parental eating behaviours played an important role in affecting dietary intakes.

A study carried out by Salmon *et al*. (2005) showed that 80% of the children exposed to television food advertisements preferred more confectionery, beverages and food products which contain large amounts of fat and sugar that increase the risk of obesity. Moreover, studies have indicated that some people eat or do not eat certain types of foods based on religious, political, or social beliefs reflecting their food choices (Rodriguez, 2009; Mhauka, 2013). Studies carried out elsewhere around the globe on factors affecting fish consumption among household members include price, availability, accessibility,

health concerns, knowledge on nutritional benefits and cultures (Erdogan *et al*., 2011; Esilaba *et al*., 2017).

Fish consumers eating habit was a component of the study conducted to assess organochlorine compounds (PCBs and OCPs) exposure to people living around Lake Victoria and/or engaged in fishing and/or fish processing where organochlorine pesticides were used to control pests and/or malaria control programs and industries and other anthropogenic activities are discharging effluents containing traces of PCBs into water bodies. The aim was to establish a baseline data, scenario of OCPs and PCBs exposure and to carryout risk characterization that reveal health status of the community in target area.

1.7 Problem Statement and Justification

In recent years Lake Victoria fisheries sector has had an abusive history as in 1998, fish exports from Lake Victoria to the European Union were temporarily banned following observations of tainted fish, which were later proved to have been harvested using endosulfan (Henry and Kishimba, 2006). There are also unsubstantiated claims that in order to extent the shelf life of products in the markets some unfaithful fish sellers store their fish products using chemicals such as pesticides and other unknown repellents which might be potential sources of OCPs and PCBs residues in fish products along the food chain from fishing to consumption. Within Lake Victoria there are also doubts that some artisanal fish processors use transformer oils for fish frying and plastic smoke for smoking fish products. This indicates a danger to human health as both transformer oil and smoke are good sources of PCBs in the environment and food items (Witczak, 2012; Polder *et al*., 2014).

Some parts of the lake are still experiencing an increasing use of pesticides of various kinds in fishing and preservation of fish products, which in turn accumulate in fish, water and sediments. The consumers of such contaminated water and/or fish will finally consume these hazardous substances in their diet. This is because the evidence available indicates that exposure to chemical residues through food consumption is higher in magnitude than other exposure routes such as inhalation and dermal absorption (Juraske *et al*., 2009).

It is therefore more likely that the lake has received a considerable amount of pollution as a result of anthropogenic activities being undertaken along the lake such as agricultural, industrial and use of persistent organochlorine pesticides during preservation and storage of fish products. Previous studies to assess the quality of fish and fishery products in Lake Victoria and other parts of the World (whether microbiological or chemical quality) focused mainly on fish products that are intended for export to Europe and other developed countries (Manirakiza *et al*., 2002; Henry and Kishimba, 2006; Kasozi *et al*., 2006; Darko *et al*., 2008; Kirema – Mukasa, 2012; Polder *et al*., 2014; Ssebugere *et al*., 2014a; Oluochi- Otiego *et al*., 2016; Baniga *et al*., 2017). Some of the studies have as well been limited in scope as they have been focusing on only few persistent organochlorine compounds.

To our knowledge, there are limited studies that show the quality of water, sediments, fish and fish products from Lake Victoria in terms of POPs and their associated human health risks. The processed fish such as trims, smoked, salted- sundried and deep fried products are highly consumed in the domestic and regional markets in countries Democratic Republic of Congo, Rwanda, Burundi, Malawi, Kenya and Uganda (LVFO, 2013).

Although in some parts of the World studies have detected low levels of undesirable chemical substances including persistent organic pollutants in water, sediments and fish species (Kasozi *et al*., 2006; Henry and Kishimba, 2006; Joseph *et al*., 2011), the concentration levels are of concern to food chain as in some cases they have exceeded the maximum World Health Organization (WHO) limits (Makokha *et al*., 2008). These substances have not been exhaustively studied on the Tanzanian side of Lake Victoria and thus currently information on the levels of POPs in different matrices of Lake Victoria and the magnitude of health risks to human beings is inadequate.

In this study, despite the fact that Organophosphorous, Carbamates and Pyrethroids are the most important pesticides for use in agriculture, were not considered as a significant problem in water and sediments. This is because high dilution of the lake and low to moderate half- lives ranging from few hours to a number of few days (Laskowski, 2002) makes it possible that their residues may not be found in the aquatic environment.

There are many organochlorine compounds that exist naturally in the environment and some originate from anthropogenic activities but those which are noted for their persistence and bioaccumulative properties in the environment are polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). This study therefore considered only OCPs and PCBs due to the fact that they are able to undergo long range atmospheric transport, thus they may be found in areas where they have never been produced or used (Afful *et al*., 2013). They also have high solubility in lipids and chemically stable; characteristics which have resulted into their widespread distribution into almost all environmental media like air, water, soil, rain and the biota (Afful *et al*., 2013; Polder *et al*., 2014).

For PCBs the study considered only indicatory PCBs because the evidence available indicates that they are known to persist and bio accumulate more in the environment and food items compared to other congeners and therefore assumed to be a suitable representative for all PCBs. There is therefore, need for data on levels of persistent organic pollutants in water, sediments and fish from Lake Victoria and associated human health risks for proper management of lake water quality, sustainability of the lake ecosystem, consumer protection and expansion of local, regional and international markets for fish products from the lake.

1.8 Objectives

1.8.1 Overall objective

The overall objective of this study was to assess fish consumers' eating habits and establish prevailing quantities of persistent organic pollutants in water, sediments, fish and processed fish products from Lake Victoria and associated human health risks.

1.8.2 Specific objectives

The specific objectives of this study were to:

i. Establish levels of indicator polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in water, sediments and fish from Lake Victoria.
ii. Assess the prevalence and quantify residual levels of indicator PCBs and OCPs in existing processed fish products from Lake Victoria.
iii. Assess the fish consumers' eating habits.
iv. Evaluate the human health risks associated with consumption of persistent organic pollutants contaminated fish products.

1.9 Organization of the Thesis

This Thesis is organized in seven chapters. The first chapter comprises of an introductory part of persistent organic pollutants (POPs) and key background information on polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and economic importance of Lake Victoria. This chapter ends with a brief literature review on POPs pollution in Lake Victoria and the rest of the world, the concept of human exposure assessment and fish consumers eating habits. Chapter two to chapter six consist of five papers (two published and three accepted) addressing the four specific objectives of the study. The seventh chapter contains the conclusions and recommendations based on the study findings.

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CHAPTER TWO

Paper One: Assessment of persistent organochlorine compounds contamination on the Lake Victoria water and sediments: a case study in Tanzania

Accepted by African Journal of Aquatic Sciences

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Abstract

This study was conducted to investigate the levels of organochlorine pesticides (OCPs) and their degradation products as well as indicatory polychlorinated biphenyls (PCBs) in the water and sediments from Lake Victoria, Tanzania. Seven indicator PCBs were detected in sediments while five were detected in water samples. The PCBs loading ranged from <LOD to 10.28 µg/kg- dw and from 0.95 to 2.24 µg/L in sediments and water, respectively. The load was dominated by CB 138 and CB 153 due to their structures, high chemical stability and higher degree of chlorination. In addition, ten OCPs were detected in sediments whereas only seven OCPs were detected in water.

Aldrin and dieldrin were detected at high concentrations in sediments than other OCPs (6.13±0.90 µg/kg- dw and 6.82±0.53 µg/kg-dw), respectively; while p, p- DDT (0.82±0.12 µg/kg- dw) had the lowest concentration. For water samples, γ- HCH (1.60±0.32 µg/L) was detected at high concentration while α- endosulphan (0.15±0.01 µg/L) was the least. The levels of organochlorine compounds in sediments were higher than the corresponding levels in water samples implying that sediments act as a sink for these compounds.

The levels of organochlorine pollutants in the lake water and sediments were far below the maximum residue limit set by the European Union (EU) and Food and Agriculture Organization (FAO) except for aldrin and dieldrin. Based on the threshold effect concentration (TEC) for fresh water ecosystems, aldrin and dieldrin are the only OCPs that seem to be a threat to the lake environment.

Key words: drins, ecosystems, indicator PCBs, metabolites, OCPs, pollutants

2.0 Introduction

Organochlorines; particularly indicatory polychlorinated biphenyls (PCBs) and organochlorine pesticides such as Dichloro diphenyl trichloroethanes (DDTs), Hexachlorocyclohexanes (HCHs), Hexachlorobenzene (HCB), Aldrin, Dieldin, Endosulfans, Heptachlor and their metabolites in the environment are characterized by their high chemical stability, low water solubility and also low vapour pressure (Bowman 2004; Waswa *et al*., 2011). As a result of these properties they are commonly referred to as persistent organic pollutants (Darko *et al*., 2008; Waswa *et al*., 2011) and they have the ability to accumulate in soils (Madadi *et al*., 2005; Ssebugere, 2010; Waswa *et al*., 2011) and sediments (Henry and Kishimba, 2002; Werimo, *et al*., 2009).

Following ingestion of sediments and water; organochlorines and the residues of their metabolites may find their way into edible fish species (Ogwok *et al*., 2009; Ssebugere *et al*., 2009) and finally accumulate and concentrate in fatty tissues of human beings as a result of fish consumption. The categories of pesticides which are currently in use on the Tanzanian side of Lake Victoria based on the survey that was carried out in 2015 include organophosphorous pesticides, carbamates, pyrethroids and partly organochlorine pesticides such as DDTs, Endosulfan, Drins and HCHs. They have been mainly used for agricultural activities in the study area like in cotton fields, horticultural production and in sugarcane plantations.

Henry and Kishimba (2002) reported the use of these pesticides for agricultural production in Lake Victoria. While most of the named pesticides are used in agriculture, DDTs are also used control mosquito populations to control malaria (Polder *et al*., 2014). There is also an increased discharge of medical wastes, industrial, leakages of transformer oils, electronics and domestic wastes that might increase PCBs loading in water and

sediments. PCBs have not been produced worldwide since 1984. Studies in Uganda and Kenya, countries with which Tanzania shares Lake Victoria reported significant quantities of PCBs in water sediments and fish (Ssebugere *et al*., 2014a; Ssebugere *et al*., 2014b; Oluoch-Otiego *et al*., 2016). The compounds are reported to be semi-volatile and this nature enables them to travel long distances in the atmosphere before they are deposited in environmental compartments (Afful *et al*., 2013). It is therefore likely that sediments and water from the Tanzanian side of Lake Victoria might also be contaminated by PCBs. The status and extent of pollution of water and sediments from Tanzanian side of Lake Victoria remains unknown.

Few studies conducted focused on eutrophication, inorganic chemicals, microbiological and suspended solids as a result of enhanced effluent discharges from various sources (Wandiga *et al*., 2002; Odada *et al*., 2004; Waswa *et al*., 2011; Kihampa and Wenaty, 2013). However, very little has been done to assess the organochlorines residue inputs to the lake's current state of pollution. For example, Henry and Kishimba (2002) assessed the levels of only few organochlorine pesticides (DDTs, HCHs and endosulfans). Limited studies have been undertaken to investigate the levels of PCBs in water and sediments on Tanzanian side of Lake Victoria.

In this study, despite the fact that organophosphorous pesticides, carbamates and pyrethroids are the most important pesticides for use in agriculture, they were not considered as a significant problem in water and sediments. This is because high dilution of the lake and low to moderate half- lives ranging from few hours to a number of few days brings no doubt that organophosphorous, carbamates and pyrethroids pesticides may not be found in the aqueous environment (Laskowski, 2002).

There are many organochlorine compounds that exist naturally in the environment and some being anthropogenic but those which are noted for their persistence and bioaccumulative properties in the environment are PCBs and organochlorine pesticides. This study therefore considered only organochlorine pesticides and PCBs because it is evident that they are able to undergo long range atmospheric transport, thus they may be found in areas where they have never been produced or used (Afful *et al*., 2013). They also have high solubility in lipids and chemically stable, characteristics which have resulted into their widespread distribution into almost all environmental media like air, water, soil, rain and the biota (Afful *et al*., 2013; Polder *et al*., 2014).

In this study, focus was on indicatory PCBs because the evidence available indicated that they persist and bio accumulate in the environment and food items and therefore assumed to be a suitable representative of all PCBs. This study therefore, assessed the levels of a wide range of organochlorine compounds in water and sediments in Lake Victoria in Tanzania.

2.1 Materials and Methods

2.1.1 Description of study area

The study was conducted in four administrative regions constituting Tanzanian side of Lake Victoria (Mwanza, Mara, Geita and Kagera). In Tanzania, the lake lies between 31°0′0″E to 35°0′0″E and 1°0′0″S to 4°0′0″S (Fig. 2.1). This area has undergone rapid ecological changes resulting from industrial and agricultural practices that have led to massive deforestation and vegetation burning within the vicinity of the lake. There is also rapid rising human population that puts mounting pressure on the lake in terms of water pollution (Mbabazi *et al*., 2010).

According to National Census of 2012, the population in the Tanzanian side of Lake Victoria has risen from 6.34 million to 8.72 million people over the period of five years (URT, 2013). With the growth rate of about 5% per annum the current population in Lake Victoria zone of Tanzania is projected to be over 10 million people, all of them will engage in different anthropogenic activities and thus contribute to POPs loading in Lake Victoria.

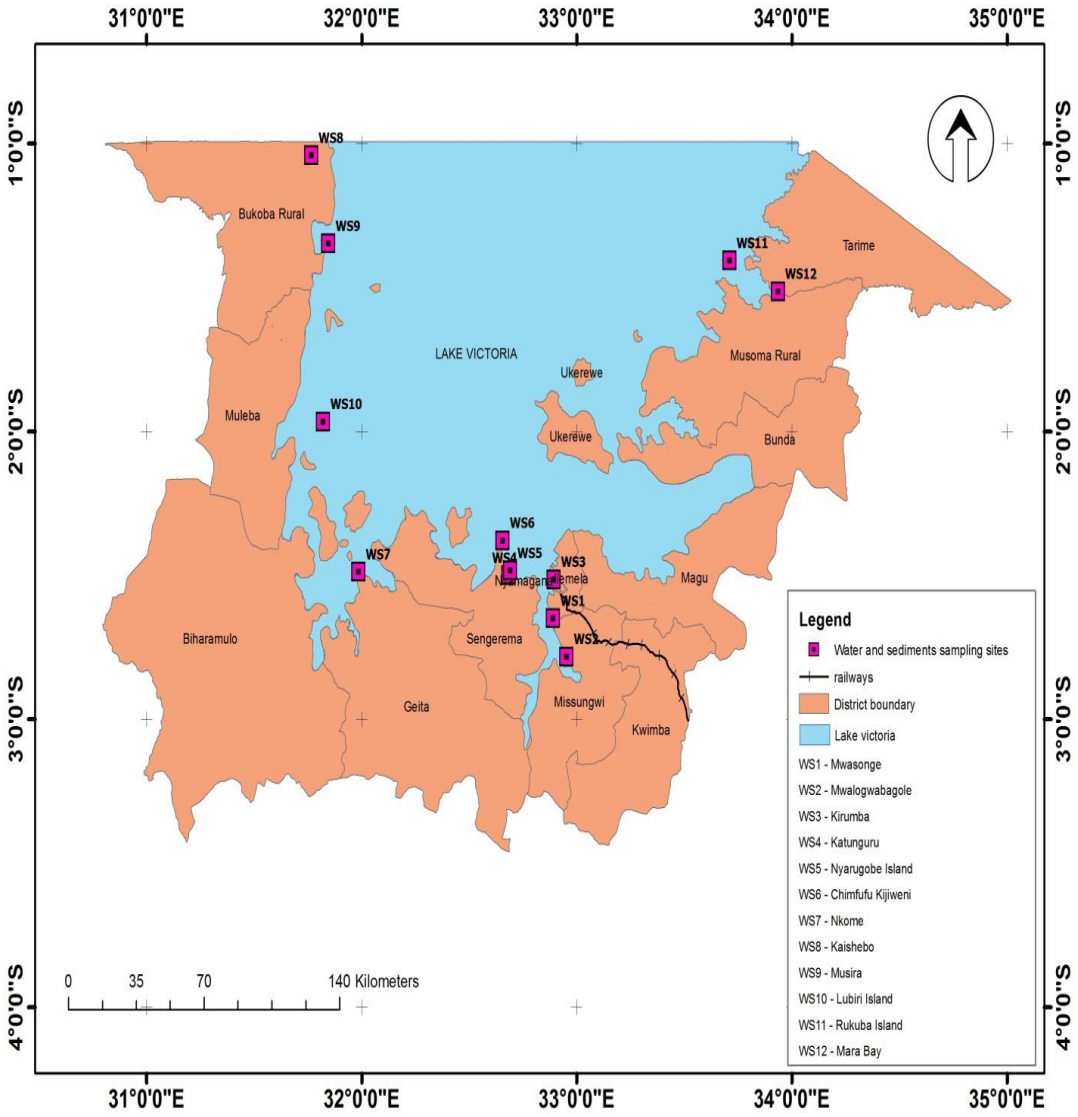

Figure 2.1: Tanzanian side of Lake Victoria and the sampling stations

2.1.2 Samples Collection

The sampling sites were selected based on large human settlements, industrial, mining and agricultural activities within their immediate vicinities that could result into pollution. The sampling stations were referenced using a geographical positioning system (GPS). Water and sediment samples were collected from twelve sampling stations on the Tanzanian side of Lake Victoria (Fig. 2.1). Sampling was conducted between April and August 2016. A total of 72 samples (36 samples of each water and sediments) were collected in triplicate using standard methods (Henry and Kishimba, 2002) and transferred in ice boxes to the National Fish Quality Control Laboratory (NFQCL) for extraction and clean up procedures.

Sediment samples were collected in triplicate at approximately 20 cm depths. This is based on the assumption that sediments at this level are expected to be the most contaminated and have the greatest potential for exchange of contaminants with the water column (Waswa *et al*., 2011). The samples were taken using a Grab sampler, wrapped in aluminium foil and placed in labelled air- tight bags. They were then kept in ice-coolers and transported to the laboratory where they were kept at -18°C before extraction and clean up.

2.1.3 Sediment and Water Samples Preparation

2.1.3.1 Extraction and clean up of water samples

Unfiltered water samples were extracted by liquid – liquid extraction (LLE) method (Henry and Kishimba, 2002). Each sample was vigorously shaken and 250 mL quantitatively transferred into a 500 mL separating funnel previously rinsed with acetonitrile. Samples to be used as control samples were spiked with analytes of interest. Then 50 mL of 10% NaCl as an extraction media and 60 mL of ethyl acetate were added

into the separating funnel and shaken vigorously for 4 min. The procedure was repeated 3 times to collect enough supernatants which were then transferred into 50 mL centrifuge tubes containing 15 g of anhydrous sodium sulphate (Na_2SO_4) and 2.5 g of sodium bicarbonate ($NaHCO_3$) and placed in a vortex mixer for 2 min.

The supernatants containing ethyl acetate were then transferred into 15 mL centrifuge tubes containing 0.125 g of primary secondary amine (PSA) and 0.75 g of anhydrous magnesium sulphate ($MgSO_4$), centrifuged at 2500 rpm for 5 minutes and left to settle for further 2 min. Supernatants were transferred into vials, evaporated to almost dryness with a steam of nitrogen and then the solvent changed to isooctane ready for GC analysis.

2.1.3.2 Extraction and clean up of sediment samples

Extraction and clean up of sediment samples was performed using a QuEChERS procedure described by Anastassides *et al*. (2003). Fifteen grams of sediment was weighed into a 50 mL centrifuge tube. Twelve mL of water was added to the sediments and centrifuged for 4 h. Twenty mL ethyl acetate was added again in the mixture and shaken vigorously for 1 min.

The supernatant was transferred quantitatively to a second centrifuge tube containing 6 g of $MgSO_4$, 1.5 g of NaCl, 1.5 g of trisodium citrate dihydrate and 750 mg of disodium hydrogen citrate sesquihydrate. This content was shaken vigorously again for 1 min. Thereafter, the content was centrifuged for 5 min at 3000 rpm and cooled down with ice water. Six mL of the supernatant was transferred into a 10 mL centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$. The content was shaken for 30 sec and then centrifuged for 5 min at 3000 rpm. The resulting extract was analyzed for POPs by a gas chromatography equipped with electron capture detectors (GC/ECDs).

2.1.4 Detection and Quantification of Organochlorines using GC/ECD

Twenty one organochlorine pesticides namely; p, p'- DDT, o, p- DDT and metabolites p, p'- DDE and p, p'- DDD, α – HCH, β – HCH, γ – HCH (lindane), HCB, Heptachlor, Heptachlor epoxide, Aldrin, Dieldrin, Endrin, Isodrin, α– endosulfan, β – endosulfan, endosulfan sulfate, Oxychlordane, γ– chlordane, α– chlordane and Transnonachlor and seven indicatory PCBs (CB 28, CB 52, CB 101, CB 118, CB 138, CB 153 and CB 180) were considered for both sediment and water samples collected from twelve sampling sites. Separation and detection of organochlorines were performed on a Hewllet Packard Gas Chromatography (Agilent 6890 Series gas chromatography system; from Agilent Technologies) equipped with an autosampler (Agilent 7683 Series; from Agilent Technologies).

For optimum separation, a 1 m long pre – column was connected to a dual capillary column system with columns of different polarity and selectivity (Chrompac CP – sil 5CB and J & W DB- 17), Nominal length 50 m & 60 m respectively, 0.25 mm ID, 0.25 µm film thickness) and coupled to two ^{63}Ni electron capture detectors (Agilent 6890 ECD). Specifications for the GC conditions were: Injector temperature: 280 $^{\circ}$C; injection volume: 2 µL; injector mode: splitless; purge flow: 42 mL/min; purge time: 0.60 min; carrier gas: Helium; constant flow: 2.0 mL/min and 1.3 mL/min respectively and make up gas: Nitrogen.

The temperature programme was 90 $^{\circ}$C held for 2.0 min; 30 $^{\circ}$C/min increased to 170 $^{\circ}$C held for 7.5 min; 2.0 $^{\circ}$C/min increased to 185 $^{\circ}$C; 3.0 $^{\circ}$C/min increased to 220 $^{\circ}$C held for 15 min; 3.0 $^{\circ}$C/min increased to 255 $^{\circ}$C held for 2 min and 5.0 $^{\circ}$C/min increased to 280 $^{\circ}$C held for 10 min. The detector temperature was 300 $^{\circ}$C.

2.1.5 Quality Control Procedures

Recovery tests were conducted by spiking sediment and water samples (200 g and 250 mL respectively) with a standard solution equivalent of 10 µg of Aldrin, α- endosulfan, p, p'- DDT, p, p'- DDE and a mixture of indicatory PCBs. The samples were extracted, cleaned up and analyzed using the same procedures like other samples and the amount recovered calculated. Recovery percentages ranged from 83 to 118% therefore needing no correction of recoveries.

The limit of detection (LOD) of each analyte was determined as concentration which peak was three times the peak of signal – to – noise ratio (S/N) whereas the limit of quantification (LOQ) was taken as that concentration which peak was ten times the peak of signal – to – noise ratio.

2.1.6 Data Analysis

Statistical analysis used for data analysis included subjecting the measured organochlorines data to descriptive statistics for the deduction of minimum, maximum, mean concentrations and standard deviations of the detected compounds. Data were further subjected to Statistical Analysis Software (SAS) Version 9.1. Data on organochlorines concentrations were presented as mean ± SD. In data processing, the concentrations of organochlorines in samples below the limit of detection (<LOD) were treated as zero. Significant difference between means was declared at $p<0.05$ for all analyses.

2.2 Results and Discussion

2.2.1 Concentrations of Organochlorines in Sediments

2.2.1.1 Concentrations of indicator PCBs

The concentrations of detected polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in sediments are presented in Table 2.1. All the seven indicatory PCB congeners (CB 28, CB 52, CB 101, CB 118, CB 138, CB 153 and CB 180) which were considered in this study were detected at varying levels from eleven out of the twelve studied sites.

The limits of detection (LODs) of PCBs in sediments (µg/kg- dw) were CB 28 (0.087), CB 52 (0.097), CB 101 (0.053), CB 118 (0.163), CB 138 (0.071), CB 153 (0.076) and CB 180 (0.035) whereas their corresponding limits of quantification (LOQs) were CB 28 (0.289), CB 52 (0.320), CB 101 (0.175), CB 118 (0.539), CB 138 (0.234), CB 153 (0.250) and CB 180 (0.115).

The composition of the detected indicator PCBs varied from one site to another due to variable sources of these compounds between the studied sites. CB 28 was detected in four of the twelve sites while CB 52 was detected in five sites. CB 101 and CB 118 were detected in seven of the twelve sites that were considered in this study. CB 138 was the most prevalent PCB congener in sediment samples being detected in ten of the twelve investigated sites. CB 153 ranked second in terms of frequencies of detection being detected in nine out of twelve sites. Trace amounts of CB 180 were also detected in six out of twelve sampling sites which were considered in the current study.

Table 2.1: Mean concentrations of organochlorines in sediments (µg/kg- dw)

Compounds	Sampling points											
	WS1	WS2	WS3	WS4	WS5	WS6	WS7	WS8	WS9	WS10	WS11	WS12
α- HCH	0.60±0.03	1.53±0.17	3.44±0.26	<LOD	<LOD	0.70±0.16	0.91±0.19	2.75±0.15	1.43±0.28	<LOD	1.39±0.13	3.63±0.31
β- HCH	0.71±0.21	1.05±0.19	0.82±0.19	<LOD	<LOD	0.73±0.22	0.54±0.07	0.65±0.29	0.50±0.12	<LOD	0.48±0.05	0.88±0.11
γ –HCH	0.37±0.07	0.77±0.05	1.18±0.13	<LOD	<LOD	0.33±0.03	0.29±0.03	1.21±0.08	0.94±0.08	<LOD	0.91±0.13	1.35±0.07
Aldrin	<LOD	0.82±0.05	4.58±0.22	<LOD	<LOD	<LOD	<LOD	2.68±0.48	<LOD	<LOD	1.73±0.08	6.13±0.90
Dieldrin	0.52±0.01	1.78±0.06	5.41±1.14	<LOD	<LOD	0.44±0.06	0.52±0.02	3.64±0.24	1.12±0.11	<LOD	1.19±0.09	6.82±0.53
p, p'-DDT	0.33±0.06	0.47±0.05	0.78±0.15	<LOD	<LOD	<LOD	<LOD	0.78±0.10	<LOD	<LOD	<LOD	0.82±0.12
p, p'-DDE	0.75±0.09	0.92±0.14	1.43±0.03	<LOD	<LOD	0.31±0.02	0.34±0.07	1.06±0.14	1.26±0.11	<LOD	1.14±0.17	1.45±0.16
p, p'-DDD	0.95±0.06	1.16±0.17	1.86±0.03	<LOD	<LOD	0.57±0.09	0.53±0.02	1.78±0.04	1.60±0.39	<LOD	1.53±0.07	1.82±0.07
α-endosulfan	1.18±0.12	1.23±0.33	1.09±0.23	<LOD	<LOD	<LOD	<LOD	1.18±0.15	<LOD	<LOD	0.93±0.24	1.54±0.32
HCB	0.53±0.02	0.69±0.08	0.78±0.13	<LOD	<LOD	<LOD	<LOD	0.91±0.22	<LOD	<LOD	<LOD	0.87±0.16
CB 28	<LOD	0.44±0.18	0.77±0.15	<LOD	<LOD	<LOD	<LOD	1.10±0.08	<LOD	<LOD	<LOD	1.38±0.15
CB 52	<LOD	0.83±0.05	0.82±0.20	<LOD	<LOD	<LOD	<LOD	0.76±0.12	0.30±0.05	<LOD	<LOD	1.16±0.37
CB 101	0.78±0.06	0.57±0.09	0.52±0.15	<LOD	<LOD	<LOD	0.31±0.06	1.22±0.06	<LOD	<LOD	0.71±0.15	0.72±0.14
CB 118	0.88±0.13	1.22±0.04	1.07±0.13	<LOD	<LOD	<LOD	0.44±0.11	1.54±0.13	<LOD	<LOD	0.89±0.21	0.54±0.08
CB 138	1.24±0.03	1.15±0.09	1.83±0.10	0.89±0.34	0.55±0.06	<LOD	0.52±0.09	1.77±0.12	0.82±0.04	<LOD	0.85±0.11	2.36±0.54
CB 153	0.86±0.03	0.85±0.22	1.76±0.23	<LOD	<LOD	0.50±0.08	0.45±0.16	1.63±0.31	0.62±0.09	<LOD	0.99±0.16	2.24±0.60
CB 180	<LOD	0.85±0.15	1.55±0.21	<LOD	<LOD	<LOD	0.40±0.07	1.89±0.52	<LOD	<LOD	0.67±0.16	1.88±0.50
∑PCBs	3.76	5.91	8.32	0.89	0.55	0.50	2.12	9.91	1.74	<LOD	4.11	10.28

WS1 = Mwasonge, WS2 = Mwalogwabagole, WS3 = Kirumba, WS4 = Katunguru, WS5 = Nyarugobe Island, WS6= Chimfufu Kijiweni, WS7 = Nkome, WS8 = Kaishebo, WS9 = Musira, WS10 = Lubiri Island, WS11 = Rukuba Island and WS12 = Mara Bay

The levels of PCB congeners established in this study were slightly higher than those which were detected in Murchison Bay in Uganda. In Murchison Bay, individual PCB congeners; CB 28 (0.063 – 0.375 µg/kg- dw); CB 52 (0.108 – 0.507 µg/kg- dw); CB 101 (0.122 – 0.585 µg/kg- dw); CB 118 (0.072 – 0.434 µg/kg- dw); CB 138 (0.154 – 0.933 µg/kg- dw); CB 153 (0.177 – 0.898 µg/kg- dw) and CB 180 (0.029 – 0.218 µg/kg- dw) were reported to be present in sediments (Ssebugere *et al*. 2014a). The concentrations of the detected indicator PCBs residues were also higher than those from Napoleon Gulf of Lake Victoria in Uganda (Ssebugere *et al*., 2014b) where CB 28 (0.072 – 0.151 µg/kg- dw); CB 52 (0.019 – 0.063 µg/kg- dw); CB 101 (0.033 – 0.097 µg/kg- dw); CB 138 (0.098 – 0.242 µg/kg- dw); CB 153 (0.051 – 0.155 µg/kg- dw) and CB 180 (0.026 – 0.154 µg/kg- dw) were reported to be present.

However, the levels of the indicator PCBs that were established in the present study were far lower than those which were established in Lake Bosomtwi in Ghana (Afful *et al*. 2013) with exception of CB 153 in which CB 28 (0.55 – 5.90 µg/kg- dw); CB 52 (4.26 – 5.79 µg/kg- dw); CB 101 (0.16 – 1.37 µg/kg- dw); CB 138 (0.95 – 3.90 µg/kg- dw); CB 153 (<LOD) and CB 180 (2.07 – 7.55 µg/kg- dw).

The total PCBs loading ranged from <LOD to 10.28 µg/kg- dw with the maximum PCBs loading at WS12 and none of the PCBs being detected at WS10. Site WS12 that indicated the highest PCBs loading (10.28 µg/kg- dw) is located at Mara Bay in Mara. It is the confluence where Mara River enters Lake Victoria. Evidence available indicates that this river is highly polluted by heavy metals from North Mara Mining, pesticides from agricultural activities that are ongoing along the river and PCBs pollution on the Kenyan side of the river.

Site WS8 ranked the second in terms of PCBs loading (9.91 µg/kg- dw). This site is located near the confluence of Kagera River and Lake Victoria where sugarcane production is significant. The PCB levels at WS8 could therefore be due to bio- mass burning of sugarcane which is done every harvesting season to facilitate the harvesting process. Studies carried by Eckhardt *et al*. (2007) and Ssebugere *et al*. (2014b) reported that bio- mass burning is one of the main sources of PCBs in environmental compartments.

The other site which indicated significantly higher PCBs loading was WS3 (8.32 µg/kg- dw) located near the mouth of Rivers Mirongo, Nyashishi and Nyakurunduma which bring in effluents from a number of chemical manufacturing industries, industrial waste treatment plants and Municipal solid incinerators which could contribute to PCBs loading. Also, in close vicinity of WS3 are the Kamanga Ferry and Kirumba International fish market where ships anchor on their way to and from other Ports such as Ukerewe, Sengerema, Kisumu in Kenya and Jinja in Uganda thereby contributing to the loading of PCBs in the lake. While shipping industry is a significant contributor to economic development in the area, it inevitably brings pollution related problems in terms of PCBs loading. Other investigated sites had low PCBs which could be attributed to their far distances from the point sources.

Similar findings for PCBs were reported in other studies elsewhere (Ssebugere *et al*., 2014a; Ssebugere *et al*., 2014b). The total PCBs loading was similarly higher than in Murchison Bay and Napoleon Gulf in the Ugandan side of Lake Victoria noted by (Ssebugere *et al*., 2014a; Ssebugere *et al*., 2014b) traduce by (0.724 – 3.95 µg/kg- dw and 0.362 – 0.848 µg/kg- dw), respectively. However; according to Afful *et al*., (2013) the

total indicatory PCBs loading was far lower than the PCBs loading that were reported in Lake Bosomtwi (4.08 – 19.17 µg/kg- dw) in Ghana.

The total PCBs loading in most of the study sites where the PCBs were detected were mainly contributed by congeners CB 138 and CB 153. The percentage contribution of CB 138 and CB 153 to total PCBs loading ranged from 34% to 100%. This trend has been observed in other studies carried out elsewhere around the globe (Ssebugere *et al*., 2014a; Afful *et al*., 2013). The domination of the two congener PCBs in sediment samples is attributed to their structures, chemical stability and high degree of chlorination.

Since PCBs have not been produced worldwide for more than three decades now, their main sources in Lake Victoria environment are attributed to their long range atmospheric transport, persistence in the environment, and release to the environment during incineration and combustion processes.

Also leakages of PCBs from dumped decommissioned transformers and capacitors in refuse dumps in the past could be another possible source of PCBs congeners that were detected in Lake Victoria environment.

2.2.1.2 Concentrations of organochlorine pesticides in sediments

Out of the 21 OCPs that were considered in the current study, only ten (α – HCH, β – HCH, γ – HCH, HCB, Aldrin, Dieldrin, α– endosulfan, p, p'- DDT, p, p'- DDE and p, p'- DDD) were detected at varying quantities from all twelve stations that were investigated. The composition of the detected OCPs varied from one site to another mainly due to variation in sources and the types of pesticides that are applied. The limits of detection (LODs) of the detected OCPs in sediments (µg/kg- dw) were as follows: α- HCH (0.231), β- HCH (0.404), γ- HCH (0.168), Aldrin (0.081), Dieldrin (0.078), p, p'- DDT (0.139), p,

p'- DDE (0.157), p, p'- DDD (0.102), α- endosulfan (0.199) and HCB (0.072) while their associated limits of quantification (LOQs) were α- HCH (0.764), β- HCH (1.335), γ- HCH (0.556), Aldrin (0.266), Dieldrin (0.259), p, p'- DDT (0.459), p, p'- DDE (0.519), p, p- DDD (0.338), α- endosulfan (0.658) and HCB (0.236).

Detectable levels of α– endosulfan were observed in six sampling sites out of twelve sites which were investigated. The lowest mean concentration of α- endosulfan was observed at WS11 while the highest level was detected at WS12 such as shown in Table 2.1. The levels of α– endosulfan from the rest of the sampling points were all below their limits of detection (<LODs). The detected residues of α– endosulfan were higher than those which were established in the Southern Lake Victoria (<LOD) (Henry and Kishimba, 2002). It has been previously reported that endosulfan is among the pesticides that are extensively used in cotton and horticultural crops in the study area (Nyanda, 2002; Henry and Kishimba, 2002).

Therefore, detection of α- endosulfan in this study suggests an ongoing use of technical endosulfan in the study area. However; the other isomer of endosulfan, β – endosulfan and the decomposition product endosulfan sulfate were not detected in any sediment sample from all sites that were considered in the present study. This observation could be because of the fact that technically endosulfan consists 70% α- endosulfan and 30% of β- endosulfan (Henry and Kishimba, 2006). In this case, higher concentrations of the former than the later as observed in this study would be expected. Non detection of β- isomer in sediment samples is due to a high rate of metabolism than α- isomer which is more stable. This trend has been observed elsewhere in environmental samples (Nowak *et al*., 1992; Nowak *et al*., 1995; Kasozi *et al*., 2006).

Hexachlorobenzene (HCB) was also detected in sediment samples from five sites (WS1, WS2, WS3, WS8 and WS12) of the twelve investigated sites. The lowest level of HCB was detected in sediments from WS1 (0.53±0.02 µg/kg- dw) and the highest concentration was observed at WS8 (0.91±0.22 µg/kg- dw) with other sampling sites having levels below their lowest limits of detection (<LODs). Evidence available regarding HCB in Tanzania indicates that there has been no historical use of the pesticide in agricultural activities and/or any other application (URT, 2005). Its detection in some of the sediment samples is therefore possibly due to its volatility and long range environmental transport.

2.2.1.3 Hexachlorocyclohexanes (HCHs) concentrations in sediments

Figure 2.2 shows the variations of HCHs in sediment samples from the twelve investigated sampling sites.

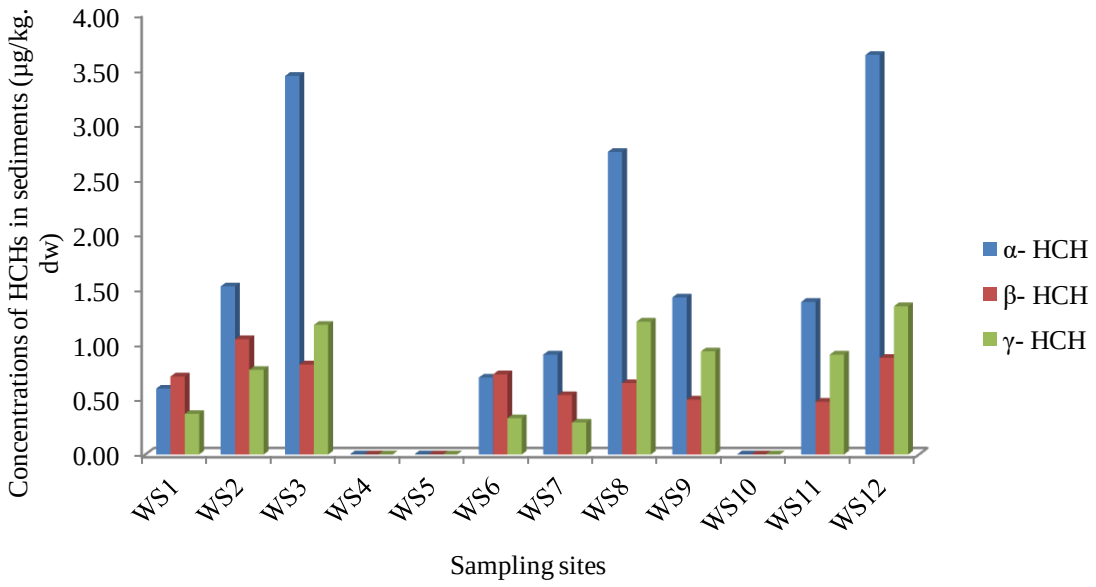

Figure 2.2: Concentrations of HCHs in sediments

Three HCHs (α – HCH, β – HCH and γ – HCH) were detected in nine out of twelve sites that were considered in this study. The other three sampling sites (WS4, WS5 and WS10) had levels of HCHs below their lowest limit of detection (<LODs). The highest mean concentration of α – HCH was 3.63±0.31 µg/kg- dw detected at WS12 while the lowest concentration was 0.60±0.03 µg/kg- dw observed at WS1. The highest level of β – HCH was 1.05±0.19 µg/kg- dw detected at WS2 while the lowest was 0.48±0.05 µg/kg- dw at WS11. The highest and lowest concentrations of γ – HCH were 1.35±0.07 µg/kg- dw and 0.29±0.03 µg/kg- dw detected at study sites WS12 and WS7, respectively.

The detected levels of HCHs are in the same range as those which were established by Afful *et al*. (2013) from sediment samples collected in Lake Bosomtwi (Ghana). In that particular study, <LOD – 0.50 µg/kg- dw for α – HCH, <LOD – 1.15 µg/kg- dw for β – HCH and 0.60 – 1.15 µg/kg- dw for γ – HCH were reported to be present in sediment samples.

The levels of HCHs in this study were far lower than those which were detected in the Southern part of Lake Victoria where 15 – 70 µg/kg- dw (α – HCH); 18 – 130 µg/kg- dw (β – HCH) and 5 – 19 µg/kg- dw (γ – HCH) were reported by Henry and Kishimba (2002) to be present in sediments. There were slightly higher concentrations of α – HCH and β – HCH isomers than the parent compound; γ – HCH (Lindane). This is attributed to the fact that, in the environment Lindane undergoes transformation into other volatile compounds such as *α* – HCH and *β* – HCH (Henry and Kishimba, 2002; Sang *et al*., 1999).

The α – HCH to γ – HCH ratio in stations where these components were simultaneously detected ranged from 1.62 to 3.14. The ratios are normally used to establish whether the

degradation of technical HCH in the environment is significant or not and also whether there is a recent or past input of technical HCH in the environment (Ssebugere *et al.*, 2014b). According to Yi *et al*. (2013) and Ssebugere *et al*. (2014b); high ratios (α – HCH/ γ – HCH) established as ranging from 4 to 7 indicates fresh inputs of technical HCH in the environment, while lower (<3) ratios (α – HCH/ γ – HCH) points that there is historical use of Lindane in the area. Low ratios (α – HCH/ γ – HCH) which were established in the current study (1.62 – 3.14) suggests past inputs of Lindane in Lake Victoria.

It is therefore evidenced by the findings that the sources of HCHs in Lake Victoria could be attributed to their historical use by farmers and long range atmospheric transport as a result of their volatile nature. However, in some parts of the lake Lindane in the form of γ – HCH was detected. This suggests that the other source of HCHs in Lake Victoria is its continued use in some parts of the catchment area.

2.2.1.4 Concentration of Drins in sediments

Of the four drins that were considered in this study (Aldrin, Dieldrin, Endrin and Isodrin), only two (Aldrin and Dieldrin) were detected at variable levels in sediments as shown in Fig. 2.3.

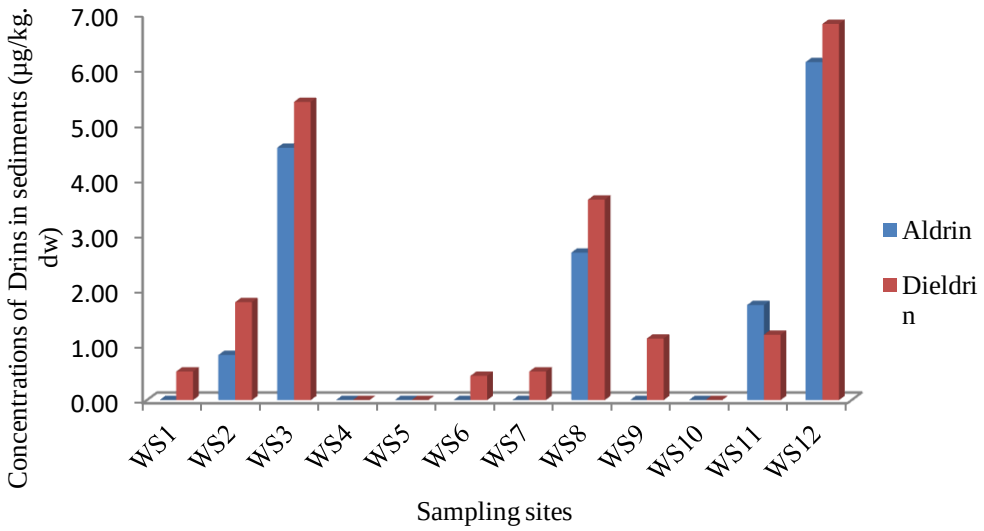

Figure 2.3: Concentration of Drins residues in sediment

Aldrin was detected in five out of the twelve sites that were investigated. The highest and lowest mean concentrations of Aldrin were 6.13±0.90 µg/kg- dw (WS12) and 0.82±0.05 µg/kg- dw (WS2), respectively. The concentrations of Aldrin in other sampling sites were below the limits of detection (<LODs). Dieldrin was detected in nine out of the twelve sites which were considered in this study. The highest mean concentration of Dieldrin was 6.82±0.53 µg/kg- dw (WS12) while the lowest was 0.44±0.06 µg/kg- dw (WS6) based on sites that indicated detectable levels.

The levels of drins that were detected in this study were far lower than the corresponding levels in sediments in the Ugandan side of Lake Victoria where the levels of Aldrin and Dieldrin ranged from 0.22 to 15.96 µg/kg- dw and 0.94 to 7.18 µg/kg- dw, respectively (Waswa *et al.*, 2011). According to Madadi *et al.* (2005), a comparison of Dieldrin/Aldrin ratio can be used to predict whether there is recent application of Aldrin or not. Values of Dieldrin/Aldrin ratios >1 implies that the detected residues are not likely from the current applications of Aldrin.

On the other hand, values <1 indicates recent applications of Aldrin. This is because Aldrin is readily converted to Dieldrin once enters either the environment or the body of an organism by the action of sunlight and bacteria (Orris *et al*., 2000; Montgomery, 2000; Afful *et al*., 2013). Dieldrin/Aldrin ratios in sites where both were detected were >1 (between 1.1 and 2.2) except WS 11 where the ratio was <1 (0.7). This observation suggests that the detected residues are not from the recent applications of Aldrin in Lake Victoria. However, based on the findings herein the drins that were detected are emanating from agricultural applications in some parts of the lake since in some places Aldrin levels were higher than Dieldrin and in some parts of the lake drins residues are attributed by their persistence and long range atmospheric transport.

2.2.1.5 Concentration of DDTs in sediments

Of the four isomeric DDTs (p, p'- DDT, o, p- DDT, p, p'- DDE and p, p'- DDD) that were considered in the present study only three were detected at varying quantities in sediment samples collected from different sites in Lake Victoria (Fig. 2.4). The parent compound p, p'- DDT was detected in five sampling sites out of twelve sites which were considered in this study. The levels of p, p'- DDT in other investigated sites were below their lowest limits of detection (<LODs). The highest and lowest concentrations of p, p'- DDT in sediments were 0.82±0.12 µg/kg- dw at WS12 and 0.33±0.06 µg/kg- dw at WS1, respectively.

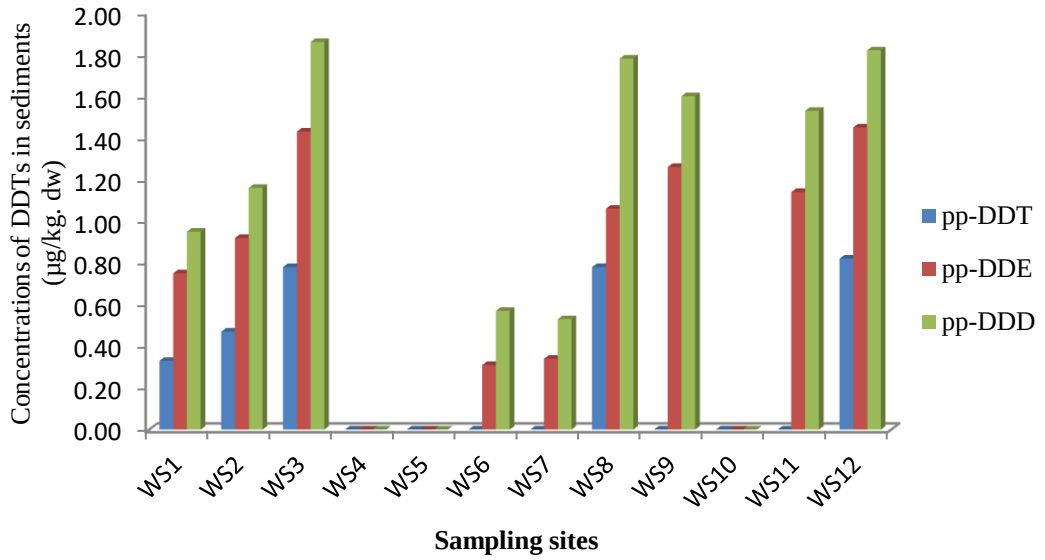

Figure 2.4: Concentration DDTs in sediment

The other DDT isomer; o, p- DDT was not detected in all sediment samples which were investigated in this study. This observation could be because of higher composition of p, p'- DDT in the technical DDT mixture that consists of 77.1% p, p'- DDT, 14.9% o, p- DDT, with p, p'- DDE and p, p'- DDD making up the balance (Waswa *et al*., 2011).

The degradation products (p, p'- DDE and p, p'- DDD) were all detected from nine sites out of twelve sites that were considered. The highest and lowest mean concentrations of p, p'- DDE and p, p'- DDD were 1.45±0.16 µg/kg- dw and 0.31±0.02 µg/kg- dw and 1.86±0.03 µg/kg- dw and 0.53±0.02 µg/kg- dw, respectively. At each of the sites where the two decomposition products (p, p'- DDE and p, p'- DDD) were detected, higher concentration of the later than the former were observed.

This observation which could also be expected, suggests that microbial decomposition of DDT in sediments is anaerobic decomposition producing p, p'- DDD rather than an aerobic breakdown to p, p'- DDE due to limited supply of oxygen, which again has been reported in other studies elsewhere around the globe (Kasozi *et al.,* 2006).

The sum of the detected decomposition products (DDE+DDD) in all sites where trace amounts were detected ranged from 0.87 to 3.29 µg/kg- dw while DDT ranged from 0.33 to 0.82 µg/kg- dw. The DDT/(DDE+DDD) ratio is mainly used to establish whether there is recent or past use of DDT in the area (Henry and Kishimba, 2006). Ratios >1 indicates that there is a recent input of DDT in the environment while established ratios <1 implies historical use of DDT (Polder *et al.,* 2014). In the current study, the DDT/(DDE+DDD) ratios were very low (between 0.19 and 0.27) suggesting a historical use of DDT in Lake Victoria.

The major sources of DDTs in Lake Victoria environmental compartments based on the findings of the study could therefore be attributed to past use of these chemicals by farmers in agriculture although there could be fresh inputs especially in controlling mosquito populations for eradicating malaria in the area since the parent compound, DDT was detected in some of the studied sites.

2.2.2 Concentrations of Organochlorines in Water

Of the seven indicator PCBs considered in the current study only five were detected and only at low levels in some sampling sites (Table 2.2) . The limits of detection (LODs) of PCBs in water samples (µg/L) were CB 28 (0.344), CB 52 (0.246), CB 101 (0.344), CB 118 (0.344), CB 138 (0.073), CB 153 (0.344) and CB 180 (0.344) whereas there corresponding limits of quantification (LOQs) were CB 28 (1.136), CB 52 (0.813), CB 101 (1.136), CB 118 (1.136), CB 138 (0.240), CB 153 (1.136) and CB 180 (1.136).

The concentrations of PCB congeners; CB 118 and CB 180 were below their limits of detection (<LODs) for all study sites. No indicator PCBs were detected at sampling sites WS1, WS4, WS5, WS7, WS9 and WS10. The highest mean concentration for the detected PCBs residues were 0.57±0.01 and 0.57±0.02 µg/L (CB 28 and CB 153, respectively) at WS12 while the lowest mean concentration was 0.34 µg/L (CB 52) at WS3. The total PCB load in water ranged from 0.95 to 2.24 µg/L. The concentrations of PCBs that were detected in the current study were far lower than those which were detected by Afful *et al*. (2013) in water from Lake Bosomtwi, Ghana in which the PCB loading ranged from 1.09 to 7.19 µg/L.

The total PCBs loading in most of the sampling sites where the compounds were detected in water was dominated by congener indicatory PCBs 138 and 153. The percentage contribution of the two congener PCBs ranged from 41% to 100% at some sampling sites. This is mainly due to structures, high chemical stability and high degree of chlorination of these compounds.

Table 2.2: Mean concentrations of organochlorines in water (µg/L)

Compounds	Sampling points											
	WS1	WS2	WS3	WS4	WS5	WS6	WS7	WS8	WS9	WS10	WS11	WS12
α- HCH	<LOD	0.28±0.07	0.33±0.0.04	<LOD	<LOD	<LOD	<LOD	0.69±0.08	<LOD	<LOD	0.27±0.04	0.71±0.03
β- HCH	<LOD	0.22±0.03	0.17±0.01	<LOD	<LOD	<LOD	<LOD	0.43±0.09	<LOD	<LOD	0.34±0.04	0.46±0.04
γ HCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1.60±0.32	<LOD	<LOD	0.34±0.08	1.16±0.12
Aldrin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dieldrin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
p, p'-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.33±0.05	<LOD	<LOD	0.30±0.02	0.64±0.08
p, p'-DDE	<LOD	0.29±0.02	0.47±0.04	<LOD	<LOD	<LOD	<LOD	0.76±0.06	<LOD	<LOD	0.54±0.11	0.84±0.04
p, p'-DDD	<LOD	<LOD	0.23±0.01	<LOD	<LOD	<LOD	<LOD	0.37±0.06	<LOD	<LOD	<LOD	0.33±0.04
α- endosulfan	0.13±0.01	0.13±0.01	0.13±0.01	<LOD	<LOD	<LOD	<LOD	0.13±0.02	<LOD	<LOD	<LOD	0.15±0.01
HCB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CB 28	<LOD	0.45±0.03	0.42±0.01	<LOD	<LOD	<LOD	<LOD	0.46±0.01	<LOD	<LOD	0.48±0.03	0.57±0.01
CB 52	<LOD	0.36±0.03	0.34±0.02	<LOD	<LOD	<LOD	<LOD	0.40±0.03	<LOD	<LOD	0.42±0.06	0.34±0.01
CB 101	<LOD	0.48±0.03	0.52±0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CB 118	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CB 138	<LOD	0.46±0.02	0.46±0.10	<LOD	<LOD	0.49±0.05	<LOD	0.52±0.08	<LOD	<LOD	0.47±0.03	0.46±0.01
CB 153	<LOD	0.45±0.06	0.50±0.02	<LOD	<LOD	0.46±0.05	<LOD	0.49±0.04	<LOD	<LOD	0.50±0.08	0.57±0.02
CB 180	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
∑PCBs	<LOD	2.20	2.24	<LOD	<LOD	0.95	<LOD	1.87	<LOD	<LOD	1.87	1.94

WS1 = Mwasonge, WS2 = Mwalogwabagole, WS3 = Kirumba, WS4 = Katunguru, WS5 = Nyarugobe Island, WS6 = Chimfufu Kijiweni, WS7 = Nkome, WS8 = Kaishebo,

WS9 = Musira, WS10 = Lubiri Island, WS11 = Rukuba Island and WS12 = Mara Bay

Of the 21 OCPs which were considered in this study, only seven OCPs (α – HCH, β – HCH, γ – HCH, p, p'- DDT, p, p'- DDE, p, p'- DDD and α– endosulfan) were detected at low levels because of their low solubility in water. The composition of the detected OCPs varied from one site to another due to variation in sources and types of pesticides used in the area. The limits of detection (LODs) of the detected organochlorine pesticides in water samples collected from different sites in Lake Victoria (µg/L) were as follows: α-HCH (0.194), β- HCH (0.256), γ- HCH (0.022), Aldrin (0.151), Dieldrin (0.227), p, p'-DDT (0.243), p, p'- DDE (0.153), p, p'- DDD (0.293), α- endosulfan (0.025) and HCB (0.116) while their limits of quantification (LOQs) were α- HCH (0.639), β- HCH (0.845), γ- HCH (0.072), Aldrin (0.498), Dieldrin (0.751), p, p'- DDT (0.803), p, p'- DDE (0.504), p, p'- DDD (0.966), α- endosulfan (0.082) and HCB (0.547).

Alfa (α) – HCH and β – HCH were detected at four study sites (WS2, WS3, WS8, WS11 and WS12) with the lowest mean concentration of 0.28±0.07 µg/L and highest concentration of 0.71±0.03 µg/L for α – HCH at WS2 and WS 12 respectively while for β – HCH the lowest concentration was 0.17±0.01 µg/L (at WS3) and the highest concentration was 0.46±0.04 µg/L (at WS 12). γ – HCH was detected at three sampling sites; WS8, WS11 and WS12 with the lowest mean concentration of 0.34±0.08µg/L at WS 11 while the highest concentration was 1.60±0.32 µg/L at WS8. Detection of γ – HCH at these sites indicates recent exposure of water to Lindane. However, the levels of γ – HCH that were detected in water in the current study are within the acceptable limits of 2 µg/L set by the World Health Organization for drinking water (WHO 1993). The levels of HCHs in all other study sites were below the limits of detection (<LODs).

The cyclodienes (Aldrin and Dieldrin) and Hexachlorobenzene (HCB) were not detected in water samples in all study sites.

Para (p), p'- DDT was detected at three study sites (WS8, WS11 and WS12). The mean concentration of p, p'- DDT ranged from 0.30±0.02 µg/L at WS11 to 0.64±0.08 µg/L at WS12. The presence of p, p'- DDT in water samples from the three named sampling sites is an indication of current exposure of water to technical DDT. The degradation products p, p'- DDE and p, p'- DDD were detected at five (WS2, WS3, WS8, WS11 and WS12) and three (WS3, WS8 and WS12) study sites respectively. The mean concentration of p, p- DDE and p, p'- DDD ranged from 0.29±0.02 µg/L to 0.84±0.04 µg/L and from 0.23±0.01 µg/L to 0.37±0.06 µg/L respectively. However, the concentrations of DDT metabolites in water for this study were within the acceptable limits of 2 µg/L as per WHO guidelines (WHO, 1993). The results emanating from this study on levels of DDT metabolites in water are similar to those which were established by Mohamed *et al*. (2014).

Alfa (α) – endosulfan residues were detected at low concentration in five sampling sites (WS1, WS2, WS3, WS8 and WS12) at a concentration ranging from 0.13±0.01 µg/L to 0.15±0.01 µg/L. The other two Endosulfan isomers which were considered in this study; β – endosulfan and Endosulfan sulfate were not detected at any of the 12 sampling sites. This is an indication of the current exposure of water to technical Endosulfan in different sampling sites in the Tanzanian side of Lake Victoria. However, the levels of OCPs which were detected in the current study are more or less in the same range or lower than other previous studies carried out around the globe such as <LOD for α – HCH, β – HCH,

aldrin, p, p'- DDE, α– endosulfan and β – endosulfan; <LOD- 0.30 µg/L for γ – HCH; <LOD-0.05 µg/L for dieldrin; <LOD-0.25 µg/L for p, p'- DDT; <LOD-6.35 µg/L for p, p'- DDD and <LOD-5.63 µg/L for endosulfan sulfate that are reported in literature (Afful *et al*., 2013).

2.2.3 Comparison of the total PCB loading and organochlorine pesticides in water and sediments from Lake Victoria

Figure 2.5 shows a comparison of total PCBs loading in water and sediment from the 12 sampling sites of Lake Victoria that were considered in this study. It appears that in all sampling sites where indicatory PCB congeners were detected the total PCBs loading in water were far lower than the corresponding levels in sediments. Similarly; the concentrations of the detected OCPs in water were lower than those which were detected in sediments at the same sampling points. This trend could be attributed to the fact that the investigated compounds have very low solubility in water and therefore they are deposited in the sediments. In this case sediments can act as a sink for these organic pollutants (Kihampa and Wenaty, 2013; Afful *et al*., 2013; Mohamed *et al*., 2014).

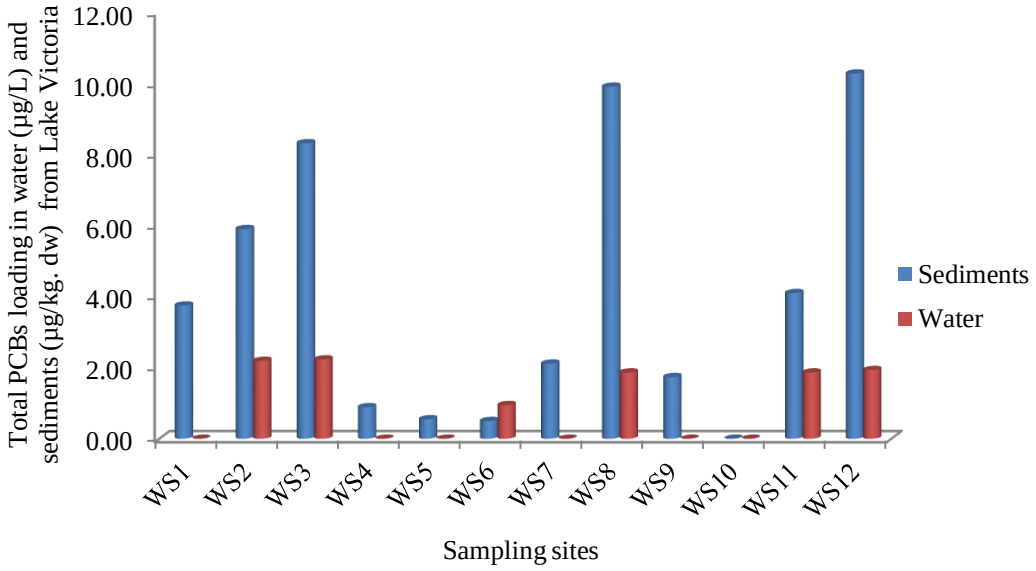

Figure 2.5: Comparison of the total PCB load in water and sediments from 12 sampling sites in Lake Victoria

In the incidences of environmental changes sediments may act as sources of persistent organic pollutants in water and other environmental compartments especially during lake's mixing.

2.2.4 Comparison of POPs concentrations established in this study to Threshold Effect Concentrations (TEC)

Table 2.3 compares the maximum concentration of POPs established in this study to the Threshold Effect Concentrations (TEC). It was found that most of the detected components in the present study had mean residue levels below TEC except Aldrin and Dieldrin whose mean residue levels were (6.13±0.90 and 6.82±0.53 µg/kg – dw) higher than the TEC (2.00 and 1.90 µg/kg – dw) respectively (McDonald *et al.*, 2000; Waswa *et al.*, 2011). With the results of the present study on the threshold effect concentration

(TEC) for fresh water ecosystems, it is evident that Aldrin and Dieldrin are the only OCPs that seem to be a threat to the lake environment as their concentrations were above TEC.

Table 2.3: Comparison of the maximum concentrations of the detected OCPs in water (µg/L) and sediments (µg/kg-dw) to the TEC

Component	Water	Sediment	TEC**
p, p'-DDT	0.64±0.08	0.82±0.12	4.20
p, p'-DDE	0.84±0.04	1.45±0.16	3.16
p, p'-DDD	0.37±0.06	1.82±0.03	3.54
Aldrin	ND	6.13±0.90***	2.00
Dieldrin	ND	6.82±0.53***	1.90
α- HCH	0.71±0.03	3.63±0.31	6.00
β- HCH	0.46±0.04	1.05±0.19	5.00
γ- HCH	1.60±0.32	1.35±0.07	2.37
α- endosulfan	0.15±0.01	1.54±0.32	NA
HCB	ND	0.91±0.22	NA

** TEC – Threshold effect concentration below which harmful effects are unlikely to be observed (McDonald *et al*., 2000)
*** - Mean residue level is above TEC; thus harmful effects are likely to be observed
ND – Not detected
NA – Not available

2.3 Conclusions and Recommendations

The results of this study indicated that some organochlorine compounds were present in Lake Victoria in Tanzania. The detection of organochlorine pesticides in water and sediments of the lake indicated either a historical use of these chemicals in the catchment of the lake or environmental transport from other places to the study area. Similarly, detection of indicatory PCBs in water and sediment samples indicated environmental persistence of these compounds. However, in most of the water samples concentrations of the compounds were below detection limits.

In general, more organochlorine pollutants were detected in the sediments than water samples. Sediments therefore, serve as a sink for organochlorine compounds and thus act as sources of these environmental pollutants in case of environmental changes. The levels of organochlorine pollutants in the lake water and sediments were far below the maximum residue limit set by European Union and Food and Agriculture Organization of the United Nations except for Aldrin and Dieldrin. Based on the threshold effect concentration (TEC) for fresh water ecosystems, Aldrin and Dieldrin were the only OCPs that seem to be a threat to the lake environment and therefore there is a necessity of a continuous long-term monitoring of the affected environment.

2.4 Acknowledgements

The authors are grateful to the Danish International Development Agency (DANIDA) through the Innovation and Markets for Lake Victoria Fisheries Project (DFC File No. 14 – P01 – TAN) for supporting the work and Mr. Malulu of Tanzania Pesticides Research Institute (TPRI) for technical assistance during samples analysis.

2.5 Conflict of interests

We, the authors would like to declare that there is no conflict of interests regarding publication of the manuscript.

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CHAPTER THREE

Paper Two: Persistent Organochlorine Compounds Levels in Selected Fish Species from Lake Victoria and Associated Human Health Risks

Published by the Journal of Agriculture, Forestry and Fisheries

CHAPTER FOUR

Paper Three: Assessment of health risks associated with organochlorine pesticides levels in processed fish products from Lake Victoria.

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Accepted by the African Journal of Food Science

Abstract

This study analyzed prevalence, levels and risks of organochlorine pesticides in processed fish products from Lake Victoria in Tanzania. Samples extractions were achieved using a QuEChERS method while detection and quantification of OCPs were done using a gas chromatography equipped with electron capture detectors (GC – ECDs). Nine organochlorine pesticides (OCPs) (α- HCH, β- HCH, γ- HCH (lindane), HCB, Dieldrin, p, p'- DDE, α- endosulfan, Oxychlordane and *α*- chlordane) were detected at measurable variable levels in the investigated fish products. Of the nine OCPs detected, the highest content was 3.73±0.31 µg/kg (p, p'- DDE) detected in deep fried *O. niloticus* while the lowest amount was 0.14 ±0.06 µg/kg (α- HCH) detected at measurable levels in smoked *O. niloticus*. In the smoked products, several of the OCPs were detected in the same sample. This is attributed to the fact that OCPs are fat soluble and therefore found in the lipid phase of the fish during smoking. However; there were unexpected high levels and prevalence of p, p'- DDE, α- endosulfan, HCB, Dieldrin and lindane in deep fried *O. niloticus* compared to other fish products which were considered in this study. This is due to environmental contamination. The decomposition product, p, p'- DDE was the most prevalent of the OCPs analyzed in this study. However, the levels of OCPs found in processed fish products as per this study were below the maximum residual limits (MRL) set by FAO and WHO for fish and other fishery products indicating that the fish products were safe for human consumption and do not pose health risks in terms of OCPs residues.

Key words: Cancer risks, Hazard index, Kayabo, Kirumba market, OCPs, Trims, Tanzania.

4.0 Introduction

Organochlorine pesticides (OCPs) such as DDTs, HCHs, HCB, Aldrin, Dieldrin and Endosulfans are products used for plant protection, fighting against pests and preservation of foodstuffs during storage and the residues derived from their use may be found in food products (Biego *et al*., 2010). They have also been used extensively in mosquito, termites and tsetse fly control programs (Farshid *et al*., 2012). In Tanzania endosulfans are still used for agricultural purposes such as in cotton fields and horticultural production while DDTs are used to control mosquito populations to fight against malaria (Henry and Kishimba, 2006; Polder *et al*., 2014; Mahugija *et al*., 2018).

These compounds are highly lipophilic (Farshid *et al*., 2012; Polder *et al*., 2014), persist in the environment for long times (El- Mekkawi *et al*., 2009; Ssebugere *et al*., 2014), readily transported over longer distances and/or tend to bioaccumulate through the food chain (Guzzella *et al*., 2005; Henry and Kishimba, 2006; Biego *et al*., 2010). Furthermore, the persistent organochlorine compounds tend to accumulate in living organisms and are commonly known to cause carcinogenic, mutagenic and teratogenic effects. The compounds affect the nervous, immune, reproductive, renal and hepatic systems as well (Biego *et al*., 2010; Polder *et al*., 2014).

Lake Victoria is one of the most important water resources in Tanzania and other East African countries. The local communities dwelling around the lake use it mainly for fishing, swimming and boat transport. Communities use the water from the lake for cooking, washing, agricultural and industrial activities. A field survey into the study area

witnessed several agricultural related activities involving the use of pesticides taking place in Lake Victoria basin.

There are also numerous rivers and streams such as Mara, Kagera, Kanoni, Butimba, Nyashishi and Mirongo rivers that are crossing the fields within the Lake Victoria basin suggesting that they may carry pesticides to the lake. Due to the solubility of the pesticides in fat and lipids, fish and other aquatic organisms concentrate and accumulate pesticide residues in their fatty tissues (Mahugija *et al*., 2018). It is therefore, likely that the lake has been polluted as a result of anthropogenic activities being undertaken along the lake such as agricultural and industrial activities (Ssebugere *et al*., 2014).

Previous studies to assess the quality of fish and fishery products in Lake Victoria and other parts of the World (whether microbiological or chemical quality) focused mainly on fish products that are intended for export to Europe and other developed countries (Manirakiza *et al*., 2002; Henry and Kishimba, 2006; Kasozi *et al*., 2006; Darko *et al*., 2008; Kirema – Mukasa, 2012; Polder *et al*., 2014; Ssebugere *et al*., 2014; Oluochi-Otiego *et al*., 2016; Baniga *et al*., 2017). Some of the studies have as well been limited in scope as they have been focusing on only few persistent organochlorine compounds.

As far as we know, there are very limited studies that show the quality of fish products such as salted- sundried Nile perch (*Kayabo*), smoked products of both Nile perch and Nile tilapia, trims and deep fried products. These processed fish products are highly consumed by communities both locally and regionally in countries including Democratic Republic of Congo (DRC), Rwanda, Burundi, Malawi, Kenya and Uganda (LVFO, 2013).

The aim of this study therefore was to provide more information on the prevalence and levels of persistent organochlorine pesticides and the associated risks to human health in processed fish products for domestic and regional markets.

4.1 Materials and Methods

4.1.1 Description of the study area

The study was conducted at the Kirumba Fish Market in Mwanza between May and August 2017. This Fish Market was purposively selected out of other markets in Lake Victoria because it is the largest fish market in the zone that collects fish products from all other regions making up the Tanzanian side of Lake Victoria. Fish folks and other processors bring their already processed fish products at the market and sell them to buyers who then deliver the products to different markets located within and outside the country, such as Uganda, DRC, Burundi, Rwanda, Zambia, Kenya and Malawi where they are used as source of protein as well as income (LVFO, 2013).

4.1.2 Fish samples collection and extraction

Six products of fish samples; salted and sundried *L. niloticus* commonly known as *Kayabo*, trims of *L. niloticus* (*Chips*), smoked *L. niloticus* and *O. niloticus* and deep fried products of both *L. niloticus* and *O. niloticus* were collected from randomly selected fish processors and sellers in the market. A total of 360 samples of fish products (that is 60 samples of each product) were collected for analysis of OCPs. Fish samples extraction and clean up for determination of OCPs was effected using a QuEChERS procedure at the National Fish Quality Control Laboratory in Mwanza, Tanzania.

Thirty grams of each sample was measured in triplicates and blended to homogenize. Fifteen grams of the homogenized samples were transferred into 100 mL centrifuge tubes. Thereafter, 2.5 g of sodium bicarbonate ($NaHCO_3$), 60 mL of ethyl acetate and 15 g of anhydrous Na_2SO_4 were added and placed in a shaking machine to homogenize for 20 min.

The supernatants were transferred into 15 mL centrifuge tubes containing 0.125 g of primary secondary amine (PSA) and 0.75 g of anhydrous $MgSO_4$ (Anastassides *et al*., 2003). The mixture was centrifuged at 2500 rpm for 10 min and left to separate for further 5 min. The supernatants were transferred into vials and transported to Denmark, Danish Technical University (DTU, Laboratory of Food Analytical Chemistry for analysis. Prior to GC analysis, the supernatants were evaporated with a gentle stream of nitrogen to almost dryness and the solvent was changed to isooctane ready for GC analysis.

4.1.3 Chemical analysis

Chemical analysis was performed at the laboratory of Food Analytical Chemistry, Technical University of Denmark (DTU), Denmark. The samples of processed fish products collected were analyzed for 19 OCPs namely; p, p'- DDT, o, p- DDT and metabolites p, p'- DDE and p, p'- DDD, α – HCH, β – HCH, γ – HCH (lindane), HCB, Heptachlor, Heptachlor epoxide, Aldrin, Dieldrin, Endrin, Isodrin, α– Endosulfan, Oxychlordane, γ– Chlordane, α– Chlordane and Transnonachlor. Most of the studied OCPs are listed in the Stockholm Convention on POPs for initial elimination and reduction in use because of their adverse effects on the environment as well as living organisms.

4.1.4 Identification and quantification of organochlorine pesticides

Separation and detection of OCPs were performed on a Hewlett Packard Gas Chromatography (Agilent 6890 Series gas chromatography system; Agilent Technologies) equipped with an autosampler (Agilent 7683 Series; Agilent Technologies). For optimum separation, a dual capillary column system with two separate columns of different polarity and selectivity were used (Chrompac CP – sil 5CB and J & W DB- 17), Nominal length 50 m & 60 m respectively, 0.25 mm ID, 0.25 µm film thickness) and coupled to two ^{63}Ni electron capture detectors (Agilent 6890 ECD).

The following GC conditions were used: Injector temperature: 280°C; injection volume: 2 µL; injector mode: splitless; purge flow: 42 mL/min; purge time: 0.60 min; carrier gas: Helium; constant flow: 2.0 mL/min and 1.3 mL/min respectively and make up gas: Nitrogen. The temperature programme was 90°C held for 2.0 min; 30°C/min increased to 170°C held for 7.5 min; 2.0°C/min increased to 185°C; 3.0°C/min increased to 220°C held for 15 min; 3.0°C/min increased to 255°C held for 2.0 min and 5.0°C/min increased to 280°C held for 10 min. The detector temperature was 300°C.

4.1.5 Analytical quality control

To maintain the quality of analytical results blanks and standards were analyzed every after analysis of five samples. The limits of detection (LODs) of the analytes were calculated as concentrations whose peaks were three times the peaks of signal to noise (S/N) ratios while their corresponding limits of quantification (LOQs) were determined as concentrations whose peaks were ten times the peaks of signal to noise (S/N) ratios.

4.1.6 Risk assessment model

The estimated dose (CDI) received through consumption of fish products was calculated using equation (i) and the cancer risk (C_R) using equation (ii), adopted from the Environmental Protection Agency (USEPA, 1997; USEPA, 2001) of the United States.

$$CDI = \frac{C * CR * EF * ED}{BW * AT} ---------------------------(i)$$

$$C_R = SF * CDI -- (ii)$$

For non-carcinogenic risks, the hazard quotients (HQs) of each organochlorine pesticide measured were calculated by using equation (iii) and the overall non-carcinogenic risk using equation (iv)

$$HQ = \frac{CDI}{RfD} ------- ------------------------- (iii)$$

$$HI = \Sigma HQs --------------------------------(iv)$$

Where;

CDI (mg/kg-day) is the estimated chronic daily intake

CR is the cancer risk via consumption of contaminated fish products

C (mg/kg) is the measured concentration of OCPs in fish products

IR (kg/day) is the consumption rate

HI (mg/kg-day) is the hazard index (overall non- cancer risk via consumption of contaminated fish products

HQ (mg/kg-day) is the hazard quotient (individual compound non- cancer risk via consumption of contaminated fish products)

EF is the exposure frequency, 365 days/year (USEPA, 2009)

ED is the exposure duration, 60 years for adults and 12 years for children (USEPA, 2009)

SF is the cancer slope factor, in this study, 2 $(mg/kg\text{-}day)^{-1}$ (Ge *et al.*, 2013) for all OCPs detected.

RfD is the Reference Dose (mg/kg-day), varied from one organochlorine pesticide to another (Ezemonye *et al*., 2015)

BW is the hypothetical average body weight, in this study, 70 kg for adults and 29 kg for children (USEPA, 2001).

AT is the averaging time, 60 years* 365 days/year = 21900 days for adults and 12 years* 365 days/year = 4380 days for children (USEPA, 2001; Ge *et al*., 2013). Qualitative descriptions of lifetime cancer risks were based on ATSDR standards as follows; very low when the estimated value is ≤10E-06, low: 10E-06<value≤10E-04, moderate: 10E-04<value≤10E-03, high: 10E-03<value≤10E-01 and very high when the estimated value is ≥10E-01 (ATSDR, 1995; Ge *et al*., 2013). For non- carcinogenic risks, hazard index (HI), calculated as sum of hazard quotients (HQs) greater than one was considered risky while HI less than one was considered no risk associated with consumption of fish products.

4.1.7 Data analysis

Statistical analysis used for the data analyses include subjecting the measured OCPs data to descriptive statistics in order to deduce the minimum, maximum, mean concentrations and standard deviations of the detected OCPs. The data were analyzed using a Statistical Package for Social Sciences (SPSS) Version 16. Data on OCP concentration were presented as mean ± SD. ANOVA was used to compare concentrations between fish

products and the means were separated using Duncan's Multiple Range Test. In data processing, the concentrations of OCPs in samples below the limit of detection (<LOD) were treated as zero. Significant difference was declared at $p<0.05$ for all analyses.

4.2 Results and Discussion

4.2.1 Concentrations of organochlorine pesticides

The concentrations of the detected organochlorine pesticides (OCPs) in processed fish products are shown in Table 4.1. Of the nineteen OCPs considered in this study, only 9 (α- HCH, β- HCH, γ- HCH (Lindane), HCB, Dieldrin, p, p'- DDE, α- endosulfan, Oxychlordane and *α*- chlordane) were detected at measurable levels. The LODs of the detected components ranged between 0.01 and 0.40 µg/kg whereas their corresponding LOQs ranged between 0.04 and 1.30 µg/kg.

Table 4.1: Mean concentration of OCPs (µg/kg) in processed fish products

	Samples					
OCPs	KK	KT	KST	KSS	NFF	NFS
α- HCH	0.54±0.02^{b}	<LOD	0.14±0.06^{a}	0.39±0.01^{b}	<LOD	<LOD
β- HCH	<LOD	<LOD	<LOD	1.43±0.18^{a}	<LOD	<LOD
Lindane	1.15±0.05^{a}	<LOD	0.44±0.08^{b}	0.99±0.05^{a}	1.00±0.04^{a}	0.52±0.02^{b}
HCB	0.66±0.10^{a}	<LOD	0.31±0.08^{b}	1.66±0.38^{a}	<LOD	2.03±0.03^{a}
Dieldrin	<LOD	<LOD	0.71±0.10^{a}	0.66±0.08^{a}	<LOD	0.78±0.08^{a}
p, p'-DDE	2.02±0.11^{bc}	1.74±0.71^{bc}	2.50±0.34^{b}	1.07±0.23^{c}	2.17±0.16^{b}	3.73±0.31^{a}
α-endosulfan	<LOD	<LOD	<LOD	0.65±0.03^{a}	<LOD	<LOD
Oxychlordane	<LOD	<LOD	<LOD	0.39±0.03^{b}	<LOD	<LOD
α- chlordane	<LOD	<LOD	0.68±0.04^{b}	0.89±0.02^{a}	<LOD	<LOD

*Means on the same row with different superscript are significantly different at $p<0.05$, <LOD- below the limit of detection (0.01- 0.40µg/kg); KK (salted and sundried Nile perch products (*Kayabo*)), KT (Nile perch trims (*chips*)), KST (smoked Nile tilapia), KSS (smoked Nile perch), NFF (deep fried Nile perch) and NFS (deep fried Nile tilapia).

Alfa (α- HCH) was detected in three fish products; salted and sundried *L. niloticus* and smoked products of *L. niloticus* and *O. niloticus*. The highest mean concentration was 0.54 µg/kg being detected in salted and sundried products while the lowest mean concentration was 0.14 µg/kg in smoked *O. niloticus*. The other three fish products; the trims, deep fried Nile perch and Nile tilapia had no detectable (<LOD) levels of α- HCH. Beta (β- HCH) was detected in only one fish product; smoked products of *L. niloticus* at the highest level of 1.43 µg/kg. The other five fish products (smoked *O. niloticus*, salted and sundried *L. niloticus*, trims, deep fried products of *L. niloticus* and *O. niloticus*) had no detectable (<LOD) concentrations of β- HCH.

Lindane (γ- HCH) was found at detectable levels in five out of six of the investigated fish products out of six. It was detected in salted and sundried products of *L. niloticus*, smoked products of both *L. niloticus* and *O. niloticus* and deep fried products of both *L. niloticus* and *O. niloticus*. The highest mean concentration was detected in salted and sundried products at 1.15 µg/kg while the lowest was 0.44 µg/kg being detected in smoked products of *O. niloticus*. The investigated fish trims were free from γ- HCH. The concentrations of Lindane established in this study for processed fish products are below the MRL of 100 µg/kg set for fishery products (Biogo *et al.*, 2010).

The ratio of α – HCH to γ – HCH in existing processed fish products where these components were detected simultaneously ranged from 0.32 to 0.47. The ratios are normally used to establish whether the degradation of technical HCH in the environment/and or food items is significant or not and also whether there is recent or past input of technical HCH in the environment/and or food items (Ssebugere *et al*., 2014). Yi *et al*. (2013) and Ssebugere *et al*. (2014) reported that high ratios (α – HCH/ γ – HCH) established as ranging from 4 to 7 are used to indicate fresh inputs of technical HCH in the environment/food items, while lower (<3) ratios (α – HCH/ γ – HCH) suggest that there is historical use of Lindane in the area. Therefore low ratios (α – HCH/ γ – HCH) established in this study (0.32 – 0.47) suggest past exposure of the fish products to Lindane.

Furthermore, the results herein showed that the quantities of HCHs obtained in processed fish products were below the extraneous maximum residue limit (EMRL) of 5000 µg/kg set by FAO/WHO Codex Alimentarius Commission for fish and other fisheries products (FAO/WHO, 1997).

Hexachlorobenzene (HCB) was observed in four out of six investigated fish products; being detected in salted and sundried *L. niloticus*, both in *L. niloticus* and *O. niloticus* smoked products and deep fried products of *O. niloticus*. There were high mean concentrations of HCB in deep fried *O. niloticus* (2.03 µg/kg) compared to other fish products considered in this study while the lowest detectable levels of HCB were detected in smoked *O. niloticus* (0.31 µg/kg). The fish trims and deep fried *L. niloticus* had no detectable (<LOD) levels of HCB.

It was noted in this study that the levels of HCB measured in fish products were comparatively far lower than the tolerable limits of 200 µg/kg recommended by FAO/WHO. HCB is used as a pesticide and is also present as a by product in the process of industrial production (Wang *et al*., 2013). Since there is no historical use of HCB in Tanzania, it is thus speculated that its presence in food items is due to long range atmospheric transport or came from by products in the process of industrial production.

Of the four cyclodienes which were considered in this study, only Dieldrin was detected at measurable quantities in three fish products; both smoked products of *L. niloticus* and *O. niloticus* and deep fried products of *O. niloticus*. The deep fried products contained higher concentrations (0.78 µg/kg) compared to other products whereas smoked *L. niloticus* had the lowest mean concentration (0.66 µg/kg) though the difference was not significant at all. The other three products (salted and sundried, trims and deep fried *L. niloticus*) had no traces of Dieldrin. Aldrin and other drin residues were not detected in all investigated fish products. This is because once it enters the environment or the body of an organism Aldrin is converted to Dieldrin by the action of sunlight and bacteria. Dieldrin is as well known to be resistant to bacterial and chemical breakdown (Orris *et al*., 2000; Afful *et al*., 2013). Other drins such as Isodrin and Endrin are also unstable under environmental conditions.

The ratios of the detected Dieldrin to Aldrin residues (Dieldrin/Aldrin) are usually used to ascertain whether the detected residues are due to current applications of Aldrin or not (Madadi *et al*., 2006). The ratios <1 are used to indicate current applications of Aldrin

while ratios >1 indicate past applications of Aldrin. Due to the fact that in the present study Aldrin was not detected in all fish products considered, the ratios were not established. However, it appears that the concentrations of Dieldrin; the only drin that was detected at measurable levels in processed fish products were lower than the maximum residue limit of 200 µg/kg recommended by the competent authorities for fish and other fishery products (Biogo *et al.,* 2010).

Of the four isomeric DDTs considered; only one p, p'- DDE was observed at measurable quantities in all six fish products at variable concentrations. The deep fried products of *O. niloticus* were found to have higher concentrations (3.73 µg/kg) as compared to all other investigated fish products while the smoked products of *L. niloticus* had the lowest concentrations. The two DDT isomers; p, p'- DDT and o, p- DDT were not detected at all; this observation indicates past use of DDT in the area. Similarly, the other decomposition product p, p'- DDD was not detected at measurable concentrations in all fish products considered in this study. According to Kasozi *et al*. (2006), this observation implies that degradation of DDT in fish is an aerobic process that produces p, p'- DDE and not p, p'- DDD due to availability of oxygen.

The ratio of DDT to (DDE+DDE) is commonly used to establish whether there is current or past application of DDT in the area in question (Liu *et al*., 2010; Kuranchie-Mensah *et al*., 2011; Bergkvist *et al*., 2012; Afful *et al*., 2013). For this study, the ratio was not established because the parent compound DDT was not detected in all fish products considered. This indicates historical use of DDT in the study area. However, the

quantities of DDTs and its metabolites as per the current study are far below the MRL of 200 µg/kg recommended for fish and other fishery products (Biogo *et al*., 2010).

For the three Endosulfans studied; α- endosulfan, β- endosulfan and the degradation product; Endosulfan sulfate, only α- endosulfan was detected in one out of six processed fish products which were considered in this study. High levels as compared to other fish products of α- endosulfan were detected in smoked products of *L. niloticus* (0.65 µg/kg) while other five fish products considered in this study had no detectable (<LOD) quantities of α- endosulfan. The presence of α- endosulfan in processed fish products indicates recent exposure of the products to the chemical through the food chain.

However, β- endosulfan was not detected; this observation is due to variation of the two components in the technical endosulfan mixture which consist of 70% α- endosulfan and 30% β- endosulfan with endosulfan sulfate being the decomposition product (Afful *et al*., 2013). Thus in processed fish products high concentrations of α- endosulfan than β- endosulfan could be anticipated.

Small concentrations of Oxychlordane were detected in only one fish product; the smoked *L. niloticus* products. The mean concentration was 0.39 µg/kg. All other fish products which were considered in this study had no detectable (<LOD) levels of Oxychlordane. The presence of Oxychlordane residues in some of the fish products is a result of their environmental persistence.

Two of the six fish products considered in the current study had trace amounts of α- chlordane (smoked products of both *L. niloticus* and *O. niloticus*). The highest mean

concentration was 0.89 µg/kg (measured in smoked *L. niloticus* products) while the lowest mean concentration was observed in smoked *O. niloticus* (0.68 µg/kg). The chemical was not detected (<LOD) in all other fish products. However, the detected Oxychlordane and α- chlordane were below the Codex Alimentarius Commission Extraneous Residual Limits (EMRL) of 0.05 mg/kg set for products of animal origin as sum of *cis*- and *trans*- isomers and Oxychlordane and expressed as chlordane (EFSA, 2007).

A study conducted to assess the effects of heat treatments such as deep frying on the levels of persistent organochlorine pesticides in fish indicated a significant reduction of the pesticides as a result of heat treatments (Witczak, 2009). It could therefore be expected that deep fried products could have lower levels of the persistent organochlorine pesticides compared to other existing processed fish products. This is because of the fact that deep frying process creates unique cooking conditions that accelerates drying of the fillets and evaporation of water and persistent organochlorine compounds such as OCPs from the fillets as a result of high temperature of the cooking oil and by transfer of organochlorine compounds to the cooking oil which itself could be acting as an extraction solvent (Witczak, 2009).

High levels of organochlorine pesticides such as HCB, Dieldrin, p, p'-DDE and Lindane were detected in deep fried products compared to other processed fish products such as trims, salted and sundried and smoked products. Occurrence of this phenomenon is due to environmental contamination that occurs along the food chain including use of pesticides for fish storage.

Another study indicated that smoking of fish tends to increase the levels of persistent organochlorine compounds. The reason for this increase in concentration was found to be due to a decrease in co- distillation with steam and the consequence of their penetration with smoke to the fish meat tissue (Witczak and Tomza- Marciniak, 2010; Witczak, 2012). The results of this study agree with this fact because most of the smoked samples had significant quantities of different persistent organochlorine pesticides.

Analysis of variance of the detected organochlorine pesticides from different existing processed fish products collected from the market are given in Table 4.2. Mean separations using Duncan's Multiple Range Test indicated significant differences ($p<0.05$) of the levels of some OCPs between fish products. For Oxychlordane, β- HCH and α- endosulfan the means were not separated as they were detected in measurable levels in only one fish product each.

Table 4.2: Analysis of Variance of the detected OCP residues in fish products

OCPs	DF	F	P
α- HCH	2	10.40	0.001**
Lindane	4	24.14	<0.001**
HCB	3	16.32	0.010**
Dieldrin	2	0.42	0.667
p, p'-DDE	5	8.87	<0.001**
α- chlordane	1	31.29	0.031**

** Means are significantly different at 0.05 level
DF- Degree of freedom, F- F Value and P- P Value

4.2.2 Comparison of Organochlorine Residue Levels to other Previous Studies in Lake Victoria and to International Standards

Table 4.3 compares mean organochlorine concentrations of the measured persistent organochlorine pesticides in the present study to maximum residue limit (MRL) recommended by some International statutory bodies for aquatic biota (Afful *et al*., 2013) and the maximum concentrations of the detected OCPs residues in fresh fish muscles in other previous studies in Lake Victoria.

Table 4.3: Comparison of mean OCPs concentrations (µg/kg) in processed fish products to other previous studies in Lake Victoria and maximum residual limits (MRL) stipulated by some statutory agencies

Compound	This work	Polder *et al*., 2014	EFSA, 2007	Henry and Kishimba, 2006	FAO/WHO, 1997
∑Chlordane	2.0	ND	50	ND	NE
∑DDT	13.2	34.7	NE	30	300
Dieldrin	2.2	ND	NE	ND	300
α- HCH	1.1	ND	NE	ND	NE
β- HCH	1.4	ND	NE	ND	300
γ- HCH	4.1	ND	NE	ND	300
α- Endosulfan	0.7	94.3	NE	200	NE
HCB	4.7	2.5	NE	ND	200

ND means Not Determined and NE means Not Established

Generally, the mean residue levels of the persistent organochlorines in the investigated existing processed fish products from Lake Victoria were far lower than the levels detected by Henry and Kishimba in the same study area (Henry and Kishimba, 2006), lower or in the same range as those detected by Polder *et al*., 2014) and far below the MRL recommended by the European Food Safety Authority and Food and Agricultural Organization (FAO) of the United Nations (Table 4.3) for fish and other fishery products.

The results herein suggest that persistent organochlorine pesticides investigated in the present study may not pose health hazards to humans.

4.2.3 Prevalence of organochlorine pesticides in processed fish products

The percentage occurrence of the detected organochlorine pesticides in processed fish products from Lake Victoria Zone and other domestic and regional markets are shown in Fig.4.1. The detected organochlorine pesticides were more prevalent in smoked fish products (*L. niloticus* and *O. niloticus*) compared to other fish products. The prevalence of the 9 OCPs detected ranged from 0% (Oxychlordane) to 100% (α- HCH and p, p'-DDE) and 10% (α- chlordane) to 70% (α- HCH and p, p'-DDE) in smoked *O. niloticus* and *L. niloticus* products, respectively.

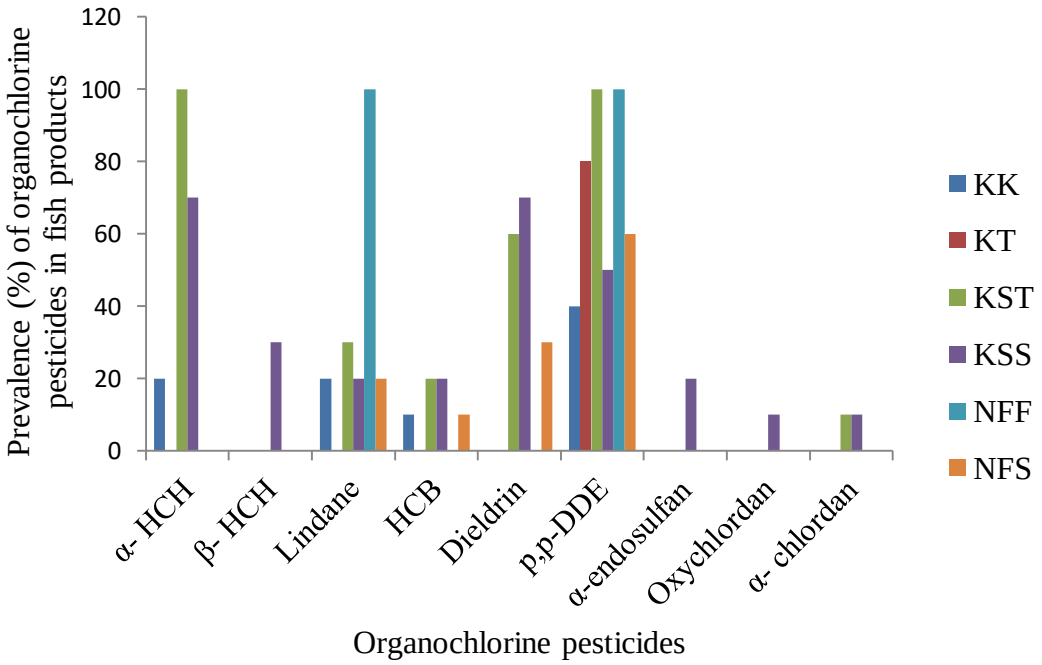

Figure 4.1: Prevalence (%) of organochlorine pesticides in processed fish products

All the 9 OCPs occurred in smoked *L. niloticus* while 6 (except Oxychlordane, β- HCH and *α*-endosulfan) occurred in smoked *O. niloticus*. High prevalence of the investigated OCPs was also observed in deep fried *O. niloticus* where 4 OCPs were detected; HCB (10%), Lindane (20%), Dieldrin (30%) and p, p'-DDE (60%) with other OCPs having being not detected. The prevalence of persistent OCPs was followed by salted and sundried *L. niloticus* products in which four OCPs were detected at measurable levels; HCB (10%), α- HCH and Lindane (20%) and p, p'-DDE (40%).

For deep fried *L. niloticus* products, only Lindane (100%) and p, p'-DDE (100%) were detected at measurable quantities. Other OCPs were not detected in these products. The prevalence of OCPs in fish trims was dominated by p, p'-DDE (80%) while other OCPs were not detected. It is noted therefore that the prevalence of p, p'-DDE in all the six fish products considered in this study was extremely high ranging from 40% (for salted and sundried *L. niloticus* products) to 100% (for smoked *O. niloticus* and deep fried *L. niloticus*). This was followed by Lindane being observed in five investigated fish products (except the trims) at a range of 20% for salted and sundried, smoked *L. niloticus* and deep fried *O. niloticus*) to 100% (for deep fried *L. niloticus*).

Oxychlordane was the least prevalent persistent organochlorine pesticide of the 9 OCPs considered being detected (10%) in only one fish product (smoked *L. niloticus*). High prevalence of OCPs in smoked and deep fried fish products is an indication that the two heat treatment techniques are good methods for preservation of fish products in the sense that almost all the water is removed. Being lipophilic in nature the OCPs are left in the fat- rich tissues of the fish mass. However, we could expect low prevalence of OCPs in deep fried products compared to other products because of the reason that at high

temperature cooking oil acts as an extracting solvent extracting most of the OCPs from the fish product. High prevalence of OCPs is probably due to reuse of oil several times thereby re- introducing the contaminants to fish products.

4.2.4 Human health risk assessment

Human health risk assessment for organochlorine pesticides in existing processed fish products considered in this study was evaluated using equations (i) to (iii) and ATSDR standard for adults and children. As indicated in Table 4.4, the cancer risks for the detected OCPs for both adults and children were between 1E-06 and 1E-04. This observation indicates that there are few cancer risks of OCPs associated with consumption of fish products from Lake Victoria. Based on ATSDR standard, the cancer risks for OCPs in this study are between very low to low. The cancer risks which were established in this study were in the same range as those which were established by Wenaty *et al*. (2019) in fresh fish muscles in which the cancer risks for adults ranged from 7.8E-6 to 1.3E-5 and from 8.6E-6 to 3.2E-5.

Table 4.5 (page 103) shows the non- carcinogenic risks of OCPs associated with consumption of fish products from Lake Victoria. The Hazard Index (HI)(sum of Hazard Quotients (HQ)) ranged between 3.7E-05 and 1.0E-01 for adults and between 4.4E-05 and 1.2E-01 for children. According to recommendations by United States Environmental Protection Agency (USEPA, 2009), the values were very low (<1), suggesting that the risks associated with consumption of the analyzed fish products from Lake Victoria are insignificant for both adults and children. The non cancer risks for both adults and children were similarly in the same range as those which were established in fresh fish

muscles from Lake Victoria. The HI of OCPs in fresh fish muscles was 4.7E-2 for adults and 5.7E-2 for children (Wenaty *et al*., 2019).

Table 4.4: Lifetime cancer risks for the 9 OCPs detected in processed fish products

Fish samples	KK		KT		KST		KSS		NFF		NFS	
OCPs	AD CR	CHI CR	AD CR	CHI CR	AD CR	CHI CR	AD CR	CHI CR	AD CR	CHI CR	AD CR	CHI CR
Oxychlordane	ND	ND	ND	ND	ND	ND	4.1E-06	1.0E-05	ND	ND	ND	ND
α-Chlordane	ND	ND	ND	ND	7.2E-06	1.7E-05	9.4E-06	2.3E-05	ND	ND	ND	ND
p, p'-DDE	2.1E-05	5.2E-05	1.8E-05	4.4E-05	2.6E-05	6.4E-05	1.1E-05	2.7E-05	2.3E-05	5.5E-05	3.9E-05	9.5E-05
Dieldrin	ND	ND	ND	ND	7.5E-06	1.8E-05	7.0E-06	1.7E-05	ND	ND	8.3E-06	2.0E-05
α- HCH	5.7E-06	1.4E-05	ND	ND	1.5E-06	3.6E-06	4.1E-06	1.0E-05	ND	ND	ND	ND
β- HCH	ND	ND	ND	ND	ND	ND	1.5E-05	3.7E-05	ND	ND	ND	ND
γ- HCH	1.2E-05	3.0E-05	ND	ND	4.7E-06	1.1E-05	1.1E-05	2.5E-05	1.1E-05	2.6E-05	5.5E-06	1.3E-05
α- Endosulfan	ND	ND	ND	ND	ND	ND	6.9E-06	1.7E-05	ND	ND	ND	ND
HCB	7.0E-06	1.7E-05	ND	ND	3.3E-06	7.9E-06	1.8E-05	4.2E-05	ND	ND	2.2E-05	5.2E-05

AD stands for adults, CHI for children, CR for lifetime cancer risk and ND for Not determined as their concentrations in fish products were <LOD

Table 4.5: Non- carcinogenic risks of the 9 OCPs measured in fish products from Lake Victoria

Fish samples	KK	KT	KST	KSS	NFF	NFS

OCPs	AD HQ	CHI HQ	AD HQ	CHI HQ	AD HQ	CHI HQ	AD HQ	CHI HQ	AD HQ	CHI HQ	AD HQ	CHI HQ
Oxychlordane	ND	ND	ND	ND	ND	ND	2.8E-05	3.3E-05	ND	ND	ND	ND
α-Chlordane	ND	ND	ND	ND	4.8E-05	5.8E-05	6.3E-05	7.6E-05	ND	ND	ND	ND
p, p'-DDE	4.3E-05	5.2E-05	3.7E-05	4.4E-05	5.3E-05	6.4E-05	2.3E-05	2.7E-05	4.6E-05	5.5E-05	7.9E-05	9.5E-05
Dieldrin	ND	ND	ND	ND	1.5E-03	1.8E-03	1.4E-03	1.7E-03	ND	ND	1.7E-03	2.0E-03
α- HCH	1.9E-02	2.3E-02	ND	ND	4.9E-03	6.0E-03	1.4E-02	1.7E-02	ND	ND	ND	ND
β- HCH	ND	ND	ND	ND	ND	ND	5.0E-02	6.1E-02	ND	ND	ND	ND
γ- HCH	4.1E-02	4.9E-02	ND	ND	1.6E-02	1.9E-02	3.5E-02	4.2E-02	3.5E-02	4.3E-02	1.8E-02	2.2E-02
α- Endosulfan	ND	ND	ND	ND	ND	ND	1.1E-05	1.3E-05	ND	ND	ND	ND
HCB	8.7E-05	1.1E-04	ND	ND	4.1E-05	4.9E-05	2.2E-04	2.7E-04	ND	ND	2.7E-04	3.2E-04
HI = ∑HQs	6.0E-02	7.2E-02	3.7E-05	4.4E-05	2.2E-02	2.7E-02	1.0E-01	1.2E-01	3.5E-02	4.3E-02	2.0E-02	2.5E-02

AD stands for adults, CHI for children, HQ for hazard quotient, HI for hazard index and ND for not determined as their concentrations in fish products were <LOD

On the other hand, the Chronic Daily Intake (CDI) and Hazard Quotients (HQs) of OCPs in this study were far lower than those which were established by Ezemonye *et al*. (2015) in *Tilapia zilli* and *Clarias gariepinus* in Edo State, Nigeria. It was reported that the HQs for Heptachlor (1.09) and Aldrin (1.41) were one (1) suggesting that the potential health risks associated with intake of Heptachlor and Aldrin, through the consumption of Tilapia zilli was high. Similarly, it was observed in the same study that the chronic daily intake (CDI) for Heptachlor epoxide, Endrin, Aldrin and Dieldrin in Clarias gariepinus were higher than their recommended reference dose (RfD) while the estimated HQs were greater than 1 suggesting potential health risks through the consumption of Clarias gariepinus (Ezemonye *et al*., 2015)

4.3 Conclusions and Recommendations

The levels of the detected organochlorine pesticides were lower than the levels previously reported in the study area and the maximum recommended limits for fish and fishery products. OCPs were more prevalent in some processed fish products such as smoked and deep fried products suggesting that further contamination takes place along the fish value chain. The cancer and non cancer risks were very low implying that the investigated fish products are therefore safe for human consumptions and that the levels of organochlorine pesticides residues in processed fish products from Lake Victoria is not a health risk. Further studies should be conducted to investigate the influence of fish processing on the levels of OCPs.

4.4 Acknowledgements

The authors acknowledge the Danish International Development Agency (DANIDA) funded project (DFC File No. 14 – P01 – TAN) “Innovations and Markets for Lake Victoria Fisheries (IMLAF)” for financial support to undertake this study.

We are also grateful to the National Fish Quality Control Laboratory (NFQCL) staff in Mwanza for technical guidance during samples collection and extraction.

4.5 Conflict of Interest

There is no conflict of interest.

4.6 References

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CHAPTER FIVE

Paper Four: Occurrence, quantities and probable human health risks of indicatory polychlorinated biphenyls in processed *Lates niloticus (L.)* products from Lake Victoria in Tanzania

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Accepted by the African Journal of Environmental Science and Technology

Abstract

A study was conducted in Lake Victoria to assess the occurrence, levels and risks of indicator PCBs in four existing processed *L. niloticus* fish products (salted and sundried, trims, smoked and deep fried). Samples extractions were done using QuEChERS method while detection and quantification of congeners was done using a GC- ECD/MS. Percentage recoveries ranged between 71.42±0.21 to 92.48±0.31% based on triplicate determinations of spiked samples. Six PCBs (CB 28, CB 52, CB 118, CB 138, CB 153 and CB 180) were detected at measurable quantities in different fish products whereas CB 101 was not detected (ND) in any of the four fish products.

The total PCBs loading were 28.46±9.35 µg/kg (salted and sundried products), 31.55±16.66 µg/kg (trims), 30.67±6.23 µg/kg (smoked products) and 16.05±3.04 µg/kg for deep fried products. PCBs loading were dominated by CB 138, CB 153 followed by CB 180 due to structures and high degree of chlorination. However, the mean concentration of ΣPCBs in this study were below MRL of 75 µg/kg set for fish by European Commission, implying that the fish products were safe for human consumption in regard to indicator PCBs. Similarly, indicator PCBs, CB 138, CB 153 and CB 180 were more prevalent (20% to 80%) in all fish products than other congeners. This is attributed to their structures, high chemical stability and high degree of chlorination compared to other congeners. For both adults and children the cancer risks were low to moderate (between 1.7E-04 and 3.3E-04 for adults and 1.6E-04 and 7.8E-04 for children) while the non- cancer risks were insignificant as the HI were less than one.

Key Words: PCBs, Salted and sundried fish, Trims, Smoked fish, Deep fried fish, Kirumba Market.

5.0 Introduction

Polychlorinated biphenyls (PCBs) are synthetic organic compounds characterized by their lipophilicity (Cok *et al*., 2007; Bordajandi *et al*., 2008; Bjermo *et al*., 2013), persistence in the environment (due to longer half-lives), toxicity, Long range atmospheric transport (LRAT) (Liu *et al*., 2007), accumulation in biota and increase in concentration with time at higher trophic levels (Polder *et al*., 2014; Ssebugere *et al*., 2014; Oluoch- Otiego *et al*., 2016). The compounds are also regarded as endocrine disruptors (EDs) (Bell, 2014) as they alter the normal functioning of the endocrine system and are difficult to degrade in the environment (Field and Sierra- Alvarez, 2008; Frouin *et al*., 2013). Most are reported as potential carcinogens being responsible of breast, liver and testicular cancers. They have also got negative reproductive effects such as low birth weights, small head circumferences, miscarriages, poor sperm quality and low sperm counts (Bell, 2014).

In recent years Lake Victoria fisheries has had an abusive history as in 1998, fish exports from Lake Victoria to the European Union were temporarily banned following observations of tainted fish, which were latter proved to have been harvested using endosulfan (Henry and Kishimba, 2006). There are also unjustified claims that in order to extend the shelf life of products in the markets some unfaithful fish sellers store their fish products using chemicals such as pesticides and other unknown repellents which might be potential sources of PCBs in fish products along the food chain from fishing to consumption. It is also suspected that some processors use transformer oils for fish frying and plastic smoke for smoking fish products endangering consumers health as both transformer oil and smoke are good sources of PCBs in the environment and environmental compartments Witczak, 2012; Polder *et al*., 2014).

A study carried out in Poland revealed that smoking fish increases the levels of PCBs specifically; CB 101, CB 118, CB 138, CB 153 and CB 180. The reason for this was found to be due to a decrease in co- distillation with steam and the consequence of their penetration with smoke to the fish meat tissue (Witczak and Ciereszko, 2006; Witczak and Ciereszko, 2012). There is therefore a need to investigate the impacts of smoking on fish products from Lake Victoria.

Another study which was done on fried fish products revealed that deep frying reduces the levels of PCBs in fish due to the fact that; (i) deep frying process creates unique cooking conditions that accelerates drying of the fillets (ii) evaporation of water and PCBs from the fillets as a result of high temperature of the cooking oil and by transfer of PCBs to the cooking oil which itself could be acting as an extraction solvent (Witczak and Ciereszko, 2012), though the same compounds are likely to be reintroduced into fish muscles as the cooking oil is reused in subsequent fish processing.

Such studies have not been undertaken in Africa and in Lake Victoria in particular thus it is necessary that a study is conducted to reveal the prevalence of PCBs and establish how safe the fish products are. Being lipophilic in nature; high levels of PCBs could be expected in fish products such as fish trims which are mainly the fatty tissues of the fish mass. We could also expect high prevalence and levels of PCBs in smoked products due to the reason that the process takes place in closed system and therefore there is no room for escape of PCBs together with water vapour. PCBs are likely to stick in the walls of smoking chambers and go back to surfaces of the fish tissues being processed.

This study therefore was designed to assess the prevalence, levels and risks of indicator PCBs in four existing processed *L. niloticus* fish products sold namely salted and

sundried, fish trims, smoked fish and deep fried. The processed fish products are mainly consumed by the communities in the domestic and regional markets and that to our knowledge we are not aware of studies regarding the prevalence, levels and risks of PCBs in such products (salted- sundried and trims) that have been reported around the globe and only limited studies on smoked and deep fried fish have been reported in developed nations.

Studies of such kind have mainly been undertaken for fish products intended for export markets such as fish fillets. Similar studies need to be done for fish products that are processed for domestic and regional markets as they are consumed by majority of the low income population; moreover, the safety of such products in terms of chemical hazards particularly PCBs is still unknown. This study focused on only indicatory PCBs because they are known to be more persistent and bio accumulative in food chain compared to other congeners. They are therefore assumed to be a suitable representative for all PCBs.

5.1 Materials and Methods

5.1.1 Description of the study area

The study was conducted at the Kirumba fish market in Mwanza between April and August 2018. The market was purposively selected out of other markets in Lake Victoria because it is the largest fish market in the zone that collects fish products from all other regions making up the Tanzanian side of Lake Victoria. Fish folks and other processors bring their already processed fish products at the market and sell them to buyers who then deliver the products to different markets located within and outside the country, such as Uganda, DRC, Burundi, Rwanda, Zambia, Kenya and Malawi where they are used as source of protein as well as income (LVFO, 2013).

5.1.2 Fish Samples Collection and Extraction

Four existing processed products of *L. niloticus* samples; salted and sundried commonly referred to as *Kayabo*, trims commonly known as *Chips*, smoked and deep fried products were collected from randomly selected fish processors and sellers at Kirumba fish market between April and August 2018. A total of 120 samples (30 samples of each product) were collected for analysis. Fish samples extraction and clean up for determination of indicator PCBs was effected using a QuEChERS procedure (Anastassiades *et al*., 2003) at the National Fish Quality Control Laboratory in Mwanza, Tanzania. Thirty grams of each sample was measured in triplicates and blended to homogenize. Thirty grams of the composite samples were transferred into 200 mL centrifuge tubes. Thereafter, 2.5 g of sodium bicarbonate ($NaHCO_3$), 60 mL of ethyl acetate and 15 g of anhydrous Na_2SO_4 were added and placed in a shaking machine to homogenize for 20 min.

The supernatants were transferred into 15 mL centrifuge tubes containing 0.125 g of primary secondary amine (PSA) and 0.75 g of anhydrous $MgSO_4$ (Anastassiades *et al*., 2003). The mixture was centrifuged at 2500 rpm for 10 min and left to separate for further 5 min. The supernatants were transferred into vials ready for GC analysis.

5.1.3 Recoveries and analytical quality control

Recovery tests were done for six indicator PCBs of interest. Blank samples were spiked with standards and were subsequently extracted and analyzed in the same way as other samples. To maintain the quality of analytical results blanks and standards were run every after 5 samples.

5.1.4 Chemical analysis

Chemical analysis was performed at the National Fish Quality Control Laboratory in Mwanza. The samples of fish collected were analyzed for Polychlorinated biphenyls (Σ-7PCBs); with IUPAC numbers: CB- 28, 52, 101, 118, 138, 153 and 180.

5.1.5 Gas chromatographic analysis of samples

A gas chromatograph (GC-2010, Shimadzu) equipped with ^{63}Ni Electron Capture Detector (ECD) and a non-polar (HP-5MS) capillary column of 30 m length × 0.25 mm i.d. × 0.25 μm film thickness was used. Nitrogen was used as both a carrier and make-up gas at a flow rate of 23.7 mL/min. The temperature programme was: initial temperature of 120 °C held for 2 min, then increased at a rate of 10 °C/min to 270 °C held for 1 min, and at a rate of 2 °C/min to the final temperature of 290 °C held for 3 min. The injector and detector temperatures were 220 °C and 290 °C, respectively. The GC was operated in a splitless mode with an injection volume of 1 μL. The standard mixture was injected in the beginning and after every five samples. Samples were injected in duplicate. The confirmation of the findings was done using gas chromatography- mass spectrometry (Shimadzu GC-MS QP 2010 Ultra equipped with a mass selective detector-MSD, fused silica capillary column Rtx-5MS of 30 m length×0.25 mm ID × 0.25 μm film and an autosampler) applying the procedures described by Mahugija *et al*. (2018).

The GC-MS was performed in splitless injection mode and the mass spectrometer was operated in electron impact (EI) ionization and full scan mode. The calibration/working standard solutions were prepared by dissolving portions of the stock solutions in the same solvents as used for the samples. Calibration curves were prepared by running series of mixtures of standard solutions and plotting the peak areas against concentrations.

Identification of the compounds involved checking the matching of the retention times and the mass spectra of the PCBs in samples to those of external reference standards that were prepared and run at the same conditions as for the samples. Quantification was carried out by linear integration of the standards and sample data based on peak areas.

5.1.6 Risk assessment model

The estimated dose (CDI) received through consumption of fish products was calculated using equation (i) and the cancer risk (C_R) using equation (ii), which were adopted from the Environmental Protection Agency of the United States (USEPA, 1997; USEPA, 2001; Man *et al*., 2013).

$$CDI = \frac{C * CR * EF * ED}{BW * AT} \quad (i)$$

$$C_R = SF * CDI \quad (ii)$$

For non-carcinogenic risks, the hazard quotients (HQ) of each congener PCBs measured were calculated by using equation (iii) and overall non-cancer risk using equation (iv)

$$HQ = \frac{CDI}{RfD} \quad (iii)$$

$$HI = \Sigma HQs \quad (iv)$$

Where;

CDI (mg/kg-day) is the estimated chronic daily intake

CR is the cancer risk via consumption of fish products contaminated with PCBs

C (mg/kg) is the measured concentration of indicator PCBs in fish products

IR (kg/day) is the fish consumption rate

HI (mg/kg-day) is the hazard index (overall non- cancer risk via consumption of contaminated fish products

HQ (mg/kg-day) is the hazard quotient (individual compound non- cancer risk via consumption of contaminated fish products)

EF is the exposure frequency, 365 days/year (USEPA, 2009).

ED is the exposure duration, 60 years for adults and 12 years for children USEPA, 2009)

SF is the cancer slope factor, in this study, 2 $(mg/kg\text{-}day)^{-1}$ (Ge *et al*., 2013) for all indicator PCBs detected.

RfD is the Reference Dose (mg/kg-day), in this study, 0.02 mg/kg-day for all PCBs (Wael and Hamada, 2017).

BW is the hypothetical average body weight, in this study, 70 kg for adults and 29 kg for children (USEPA, 2001).

AT is the averaging time, 60 years* 365 days/year = 21900 days for adults and 12 years* 365 days/year = 4380 days for children (Ge *et al*., 2013; USEPA, 2001).

Qualitative descriptions of lifetime cancer risks of PCBs were based on ATSDR standards as follows; very low when the estimated value is ≤10E-06, low: 10E-06<value≤10E-04, moderate: 10E-04<value≤10E-03, high: 10E-03<value≤10E-01 and very high when the estimated value is ≥10E-01 (Man *et al*., 2013; Ge *et al*., 2013; ATSDR, 1995). For non-carcinogenic risks, hazard index (HI) greater than one was considered risky while HI less than one was considered no risk associated with consumption of fish products.

5.1.7 Data Analysis

Statistical analysis used for data analysis includes subjecting the measured PCBs data to descriptive statistics for the deduction of minimum, maximum, mean concentrations and standard deviations of the detected PCBs. Data were analyzed by using SPSS, Version 16.0. Data on PCB concentration were presented as mean ± SD. One – way ANOVA was used to compare concentrations between products. In data processing, the concentrations

of PCBs in samples below the limit of detection (<LOD) were treated as zero. Separation of means was done using Duncan's Multiple Range Test. Significance was declared different at $p<0.05$ for all analyses.

5.2Results and Discussion

5.2.1 Recovery experiment for PCBs extraction procedure

The results for the recovery experiment are shown in Table 5.1. The mean percentage (%) recoveries for PCBs extraction procedure ranged from 71.42±0.21 to 92.48±0.31% based on triplicate determinations. Studies have indicated that recoveries ranging between 70 and 120%, the extraction procedure is considered perfect (Afful *et al*., 2013a; Afful *et al*., 2013b). Results herein suggest a perfect extraction method that is recommended for use in further PCBs studies, hence needing no corrections for the recoveries.

Table 5.1: Results of the percentage recoveries for PCBs extraction procedure

	Amount spiked	Amount calculated	Recoveries
PCBs	(µg/kg)	(µg/kg)	(%)
CB 28	75	69.36±0.23	92.48±0.31
CB 52	50	40.90±0.46	81.79±0.93
CB 118	200	166.87±0.46	83.44±0.23
CB 138	260	202.80±0.35	78.00±0.13
CB 153	280	209.43±0.48	74.80±0.17
CB 180	390	278.54±0.82	71.42±0.21

5.2.2 Concentrations of PCBs in *L. niloticus* products from Lake Victoria

The concentrations (µg/kg) of individual indicator PCBs and the sum (∑PCBs) measured in processed *L. niloticus* products from Lake Victoria are shown in (Table 5.2).

Table 5.2: Mean concentrations (µg/kg) of individual PCBs and ∑PCBs in processed fish products from Lake Victoria in Tanzania

	Samples			
PCBs	KK	KT	KSS	NFF
CB 28	6.08±1.95	4.92±1.38	5.20±1.58	1.75±0.35
CB 52	3.04±3.00	3.72±0.87	3.62±0.77	1.25±0.21
CB 101	ND	ND	ND	ND
CB 118	4.24±3.04	1.88±0.76	5.50±0.98	3.40±0.57
CB 138	5.43±3.58	7.13±3.48	5.81±1.86	3.00±1.84
CB 153	5.74±5.18	7.83±4.65	6.46±4.05	3.30±0.14
CB 180	3.93±3.37	6.07±5.15	4.08±2.55	3.35±0.07
∑PCBs	28.46±9.35	31.55±16.66	30.67±6.23	16.05±3.04

KK: Salted- sundried products, KT: Trims, KSS: Smoked products and NFF: Deep fried products and ND: Not detected

Six indicator PCBs (CB 28, CB 52, CB 118, CB 138, CB 153 and CB 180) were detected at measurable quantities in different fish products whereas CB 101 was not detected (ND) in any of the four fish products. The mean levels of individual indicator PCBs in different fish products were in the following ranges: 1.75±0.35 µg/kg (deep fried products) to 6.08±1.95 µg/kg (salted- sundried products) for CB 28, 1.25±0.21 µg/kg (deep fried products) to 3.72±0.87 µg/kg (trims) for CB 52, 1.88±0.76 µg/kg (trims) to 5.50±0.98 µg/kg (smoked products) for CB 118, 3.00±1.84 µg/kg (deep fried products) to 7.13±3.48 µg/kg (trims) for CB 138, 3.30±0.14 µg/kg (deep fried products) to 7.83±4.65 µg/kg (trims) for CB 153 and 3.35±0.07 µg/kg (deep fried products) to 6.07±5.15 µg/kg (trims) for CB 180.

Analysis of Variance (Mean separation using Duncan's Multiple Range Test) showed significant differences for individual indicator PCBs between fish products with trims, smoked products and salted and sundried products having higher levels than deep fried products (Table 5.3).

Table 5.3: Analysis of Variance for the detected PCBs in processed fish products from Lake Victoria

	Sources of variation		
PCBs	DF	F	P
CB 28	3	3.54	0.045**
CB 52	3	3.34	0.043**
CB 118	3	10.69	0.001**
CB 138	3	1.91	0.022**
CB 153	3	0.66	0.036**
CB 180	3	0.53	0.665

** Means are significantly different at 0.05 level
DF: Degree of freedom, F: F -Value and P: P –Value

The total PCBs loading were 28.46±9.35 µg/kg (salted- sundried products), 31.55±16.66 µg/kg (trims), 30.67±6.23 µg/kg (smoked products) and 16.05±3.04 µg/kg for deep fried products. The pattern of the total PCBs loading was NFF<KK<KSS<KT. The loading for deep fried products was significantly different (DF = 3.00, F = 1.53 and P = 0.038) from the rest of the products. The total PCBs loading for other investigated products were quite similar.

Low levels of PCBs in deep fried products could be attributed to the fact that at high temperatures the cooking oil acts as an extracting solvent, thus high levels of PCBs are expected to be left with the oil (Witczak, 2009a; Witczak, 2009b). The trims are the fatty parts of fish, thus PCBs being highly lipophilic are mainly concentrated in fatty tissues (Bjermo *et al.*, 2013; Polder *et al.*, 2014). For smoked products, high concentrations of PCBs could be due to the reason that there is reduced co-distillation of the components with water vapour (Witczak and Ciereszko, 2006) and removal of water from the product as the PCBs are soluble in fat and lipids. Similarly, studies have shown that smoke contain certain amounts of PCBs (Witczak, 2012) and therefore acting as sources of these persistent organochlorine compounds in foods.

For all fish products considered in this study, the PCBs loading was dominated by CB 138, CB 153 followed by CB 180. Comparable results are also reported in previous studies (Polder *et al*., 2014; Ssebugere *et al*., 2014; Oluoch- Otiego *et al*., 2016). The domination tendency of CB 138, CB 153 and CB 180 are also reported in other previous studies (Polder *et al*., 2014; Oluoch- Otiego *et al*., 2016). This is due to the fact that CB 138, CB 153 and CB 180 are not metabolized by certain organisms compared to the rest of congeners (Ssebugere *et al*., 2014). Boon *et al*. (1997) also reported that the rate of metabolisms of PCBs depends mainly on structure and the degree of chlorination of the molecule. Being highly chlorinated, CB 138, CB 153 and CB 180 tend to have longer half-lives, persistent to biodegradation and therefore easily detected in environmental samples. The contribution of the three congener PCBs to total loading was 53.1% for salted and sundried products, 66.7% for trims, 53.3% for smoked products and 60.1% for deep fried products.

However, the mean concentration of ΣPCBs in this study were within the limit of 75µg/kg set for fish by European Commission (EC, 2011), implying that the fish products were safe for human consumption in regard to indicator PCBs. Furthermore the total loading observed were far higher (0.57 µg/kg) than that found by Polder *et al*. (2014) from Lake Tanganyika for fresh *O. niloticus* samples, higher (0.229 to 0.716 µg/kg) than those detected by Ssebugere *et al*. (2014) in fresh *L. niloticus* from the Ugandan side of Lake Victoria. This indicates that processed products have higher levels than fresh fish. It is therefore confirmed that some fish processing technologies such as smoking are main sources of PCBs in food products.

5.2.3 Prevalence of indicator PCBs in processed *L. niloticus* fishery products

The prevalence (%) of the indicator PCBs detected in different *L. niloticus* existing processed products are shown in Fig. 5.1. For the six indicator PCBs that were detected in four fish products, all were less prevalent in deep fried products being detected in 20% of all samples considered in this study. Generally, for the rest of the products the prevalence was in the following ranges: 40 to 60% for salted and sundried fish products, 50 to 70% for trims and 40 to 80% for smoked products. The mean percentage prevalence followed the trend: NFF<KK<KT<KSS. Indicator PCBs, CB 138, CB 153 and CB 180 were more prevalent (20 to 80%) in all fish products than other congeners. This may be attributed to their structures, high chemical stability and high degree of chlorination.

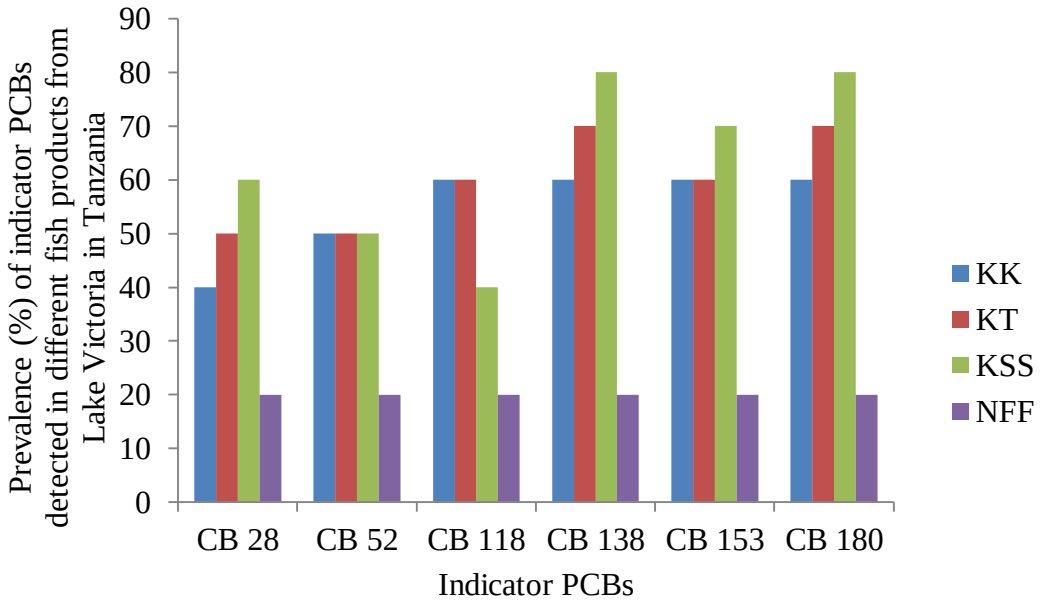

Figure 5.1: Prevalence (%) of indicator PCBs in fish products

5.3 Human Health Risk Assessment

Human health risk assessment results for the indicator PCBs measured in four processed fish products are shown in Table 5.4. The cancer risks based on indicator PCBs loading for adults were in a range between 1.7E-04 and 3.3E-04 while for children were between 1.6E-04 and 7.8E-04. These values are within the range 10E-04≤value<10E-03 and classified as low to moderate risk (Man *et al*., 2013; ATSDR, 2007). This observation suggests that there are only few cancer risks of indicator PCBs associated with consumption of *L. niloticus* fish products from Lake Victoria. Based on ATSDR standard, the cancer risks for PCBs in this study are between very low to low. Life time cancer risks for total PCBs were comparable to those reported in fish from River Nile in Egypt where the cancer risks between 9.81E-6 to 6.02E-4 were reported by Wael and Hamada (2017).

Table 5.5 shows the hazard quotients (HQs) and hazard indices (HI) defining the non-cancer risks of indicator PCBs associated with consumption of *L. niloticus* fish products from Lake Victoria. The Hazard Indices (HI)(sum of Hazard Quotients (HQs)) ranged between 8.5E-03 and 1.8E-02 for adults and between 1.0E-02 and 2.0E-02 for children. In both cases, the HI values were very low. The United States Environmental Protection Agency (USEPA, 2009), recommends that HI values less than one indicates no risk. Therefore results from this study suggest that the risks associated with consumption of the analyzed fish products from Lake Victoria are insignificant for both adults and children in regards to indicator PCBs.

The results herein are comparable to the previous study by Wenaty *et al*. (2019) that assessed the risks of POPs associated with consumption of fish species from Lake Victoria. The non cancer risks were 5.7E-2 and 4.7E-2 while the cancer risks ranged from 8.6E-6 to 3.2E-5 and 7.8E-6 to 1.3E-5 for children and adults, respectively.

Table 5.4: Lifetime cancer risks for the indicator PCBs in processed *L. niloticus* products from Lake Victoria

Samples	KK		KT		KSS		NFF	
	AD	CHI	AD	CHI	AD	CHI	AD	CHI
PCBs	CR	CR	CR	CR	CR	CR	CR	CR
CB 28	6.4E-05	1.6E-04	5.2E-05	1.3E-04	5.5E-05	1.3E-04	1.9E-05	4.5E-05
CB 52	3.2E-05	7.8E-05	3.9E-05	9.5E-05	3.9E-05	9.5E-05	1.3E-05	3.2E-05
CB 101	ND	ND	ND	ND	ND	ND	ND	ND
CB 118	4.5E-05	1.1E-04	2.0E-05	4.8E-05	5.8E-05	1.4E-04	3.6E-05	8.7E-05
CB 138	5.7E-05	1.4E-04	7.5E-05	1.8E-04	6.1E-05	1.5E-04	3.2E-05	7.7E-05
CB 153	6.1E-05	1.5E-04	8.3E-05	2.0E-04	6.8E-05	1.7E-04	3.5E-05	8.4E-05
CB 180	4.2E-05	1.0E-04	6.4E-05	1.6E-04	4.3E-05	1.0E-04	3.5E-05	8.6E-05
∑PCBs	3.0E-04	7.3E-04	3.3E-04	1.6E-04	3.2E-04	7.8E-04	1.7E-04	4.1E-04

AD stands for adults, CHI for children, CR for lifetime cancer risk and ND for Not Determined as the concentration was <LOD

Table 5.5: Non- carcinogenic risks of the indicator PCBs measured in processed *L. niloticus* products from Lake Victoria

Samples	KK		KT		KSS		NFF	
	AD	CHI	AD	CHI	AD	CHI	AD	CHI
PCBs	HQ	HQ	HQ	HQ	HQ	HQ	HQ	HQ
CB 28	3.2E-03	3.9E-03	2.6E-03	3.1E-03	2.8E-03	3.3E-03	9.3E-04	1.1E-03
CB 52	1.6E-03	1.9E-03	2.0E-03	2.4E-03	2.0E-03	2.4E-03	6.6E-04	8.0E-04
CB 101	ND	ND	ND	ND	ND	ND	ND	ND
CB 118	2.2E-03	2.7E-03	9.9E-04	1.2E-03	2.9E-03	3.5E-03	1.8E-03	2.2E-03
CB 138	2.9E-03	3.5E-03	3.8E-03	4.6E-03	3.1E-03	3.7E-03	1.6E-03	1.9E-03
CB 153	3.0E-03	3.7E-03	4.1E-03	5.0E-03	3.4E-03	4.1E-03	1.7E-03	2.1E-03
CB 180	2.1E-03	2.5E-03	3.2E-03	3.9E-03	2.2E-03	2.6E-03	1.8E-03	2.1E-03
HI =∑HQs	1.5E-02	1.8E-02	1.7E-02	2.0E-02	1.6E-02	2.0E-02	8.5E-03	1.0E-02

AD stands for adults, CHI for children, HQ for hazard quotient, HI for hazard index and ND for not determ

Similarly, the hazard index (HI) calculated for total PCBs in processed fish products in this study were far lower than the HI which were established *Oreochromis niloticus* from River Nile in Egypt. Wael and Hamada (2017) reported the HI for total PCBs from 1.12 to 15.05 and it was declared that the fish from River Nile were not safe for human consumption with regards to PCBs as the chronic daily intake were higher than the recommended reference dose (RfD) and that the HI were greater than one (1).

5.4 Conclusion and Recommendations

The levels of the detected PCBs were below the maximum recommended limits for fish and fishery products. PCBs were more prevalent in smoked, trims and salted- sundried compared to deep fried products. The cancer and non cancer risks were very low for both adults and children. The investigated fish products are therefore safe for human consumptions in regards to indicator PCB residues. Follow up studies to assess the cooking oil and the influence of fish processing such as deep frying and smoking on levels of indicator PCBs in fish products are hereby recommended.

5.5 Acknowledgements

The authors acknowledge the financial support from the Danish International Development Agency (DANIDA) through the Innovations and Markets for Lake Victoria Fisheries (IMLAF) Project (DFC File No. 14 – P01 – TAN). We also thank Mr. Michael Mhina, Mr. Hassan Mengi and Ms. Anna Uswege of National Fish Quality Control Laboratory for technical assistance during samples collection, extractions and analysis for PCBs analysis.

5.6 Conflict of interests

The authors declare no conflict of interest.

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CHAPTER SIX

Paper Five: Fish consumers' preferences, quantities of fish consumed and factors affecting fish eating habits: A Case of Lake Victoria in Tanzania

Published by the International Journal of Fisheries and Aquatic Studies

CHAPTER SEVEN

7.0 Conclusions and Recommendations

7.1 Conclusions

This study assessed the occurrence, concentrations and risks of indicator polychlorinated biphenyls (PCBs) and Organochlorine pesticides (OCPs) in water, sediments, fish and fish products from Lake Victoria in Tanzania. The stations closest to urban centers, agricultural activities, commercial and industrial establishments indicated the highest persistent organic pollutant concentrations compared to those far from point sources. At the same sampling point, sediments were found to have higher concentrations of the pollutants than water samples indicating that sediments serve as a sink for organochlorine compounds and act as sources of these pollutants in the environment in case of environmental changes.

L. niloticus were observed to have higher concentrations of POPs than *O. niloticus* due to their differences in feeding habits and trophic levels. Smoked fish products and the fish trims were observed to have higher levels of PCBs and OCPs due to a reduced co-distillation with water vapour in smoked products and high fat content in trims as the studied POPs are lipophilic in nature. There were also high levels and prevalence of OCPs in deep fried products indicating a use of some of OCPs for preservation during storage of fish products in order to extent their shelf lives in the value chain.

The OCPs and indicator PCBs levels in water, sediments, fish and fish products were below the extraneous maximum residue limit (EMRL) recommended by FAO/WHO

Codex Alimentarius Commission, European Union (EU) and other international statutory organs indicating that currently POPs pollution in Lake Victoria has not reached alarming levels and that fish and fish products are safe for human consumption. However, given that these compounds are toxic, stable, soluble and persistent in the environment, their presence in food, even in minute quantities should indeed be avoided.

Assessment of fish consumers' preferences indicated that *L. niloticus, O. niloticus* and *R. argentea* were frequently consumed fish species in the study area. The reasons for their preferences were observed to be easy accessibility and availability, knowledge on nutritional and health benefits of fish consumption and economic reasons. The per capita fish consumption per day was found to be 0.37 kg (135 kg/year). This was found to be far greater than the national annual per capita fish consumption of approximately 7.5 kg/year, worldwide per capita fish consumption of 20 kg and about 2.5 times higher than the annual per capita fish consumption of a great fish consumer of 150 g per day (55 kg/year). For establishing the actual human health risk in the study area, the per capita fish consumption obtained in this study has to be applied rather than using the theoretical per capita fish consumption of 20 g/day (0.02 kg/day) reported elsewhere in literature.

Based on the Agency for Toxic Substances and Disease Registry criteria, an average adult person of 70 kg and children weighted 29 kg were found to have very low to moderate cancer risks and insignificant non- cancer risks associated with dietary exposure in regard to indicator PCBs and OCPs.

7.2 Recommendations

i. Regulatory controls for monitoring and mitigating waste water emissions into Lake Victoria need to be implemented and emphasized by the relevant governmental agencies in Tanzania

ii. Community education though risk communication need to be given to fisher folks on the adverse effects of POPs in fishing and storage of fish products in order to reduce the levels of exposure to these toxic chemicals

iii. Future efforts should directed at ascertaining the levels and trends of known POPs as well as novel pollutants such as flame retardants, poly aromatic hydrocarbons and dioxins in other ―hot spots of Lake Victoria and surrounding water bodies since data in Tanzania and Africa is scarce. Lakes and rivers should be considered as important hot spots for exposure studies because they are final destinations for most pollutants.

iv. The study should also be extended to cooking oils and smoke to find out whether they significantly contribute to levels of POPs in smoked and deep fried fish products respectively as the two products were revealed to contain high levels of the toxicants compared to other studied products.

v. Finally, risk assessment studies of POPs in human subjects need to be performed given the fact that we eat a lot of fish species and we are therefore exposed to a mixture of chemicals that could be harmful even at low levels. Humans being at the top of trophic levels are likely to accumulate high levels of lipophilic POPs.

APPENDICES

Appendix 1: Description and characteristics of water and sediments sampling sites

S/N	Sampling site	Coordinates	Site characteristics
WS_1	Mwasonge	S02^{0}38.994' E032^{0}53.406'	Human settlements, farming activities, river Nyashishi enters the lake and domestic activities (fishing, fetching water and washing).
WS_2	Mwalogwabagole	S02^{0}46.962' E032^{0}57.113'	Discharge of waste water to the lake from different treatment plants, domestic activities (fishing, fetching water and washing), discharge of fuel from the boats and farming activities. Agricultural activities (cotton, horticultural crops), industrial activities (cotton processing), human settlement and fishing.
WS_3	Kirumba	S02^{0}30.875' E032^{0}53.606'	Fish marketing area, Mirongo river enters the lake, discharge of wastes from the City Centre (hospitals, industries and human settlement) and farming of horticultural crops. Discharge of fuel from the boats (Kamanga Ferry and Kirumba market) and farming activities. Butimba river characterized by intensive agricultural activities enters the lake, human settlement, discharge of wastewater from hospitals, prisons, colleges and domestic activities. The site is also characterized by production of vegetables.
WS_4	Katunguru	S02^{0}30.022' E032^{0}40.970'	Human settlement, farming activities, domestic activities and a historical site for illegal fishing
WS_5	Nyarugobe Island	S02^{0}29.008' E032^{0}41.430'	Agricultural activities, domestic activities (fetching water, washing and fishing), human settlement and illegal fishing.
WS_6	Chimfufu Kijiweni	S02^{0}22.783' E032^{0}39.358'	Fish and sardines marketing area, human settlement, discharge of fuel from the boats and farming activities.
WS_7	Nkome	S02^{0}29.275' E031^{0}59.118'	Human settlement, fish landing site, domestic activities (fetching water, washing and fishing) and illegal fishing.
WS_8	Kaishebo	S01^{0}02.479' E031^{0}45.949'	Kagera river characterized by intensive sugar cane production enters the lake, human settlement, farming activities, fishing and illegal fishing. Discharge of wastes from fishing boats, fish landing site, domestic activities (fetching water, washing and fishing).
WS_9	Musira	S01^{0}20.853' E031^{0}50.679'	Human settlements, fish landing site, domestic activities and agricultural activities. Kanoni river enters the lake and it is an industrial area.
WS_{10}	Lubiri Island	S01^{0}57.994'	Farming activities, domestic activities (fetching water, washing and fishing), illegal fishing and sardines and

		E031^{0}49.269'	fish marketing area.
WS_{11}	Rukuba Island	S01^{0}24.413' E033^{0}42.599'	Human settlements, farming activities and discharge of wastes from human settlements and fishing boats.
WS_{12}	Mara Bay	S01^{0}30.882' E033^{0}56.161'	The River Mara characterized by high levels of heavy metals and agrochemicals enters the lake, farming activities, mining activities, human settlements, domestic activities and illegal fishing.

WS = Water and sediments sampling points

Appendix 2: A questionnaire to assess fish consumer's preferences, quantities of fish consumed and factors affecting fish eating habits among the household individuals living in the Lake Victoria basin

1. Date of the interview ……..
2. Region ……..
3. District ……..
4. Ward …….
5. Village ……...

Part A: Personal information

Respondent name ……

Sex		Age	Marital status	Family size
M	F			

In this questionnaire fish means different species of fish found in the Lake Victoria which are commonly consumed by the majority in the study area

1. Does your household use fish in daily meal? Yes/No
2. If the answer is yes, what species do you commonly use? Nile perch/Nile tilapia/others ……. (specify)
3. What is the reason for the answer in question (2) above?
4. If the answer in question (2) above is no, what is/are the reason(s)?
5. How much fish (in kg) on average do you consume per day?
6. How often a member of your household;
    a) Attempt to eat fish as a healthy diet?
        i. Often
        ii. Sometimes
        iii. Seldom

iv. Never

b) Feel bad for not eating fish as a healthy diet?

i. Often

ii. Sometimes

iii. Seldom

iv. Never

c) Encourage fishing and eating fish?

i. Often

ii. Sometimes

iii. Seldom

iv. Never

d) Encourage buying and eating fish?

i. Often

ii. Sometimes

iii. Seldom

iv. Never

5. In general, how willingly are members of household eat fish as part of healthy diet?

a. Very willing

b. Willing

c. Slightly willing

d. Not at willing

6. How important do you feel to include fish diet in providing health diet?

a. Very important

b. Important

c. Not important

d. Not very important

7. Fish dietary assessment:
For each fish species listed, circle a correct response indicating how often on average the household used last month

Type of fish	< 1/month	1 – 3/ month	1/ week	2-4/ week	5-6/ week	1/ day	2-3/ day	4– 5/day	>6/ day
Nile perch									
Nile tilapia									
Others									

8. The next questions measures how many servings of fish normally the household members eat.

a. How many servings of fish does your household eat each day?

i. 1- 2
ii. 3 – 4
iii. 5 – 6
iv. 7 or more

b. About how long does the household have been eating this number of daily servings of fish?

i. Less than one month
ii. 1 -3 months
iii. 4 -6 months
iv. Longer than 6 months.

c. Are the members of household seriously thinking about eating more servings of fish?
Yes / No

d. How many servings of fish do the members of household think a person should eat per day for good health?

i. 1 – 2
ii. 3 – 4

iii. 5 – 6

iv. 7 or more

Daily fish consumption records

Day	Date	Food (Fish)			Specifications			
		Type of fish	Quantity(g)	Number of meals	Time consumed	Place consumed	Amount consumed (g)	Amount left (g)

THANKS!

Appendix 3: Human exposure assessment calculation parameters

Parameter	Adults	Child age (6 – 12) years
Cancer risk		
Average body weight (kg)	70	29
Fish intake (g/day)	370	370
Exposure frequency (days)	365	365
Exposure duration (years)	60	12
Non- Cancer risk		
Average body weight (kg)	70	29
Fish intake (g/day)	370	370
Exposure frequency (days)	365	365
Exposure duration (years)	30	6

(USEPA, 2009; FAO, 2001; Field survey, 2017)

Appendix 4: Pictures of some fish products that were investigated for persistent organic pollutants

Photo 1: Salted- sundried *L. niloticus* products (Kayabo)

Photo 2: Trims (Chips) of Nile perch

Photo 3: Dried trims ready for marketing

Photo 4: Piles of salted- sundried (*Kayabo*) products ready for marketing

Photo 5: Fresh catches of *L. niloticus*

Photo 6: *L. niloticus* preparation for smoking

Appendix 5: Pictures of different types of locally constructed smoking chambers

Photo 1: A fish smoking chamber at Sweya in Mwanza

Photo 2: A fish smoking chamber at Kayenze in Mwanza