#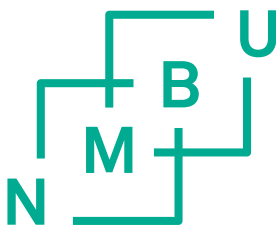 Levels of persistent organic compounds and heavy metals in farmed and wild fish from Zambia and Tanzania, and assessment of possible health effects

Nivåer av persistente organiske forbindelser og tungmetaller i oppdrettsfisk og villfisk fra Zambia og Tanzania, og vurdering av mulige helseeffekter

Chalumba Kachusi Simukoko

Thesis for the degree of Philosophy Doctor

Department of Paraclinical Sciences

Faculty of Veterinary Medicine

Norwegian University of Life Sciences

Ås (2023)

Table of Contents

3

Acknowledgements

Acronyms/Terminology

As   Arsenic

ATSDR   Agency for toxic substances and disease registry

BDE   Brominated biphenyl ether

Cd   Cadmium

CHLs   Chlordanes

Cr   Chromium

CR   Cancer risks

CRM   Certified Reference Material

Cu   Copper

DDD   Dichlorodiphenyldichloroethane

DDE   Dichlorodiphenyltrichloroethylene

DDT   Dichlordiphephyyltrichloroethane

EDI   Estimated daily intake

EFSA   European food safety authority

EWI   Estimated weekly intake

FAO   Food and Agriculture Organisation

Fe   Iron

HBCDD Hexabromocyclododecane

HCB   Hexachlorobenzene

HCH   Hexachlorocyclohexane

Hg   Mercury

HI   Hazard index

IPEN   International POPs Elimination Network

IRM   Internal reference material

JECFA   Joint FAO/WHO Export Committee on Food Additives

LC   Liquid Chromatography

LOD   Limit of detection

LOQ   Limit of quantification

MALF   Ministry of Agriculture Livestock and Fisheries

mg   microgram

Mg   Magnesium

Mo   Molybdenum

MS   Mass Spectrometry

ng   nanogram

OC   Organochlorines

OCPs   Organochlorine pesticides

Pb   Lead

PBFs   Polybrominated Flame Retardants

PBDEs   Polybrominated diphenyl ethers

PCBs   Polychlorinated biphenyls

PFAs   Perfluoroalkyl substances

PFDA   Perflourodecanoic acid

PFNA   Perflourononanoic acid

PFOA   Perfluorooctanoic acid

PFOS   Perfluorooctanesulfonic acid

PFRs   Perfluorinated Flame Retardants

POPs   Persistent Organic Pollutants

SADC   South African Development Community

Se   Selenium

TBBPA   Tetrabromobisphenol A

TCR   Total Cancer Risk

THQ   Target Hazard Quotient

TTHQ   Total Target Hazard Quotient

US-EPA   United states Environmental Protection Authority

WHO   World Health Organisation

Zn   Zinc

Summary

Pollution of water bodies due to human activities is a threat to sustainable fisheries. Pollutants like heavy metals and persistent organic pollutants (POPs) can affect biological functions in fish and human. This leads to reduced fish numbers and makes fish unsafe for human consumption. POPs are manmade chemicals that are toxic, persist and accumulate in the environment and organisms, due to their resistance to environmental and biodegradation. Heavy metals are naturally occurring elements in the earth's crust. Essential metals (Fe, Zn, Cu, Se and Ni) are required for normal metabolism but are toxic in high amounts, while non-essential metals (As, Hg, Pb and Cd) are toxic in low amounts and have no role in metabolism. This study compared levels of heavy metals and POPs in wild and farmed fish (Nile tilapia: *Oreochromis niloticus*) from Zambia and Tanzania and assessed the effects of aquaculture on wild fish stocks.

On lake Kariba Zambia, farmed tilapia had lower levels of POPs compared to wild ones. This is because farmed tilapia has lower exposure to POPs, as they are fed on commercial feed and reach market weight faster. DDT and its metabolites were the dominant organochlorines. Low p,p'-DDE/p,p'-DDT ratio was observed in wild tilapia indicating recent exposure to DDT, which is still permitted by WHO for vector in countries where malaria is endemic. PCB-118, -138, -153 and -180 and BDE-47, -99, -154 and -209 were the dominant congeners. PFASs compounds, PFOS, PFDA and PFNA were only detected in wild fish. Only PBDE exceeded the EQS_{biota} limits and may therefore pose health risk to wild and farmed tilapia.

Heavy metals in Tilapia muscle from Lake Kariba were lower than the maximum limits (ML) set by WHO and therefore safe for human consumption. Hg levels (0.021 mg/kg) in some wild tilapia were higher than the Environmental quality standard (EQS=0.020 mg/kg) set by EU. This could have adverse effects on fish. Essential metals (Cu, Fe, Zn and Mo) were significantly higher in farmed tilapia, while non-essential metals (Al, As, V, Hg, Pb and Cd) were higher in wild ones. Commercial feed contains essential metals to enhance fish performance, and this leads to higher levels observed in farmed tilapia. Wild tilapia near the fish farms access feed and waste spillage rich in trace elements from the farms, and therefore had higher essential metals compared to those away from the farms.

Milkfish and mullet fish from Tanzanian coastal areas had Pd levels higher than the ML (0.3 mg/kg ww) set by WHO/FAO and were therefore not safe for human consumption. Pb was high in both muscle (up to 1.44 mg/kg) and liver (up to 47.37 mg/kg) tissue, with liver having significantly higher Pb and Cu levels. Industrial activities and late phasing out of leaded gasoline could be responsible for the high levels of Pb observed. Wild fish had higher levels of Pb than farmed ones. Different geological conditions, fish management practices and economic activities affected the levels of metals observed from one location to another. Median Hg levels in fish muscle were below the EQS (0.02 mg/kg) set by the EU, indicating no risk to fish.

It can be concluded that tilapia from lake Kariba in Zambia is safe for human consumption as it had low levels of POPs and heavy metals. It was also noted that farmed fish had lower levels of pollutants than wild fish. On the other hand, fish from Tanzanian coastal areas might not be safe for human consumption, due to the high Pb levels observed. PBDE and Hg from some fish on lake Kariba exceeded the EQS and could have adverse effects on the fish. The study also showed that fish farming can affect wild fish near the farms, through spillage of feed and waste. Monitoring of practices in fish farming is therefore vital to avoid contamination of wild fish stocks.

Sammendrag

Forurensning av det akvatiske miljø på grunn av menneskelige aktiviteter er en trussel mot bærekraftig fiskeri og fiskeoppdrett. Forurensninger som tungmetaller og persistente organiske miljøgifter (POPs) kan påvirke biologiske funksjoner hos fisk og mennesker. Dette fører til reduserte fiskebestand og gjør fisk utrygg som matvare. POPs-er menneskeskapte kjemikalier som er giftige, vanskelig nedbrytbare og akkumuleres i miljøet og organismer. Tungmetaller er naturlig forekommende grunnstoffer i jord. Essensielle metaller (Fe, Zn, Cu, Se og Ni) er nødvendige for normale fysiologiske funksjoner, men er giftige i store mengder, mens ikke-essensielle metaller (As, Hg, Pb og Cd) er giftige i lave mengder og er ikke nødvendige for normale kroppsfunksjoner. Denne studien sammenlignet nivåer av tungmetaller og POPs i viltlevende fisk og oppdrettsfisk (Nile tilapia: Oreochromis *niloticus*) fra Zambia og Tanzania og vurderte effekten av akvakultur på ville fiskebestander.

I oppdrettsfisken tilapia fra innsjøen Kariba i Zambia ble det målt målt nivåer av POPs sammenlignet med viltlevende tilapia. Dette kan forklares med at oppdrettsfisk fôres med kommersielt fôr og når markedsvekten raskere (4 måneder). Viltlevende fisk, derimot, kan innta føde som er forurenset fra omgivelsene over opptil flere år og vil derfor akkumulere høyere nivåer. DDT og dets metabolitter var de dominerende POP. Lavt p,p'-DDE/p,p'-DDT-forhold ble observert i viltlevende tilapia, noe som indikerer nylig eksponering for DDT. DDT er fortsatt tillatt for å bekjempe malaria i land der malaria er endemisk. PCB-118, -138, -153 og -180 og BDE-47, -99, -154 og -209 var de dominerende PCB – og PBDE variantene. PFAS-forbindelsene, PFOS, PFDA og PFNA ble påvist i viltlevende fisk, men ikke i oppdrettsfisk. Bare PBDE overskred EQSbiota-grensene og kan derfor utgjøre helserisiko for viltlevende fisk og oppdrettsfisk.

Tungmetaller i Tilapia-muskelen fra Kariba-sjøen var lavere enn maksimumsgrensene (ML) satt av WHO og derfor trygge for konsum. Hg-nivåer (0,021 mg/kg) i noen viltlevende tilapia var høyere enn miljøkvalitetsstandarden (EQS=0,020 mg/kg) satt av EU. Dette kan gi negative effekter på fiskehelsen. Essensielle metaller (Cu, Fe, Zn og Mo) var signifikant høyere i oppdrettsfisk, mens ikke-essensielle metaller (Al, As, V, Hg, Pb og Cd) var høyere i viltlevende fisk. Kommersielt fôr inneholder essensielle metaller for å forbedre ernæringsveriden, noe som kan

forklare de høyere nivåene observert i oppdrettsfisk. Viltlevende tilapia nær oppdrettsanleggene får tilgang til fôr og avfall rikt på sporstoffer fra oppdrettsanleggene, og hadde derfor høyere verdier av essensielle metaller sammenlignet med de som kom fra områder borte fra oppdrettsanleggene.

Melkefisk og multefisk fra Tanzaniske kystområder hadde Pd-nivåer høyere enn Maksimal Limit (ML= 0,3 mg/kg ww) satt av WHO/FAO og kan derfor være utrygge for konsum. Pb var høy i både muskel (opptil 144 mg/kg) og lever (opptil 4737 mg/kg). Industriell virksomhet og sen utfasing av blyholdig bensin kan være årsaken til de høye nivåene av Pb som er observert. Villfisk hadde høyere nivåer av Pb enn oppdrettsfisk. Ulike geologiske forhold, fiskeforvaltningspraksis og økonomiske aktiviteter påvirket nivåene av metaller observert fra et sted til et annet. Median Hg-nivåer i fiskemuskler var under EQS (0.02 mg/kg) satt av EU, noe som indikerer ingen risiko for fisk.

Det kan konkluderes med at tilapia fra innsjøen Kariba i Zambia er trygg for konsum ettersom den hadde lave nivåer av POP-er og tungmetaller. Det ble også bemerket at oppdrettsfisk hadde lavere nivåer av forurensninger enn villfisk. Både viltlevende fiks og oppdrettsfisk fra Kariba hadde trygge nivåer av miljøgifter og toksisk metaller i fiskekjøttet. På den annen side kan det hende at fisk fra tanzaniske kystområder ikke er trygge for konsum, på grunn av de høye Pb-nivåene som er observert. PBDE og Hg fra noen fisk på innsjøen Kariba overskred EQS og kunne ha negative effekter på fisken. Studien viste også at fiskeoppdrett kan påvirke villfisk nær anleggene, via fôrspill og avfall. Overvåking av praksis i fiskeoppdrett er derfor avgjørende for å unngå forurensning av ville fiskebestander.

List of papers

i.   First findings of per- and polyfluoroalkyl substances (PFASs) in wild tilapia (Oreochromis niloticus) from Lake Kariba, Zambia, and higher levels of persistent organic pollutants (POPs) in wild versus farmed fish - implications for fish health.

ii.   Assessment of heavy metals in wild and farmed Nile tilapia (*Oreochromis niloticus*) on lake Kariba, Zambia - implications for human and fish health.

iii.   Heavy metals in farmed and wild milkfish (*Chanos chanos*) and wild mullet (*Mugil cephalus*) along the coasts of Tanzania and associated health risk for humans and fish.

1. Introduction

1.1 Aquaculture

From ancient time, fish has been an important part of the human diet. Fish provides much needed proteins, essential fatty acids, minerals and vitamins (Béné et al., 2015; Chan et al., 2019; Vicente-Zurdo et al., 2019). In 2015, fish account for 17% of total animal protein consumption of worldwide human population (FAO, 2018). However, in rural communities depending on fish as their main source of protein, the intake from fish can be as high as 70% of the total animal protein consumption (Hap et al., 2016; Morato et al., 2006). Unfortunately, the rapid growth of the human population has resulted in increased exploitation of fish and other aquatic resources in both fresh and marine waters. Since 1961, fish consumption grew from 9kg to 20.2kg per capita in 2015 (FAO, 2018). As of 2018, FAO reported (FAO, 2020) that global human consumption of fish had reached 156 million tonnes. The increasing demand for fish, have led to overfishing, which has caused a substantial decline in natural fish stocks. Since the late 1980s, global fisheries catches have declined at a rate of 0.4 million tonnes per year (Morato et al., 2006; Zeller & Pauly, 2005). In 2005, FAO (FAO, 2007) estimated that three quarters of the world's wild fish stocks had either been fully or over exploited. The increasing demand for fish and diminishing levels of natural fish stocks, has made it necessary to find alternatives sources of fish, and aquaculture has emerged as a substitute. Aquaculture or aquafarming is the farming/rearing of aquatic organisms (fish, crustaceans, mollusks, aquatic plants, algae, and other organisms) in both coastal and inland areas. Today, aquaculture is practiced in ponds, cages and tanks in fresh or marine waters.

1.1.1 World aquaculture

Since 1970, world aquaculture has grown by an average of 7.5 percent per year (FAO, 2020). In the past 20 years, the fastest growth in the sector has been observed in Africa and Asia, which have recorded double digit growth (FAO, 2018, 2020). Fish production from both fisheries and aquaculture reached 179 million tonnes in 2018 (FAO, 2020), and aquaculture contributed 46 percent (82 million tonnes) of the total fish production (FAO, 2020). In the same year (2018) FAO (FAO, 2020) estimated World fish trade to be worth USD 401 billion USD, with 250 billion coming from aquaculture. Some of the main fish species involved in aquaculture are Grass carp

(*Ctenopharyngodon idellus*), Silver carp (*Hypophthalmichthys molitrix*), Nile tilapia (*Oreochromis niloticus*), Common carp (*Cyprinus carpio*), Bighead carp (*Hypophthalmichthys nobilis*), Atlantic salmon (*Salmo salar*), Striped catfish (*Pangasianodon hypophthalmus*), Milkfish (*Chanos chanos*), Torpedo-shaped catfishes nei (*Clarias spp.)* and Rainbow trout (*Oncorhynchus mykiss*) (FAO, 2020). Asia account for nearly 70 percent of global fish production (Figure 1), with China being the leading producer of both capture and aquaculture fisheries (Figure 1). China's contribution to the total global fish production was 35 percent in 2018 (FAO, 2020; R. Sun et al., 2018). The Asian region also dominates in fish farming, accounting for 89 percent of global aquaculture output in 2018, and China accounts 57.9 percent of global aquaculture production (FAO, 2020). Other large-scale producers are Indonesia, India, Vietnam, USA, Russia, Japan, Philippines, Bangladesh, Norway, Egypt, Chile and Brazil (FAO, 2020).
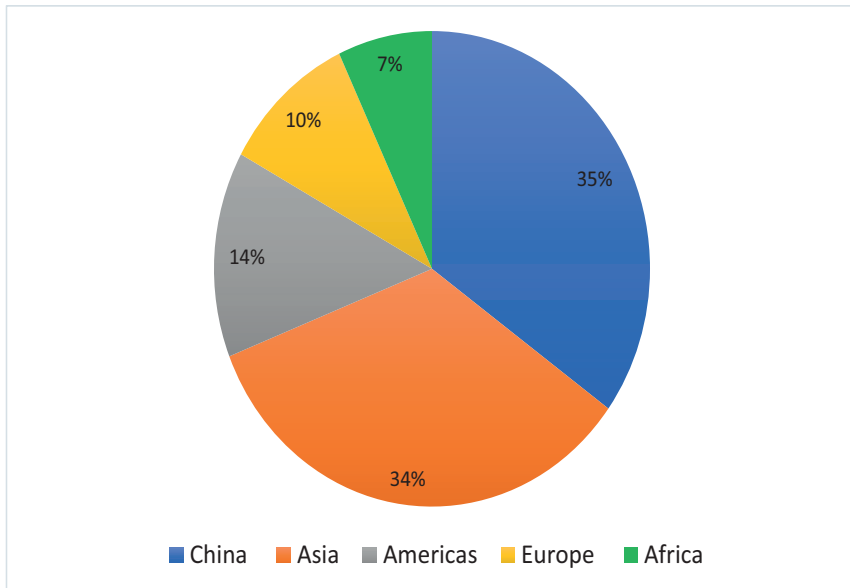
Figure 1: World fish production in 2018 and contribution from regions as reported by FAO (FAO, 2020)

1.1.2 Aquaculture in Africa

Today, Africa accounts for about 2.7 percent of global aquaculture (FAO, 2020), and in 2018, African aquaculture contributed 17.9 percent of total fish production on the continent (FAO, 2020).

Egypt is the leading producer, contributing 70.8 percent of the Africa's fish production , and aquaculture contributes 77 percent of Egypt's total fish production (Mohamed Shaalan et al., 2018). Other major producers of farmed fish in Africa are Nigeria, Uganda, Ghana, Kenya Zambia, Tunisia, Madagascar, Malawi and South Africa (Adeleke et al., 2020; Genschick et al., 2017). The main species cultured in Africa are tilapia, carp, mullet, gilthead seabream, European seabass, and African catfish. Over the past decades, a lot of effort has gone into the promotion of aquaculture in Africa, with significant investment being made, which are reflected in the recent average annual growth of 12.6 percent in sub-Saharan Africa aquaculture (Genschick et al., 2017). Countries in sub-Saharan Africa where aquaculture is seeing significant investment are Tanzania, Kenya and Zambia.

Statistics from 2014, show that Zambia was the sixth-largest producer of farmed fish in Africa, and the biggest producer of tilapia in the South African Development Community (SADC) (Genschick et al., 2017). Aquaculture made up 20% (20, 000 tonnes) of the country's total fish production in 2014, with the rest (80,000 t) from Capture fisheries (Fig. 2) (Tran et al., 2019). The main specie being cultured is Nile tilapia (*Oreochromis niloticus*). Currently aquaculture is either land-based in ponds or water-based using cages. Lake Kariba located at the border of Zambia and Zimbabwe, is one of the locations where cage fish farming is practiced, producing 4000 tonnes annually, which is 1 percent of Zambia's fish production (MALF et al., 2016; Mosha & Daudi, 2020). In southern Africa, aquaculture is practiced in cages on freshwater and marine water and on land in ponds. The dominant species are tilapia (*O. niloticus*) and catfish (*C. gariepinus*) in freshwater and milkfish (*Chanos chanos*) and grey mullet (*Mugil cephalus*) in marine water.
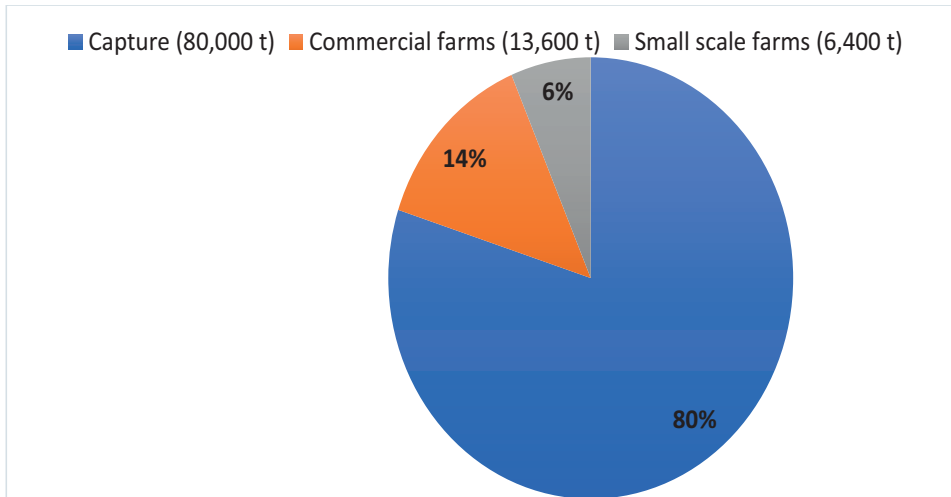
Figure 2: Fish production in Zambia and contribution from different sectors as reported by Tran *et al* (Tran et al., 2019)

1.1.3 Effects on aquaculture on the environment

The growth of aquaculture may have detrimental impact on the aquatic environment. Current aquaculture practices are a possible source of antibiotic residues, antibiotic-resistant bacteria, persistent organic pollutants, metals, parasites, and viruses in natural waters and fish (Basaran et al., 2010; Sapkota et al., 2008). Commercialization of aquaculture is characterized by intensification; high stocking densities and use of large quantities of artificial feed (Rico et al., 2012). Intensification of aquaculture has led to an increase in fish diseases (viral, bacterial, fungal and parasitic) (Bondad-Reantaso et al., 2005; Rico et al., 2012). Large quantities of feed and chemicals (antibiotics, disinfectants, fertilizers, pesticides, and antifouling agents) used in fish farms result in spillage into the surrounding water causing chemical contamination of aquatic environments (Rico et al., 2012; Simukoko et al., 2021). Another effect is genetic contamination of wild species due to escapes from fish farms (Li et al., 2011). Escaped fish may harm wild fish populations through competition and interbreeding or by spreading diseases and parasites (Li et al., 2011).

1.2 Pollution of the aquatic environment

The aquatic environment is one of the most affected environments by pollution (Baines et al., 2021). Pollution occurs when substances in the environment occur at levels above normal or that cause adverse effects in living organisms (Nazir et al., 2015). Water bodies serve as a sink for chemical contaminants from human activity (Abbassy, 2018; Pritchard, 1993) and accumulation of potentially harmful chemical contaminants in water bodies may reach levels, which are toxic to aquatic organisms. Pollution occurs due to natural sources like erosion and volcanic eruption, and anthropogenic activities (Adriano, 2001; Mansour & Sidky, 2002; Nazir et al., 2015). Anthropogenic sources of pollution include mining, industrial effluents, domestic waste disposal, sewage, vector control pesticide spraying, and agriculture runoffs (Baldantoni et al., 2018; Covaci et al., 2008; Maurya et al., 2019; Olisah et al., 2020; R. Sun et al., 2018; Xiao et al., 2014). In addition to a wide variety of known and unknown contaminants, Persistent Organic Pollutants (POPs) and heavy metals are some of the widely distributed pollutants in the environment.

1.2.1 Persistent organic pollutants (POPs)

POPs can be biproducts (E.g. dioxins from forest fires, waste burning, etc.) but are mainly manmade chemicals that have toxic potential, persist and accumulate in the environment and organisms, due to their resistance to environmental and biodegradation (Abbassy, 2018; Q. Liu et al., 2017; X. P. Nie et al., 2006; Panseri et al., 2019). POPs with exemption of Perfluoroalkyl substances (PFASs) are lipophilic and therefore bioaccumulate in fatty tissues of organisms like fish, terrestrial animals and humans (Letcher et al., 2010; X. P. Nie et al., 2006; Squadrone. et al., 2013). POPs magnify as they pass along food chains, leading to the highest concentrations in top predators and on top of food chains (Verhaert et al., 2013; Verhaert et al., 2017). Aquatic top predators tend to have higher levels than terrestrial top predators because POPs end up in water bodies (ultimate sink) and the aquatic food chains are longer than the terrestrial (Vallack et al., 1998). The volatile nature of POPs coupled with long environmental half-lives results in long-range atmospheric transport and global distribution (Fernandez & Grimalt, 2003; Wania & Mackay, 1993). Adverse effects of POPs include neurological, reproductive, immune and endocrine disruption (Letcher et al., 2010; Xie et al., 2020). POPs include organoclorinated pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) such

as, polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD), and Perfluoroalkyl substances (PFAS) like perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA).

1.2.1.1 Organochlorine pesticides (OCPs)

Organochlorine pesticides are synthetic chlorinated hydrocarbon pesticide that were widely used from 1940s to 1960s for agricultural and domestic vector control (Aktar et al., 2009; Jayaraj et al., 2016). Due to their high toxicity, persistence in the environment and bioaccumulation in fatty tissue of organisms, their use has been restricted or banned in most countries (Stockholm Convention 2009; Aktar et al., 2009). However, due to their affordability some OCPs are still used in developing countries mainly for vector control to prevent diseases like malaria (Gupta, 2004; Jayaraj et al., 2016). Dichloro-diphenyl-trichloroethane (DDT) is one OCP still permitted for use in malaria vector control in endemic areas like sub-Saharan Africa (Olisah et al., 2020; Stockholm Convention, 2009; WHO, 2011). Other examples of OCPs are hexachlorocyclohexane (HCH) like lindane, Hexachlorobenzene (HCB), Mirex, *trans*-nonachlor chlordane, endosulfan, aldrin and dieldrin (figure 3).

p'p'DDT   Lindane (γ-HCH)   HCB
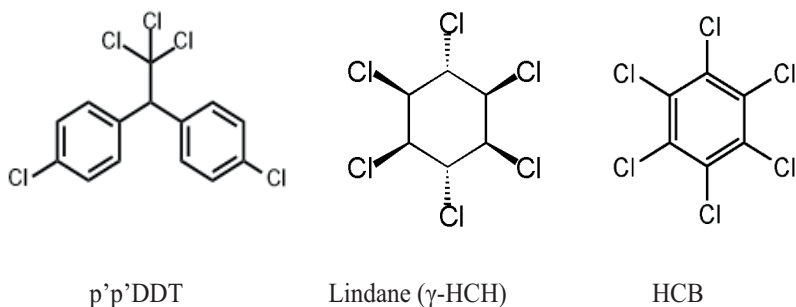
Figure 3: Structure of some organochlorinated pesticides.

1.2.1.2 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls are synthetic compounds consisting of 2 linked benzene rings with a vary degree of chlorination from 1-10 atoms (Anh et al., 2021; Stringer & Johnston, 2002). There are 209 congeners of PCBs (Xie et al., 2020). PCBs were used in insulation, capacitor dielectrics,

coolants, transformer oils, hydraulic fluids, plasticisers and carbonless copy papers. They were also used in heat transfer fluids, lubricating and cutting oils, organic diluents, sealing liquids, adhesives, dedusting agents, waxes, flame retardants and in paints and printing inks (Erickson & Kaley, 2011; Stalling & Mayer, 1972; Stringer & Johnston, 2002; Xie et al., 2020). PCBs are also toxic, persistent and lipophilic (Xie et al., 2020) and due to this, production of commercial grade PCBs were prohibited in Europe and USA in the 1970s and ceased globally in the 1990s (Xie et al., 2020). The Stockholm Convention, which was adopted in 2001 and entered into force in 2004, prohibits the production of PCBs and also the OCPs, requires phasing out of PCB containing equipment such as transformers and capacitors (Lallas, 2001). Despite their production being stopped, PCBs are still released into the environment as byproducts of thermal industrial processes (waste incineration, cement production and metallurgy) (Xie et al., 2020). Structural formulas of PCB congeners PCB153 and PCB 209 are shown in figure 4.

PCB-153   PCB-209
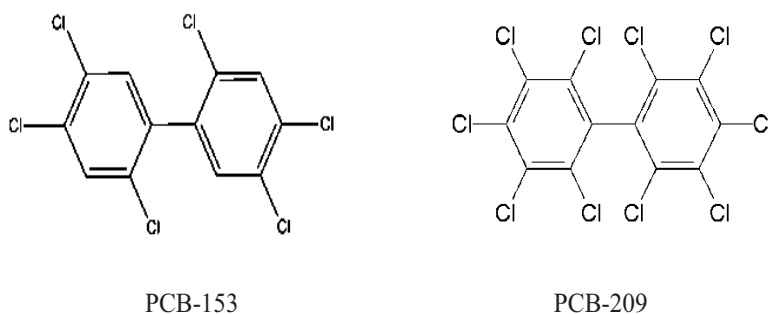
Figure 4. Structures of some PCBs

1.2.1.3 Brominated flame retardants (BFRs)

Brominated flame retardants are used to impart fire resistance properties in products like paints, textiles, furniture, building materials, automobiles, airplanes, electronics and plastics (Roberts et al., 2011b; M. A. Siddiqi et al., 2003; Wu et al., 2019).   They include compounds like Polybrominated diphenyl ethers (PBDEs), Hexabromocyclododecane (HBCDD), tetrabromobisphenol A (TBBPA) and polybrominated biphenyls (PBBs). PBDEs are synthetic brominated diphenyl ethers with 1 to 10 bromine atoms, constituting 209 congeners. Like PCBS, PBDEs are persistent, bioaccumulate in tissues, undergo long range transfer and cause adverse

23

health effects in organisms (Wu et al., 2019). PBDEs are not covalently bonded to materials that contain them and due to this, PBDEs are easily released into the environment during production, usage, recycling and disposal of materials containing them (Wu et al., 2019). HBCDD is a 12-carbon ring containing 6 bromine atoms. It was used an alternative to banned PBDEs until it was also shown to be persistent and toxic (Nie et al., 2015). Use of some PBDEs and HBCDD is banned under the Stockholm Convention (Jensen, 2015; Roberts et al., 2011b; Stockholm Convention, 2009). The European Food Safety Authority classifies TBBPA as a safe compound and its use is still permitted (Chain, 2011). Figure 5 shows some BFRs.

HBDD   TBBPA   PBDE-47
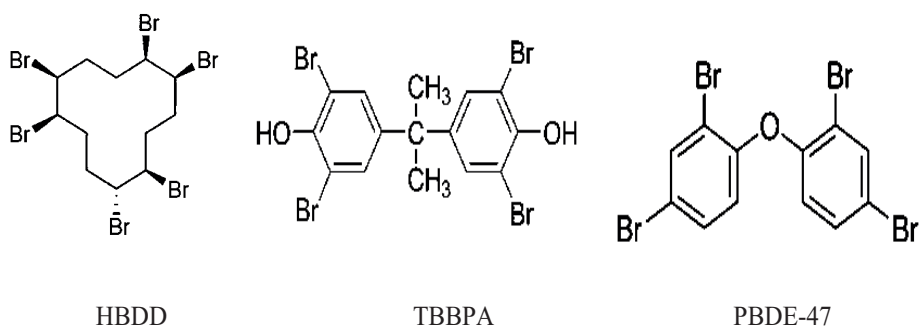
Figure 5. Structures of different types of Brominated Flame Retardants.

1.2.1.4 Perfluorinated compounds (PFCs)/Perfluoroalkyl substances (PFASs)

Polyfluorinated or perfluorinated alkyl substances (Figure 6) are synthetic compounds consisting of a partially or fully fluorinated hydrophobic alkyl chain attached to a hydrophilic end group (Thomaidi et al., 2020). PFASs have been extensively used since the 1950s as surfactants, water and oil repellants in textile, cosmetics, cookware, paper products, firefighting foam, food packaging and furnishing (Birnbaum & Grandjean, 2015; Espana et al., 2015; Thomaidi et al., 2020). PFASs are also persistent, bioaccumulate, toxic and have a global distribution (Espana et al., 2015; Kannan et al., 2001; Wang et al., 2015). Due to their persistence in the environment, they are referred to as 'forever chemicals' (Pelch et al., 2019). Unlike other POPs, PFASs are water soluble making them an important water contaminant including drinking water (Cordner et al., 2019; Post et al., 2017). PFASs are not fat soluble like other POPs (Geiger et al., 2021; Steenland

et al., 2010), but are bound to proteins in tissues. Production and use of commercial PFASs mixtures containing a multitude of different PFASs is still in use. However, two PFASs are regulated under the Stockholm Convention (Espana et al., 2015; Stockholm Convention, 2009; Z. Wang et al., 2015) and include Perfluorooctane sulfonate (PFOS) which was listed in 2009 and Perfluorooctanoic acid (PFOA) listed in 2020 (Heidelore Fiedler & Sadia, 2021; H. Fiedler et al., 2020).
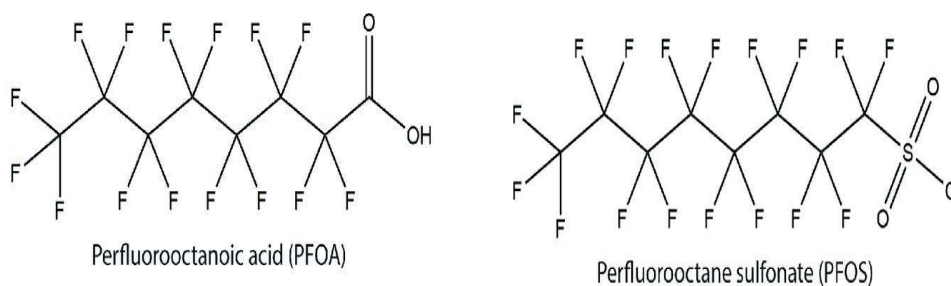
Figure 6: Structure of some PFASs (Adopted from Post et al 2017 (Post et al., 2017)).

1.2.2 Heavy metals

Heavy metals are naturally occurring elements in the earth's crust, with densities 5 or more times than water (Tchounwou et al., 2012). Pollution occurs when metals exceed naturally occurring (background) levels or are at levels causing adverse effects in humans and animals (Nazir et al., 2015). Natural processes like erosion and volcanic eruption and anthropogenic activities (Adriano, 2001; Mansour & Sidky, 2002; Nazir et al., 2015) such as mining, industry, improper waste management may cause pollution (Adriano, 2001; Maurya et al., 2019; Xiao et al., 2014). Metals can either be essential or nonessential. Essential metals (Mg, Fe, Zn, Cu, Se, Co, Mo, Cr, and Ni) are required for normal biological functions in humans and animals, but can be toxic when levels exceed threshold levels for toxicity (Javed & Usmani, 2017; Marengo et al., 2018; Waseem et al., 2014). On the other hand, non-essential metals (Al, As, V, Hg, Pb, Li, Cd, and Ag) have no known biological functions and are toxic even in minute levels (Tchounwou et al., 2012; Waseem et al., 2014). Metals can disrupt biological processes such as endocrine signaling and enzyme activity (Arisekar et al., 2020; Javed & Usmani, 2017; Tchounwou et al., 2012).

25

1.2.3 Control of pollutants

Measures to reduce spread of pollutants to the environment are regulated by international agencies such as the EU, WHO, US-EPA and nationally in Zambia and Tanzania (Zambia Environmental Management Agency) ZEMA and (Tanzanian regulator) is the regulating bodies, respectively. Countries have come up with international treaties that govern the use, production, and acceptable levels of pollution in environment and biota. One such treaty is the Stockholm Convention on Persistent Organic Pollutants adopted by partners in 2001 and came into force in 2004. The Stockholm Convention aims to eliminate, replace, or reduce the use of POPs (Stockholm Convention, 2009). Table 1 lists the POPs regulated under the Stockholm Convention. The United Nations in 1979 established the Convention on long-range transboundary transfer of air pollution focusing on acidification and eutrophication of lakes. In 1998, a protocol to control heavy metals emissions was add to the Convention (UNECE, 2015). Its objective is to control emissions of heavy metals from anthropogenic activities that are subject to long-range transboundary atmospheric transport and have adverse effects on human health or the environment (UNECE, 2015). The protocol focuses mainly on Cd, Hg and Pb. The WHO, EU and other international and national organizations have also set limits for heavy metals levels in the environment and biota.

Table 1: List of POPs included in the Stockholm Convention

Initial 12 POPs (2004)	POPs included in 2009	POPs included in 2011-2018	Under consideration
Aldrin	Chlordecone	Endosulfan	Dicofol
Chlordane	HBB	HCBDD	PFOA and related compounds
Dieldrin	HCH	HCBD	PFHxS and related compounds
Endrin	HCH	PCP	
Heptachlor	Lindane (HCH)	PCN	
HCB	PeCB	Deca-PBDE	
Mirex	Penta-PBDE	Hexa- and Hepta-PBDE	
Toxaphene	Octa-PBDE	SCCP	
DDT	PFOS	Tetra-PBDE	
PCB			
PCDD			
PCDF			

1.3 Exposure to POPs and heavy metals

1.3.1 Sources

Pollutants are a result of various anthropogenic activities as show in figure 7. Historic and current use of organochlorine pesticides for agricultural and domestic purposes remains a source of contamination in the environment and organisms (Affum et al., 2018; Jurgens et al., 2016; R. Sun et al., 2018). The source of DDT is from legacy use but also from continued use as indoor residual spraying (IRS) for malaria vector control (Banda. & Mundia., 2009; Jayaraj et al., 2016; WHO, 2011). Another source may be illegal use in agriculture and obsolete stockpiles of DDT (Azab et al., 2013; Jayaraj et al., 2016; Konradsen et al., 2003; Müller et al., 2017; Olisah et al., 2020). Sources of PCBs and BFRs are from legacy use (R. Sun et al., 2018), but also from old PCB containing equipment like electric transformers and capacitors still in use (Lallas, 2001), and improper disposal of PCB and BFR containing materials like textile, electronics (e-waste) and domestic waste (Cristale et al., 2016; Kefeni et al., 2011; J. Zheng et al., 2011). PFASs are released into the environment from PFASs containing products such as cookware, paper packaging, textile, furnishings, and electronics (Birnbaum & Grandjean, 2015; Valdersnes et al., 2017). Leaks of waste from landfills may also be a significant source of PFASs in water bodies including drinking water (Domingo & Nadal, 2019; Hepburn et al., 2019).

Mineral mining and processing activities are well documented sources of leakage of heavy metals to aquatic environments (Adriano, 2001; Maurya et al., 2019). Industrial waste spills into water, air and land are also significant sources (Xiao et al., 2014). Use of pesticide and fertilizer containing metals in agriculture have also added to environmental pollution of heavy metals (Carolin et al., 2017; Defarge et al., 2018; Gimeno-García et al., 1996).
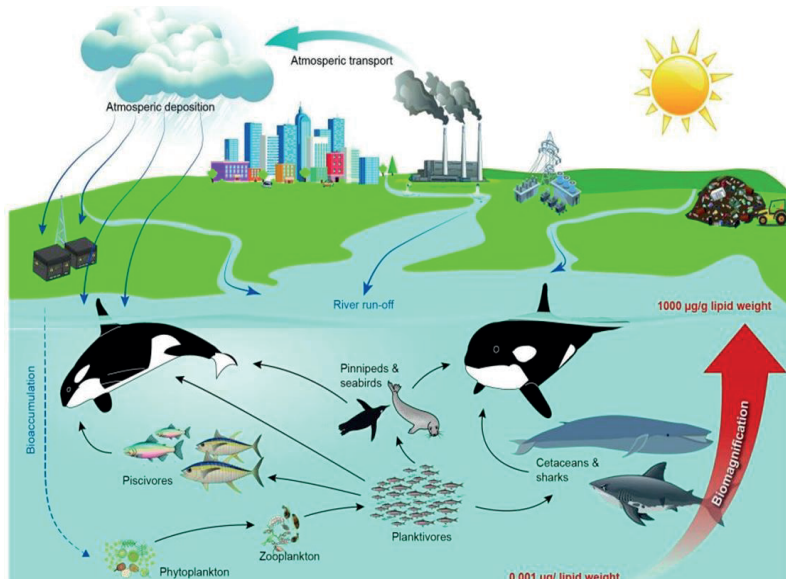
Figure 7: Sources of pollutants in the environment. (Adopted from https://theconversation.com/killer-whales-why-more-than-half-worlds-orcas-are-threatened-by-leftover-industrial-chemicals-104020)

1.3.2 Routes of exposure

Humans and animals are exposed to pollutants through the oral (ingestion), nasal (inhalation), and dermal (absorption) (David, 2006; Rhind, 2002; Wu et al., 2019) exposure routes (Fig. 8). Consumption of contaminated food and water is the main route of exposure. Plants and lower organisms bioaccumulate pollutants through uptake from soil, water and air. Humans and animals are exposed by feeding on contaminated plants and animals. Fat soluble pollutants (OCP, PCBs and BFRs) accumulate in fatty tissues of fish (David, 2006), exposing humans and other predators to higher levels of pollutants than species lower in the food chains. Water soluble pollutants (PFASs and heavy metals) are also taken up orally through food and drinking water (Cordner et al., 2019; Hu et al., 2019). Volatile and gaseous pollutants, and those suspend in dust particles are taken up by inhalation (David, 2006; Wu et al., 2019). Dermal absorption and absorption via the gills are important exposure routes for fish when they are in contact with polluted water. Injection routes are less common, occurring when pollutants are introduced into the body through a break in the skin.

28
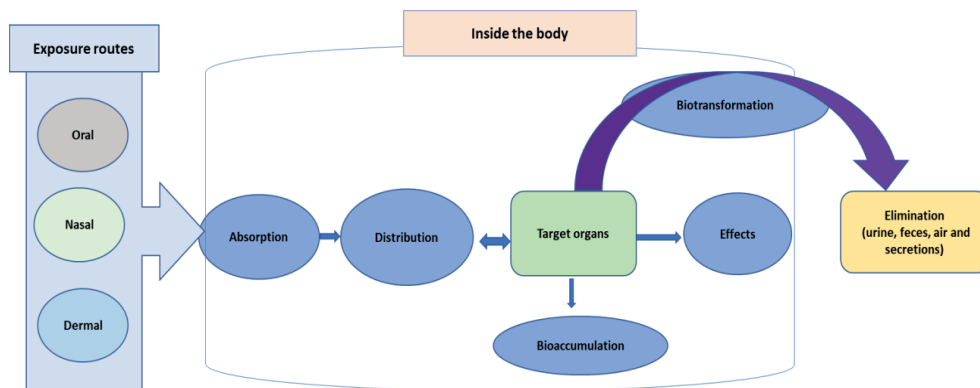
Figure 8. Exposure routes and fate of pollutants in the body

1.3.3 Fate of pollutants in the body

1.3.3.1 Absorption and bioaccumulation

After oral, nasal or dermal exposure, pollutants are absorbed through the membrane and distributed around the body (figure 8). Distribution in tissues is determined by size, concentration gradient, fat content and degree of blood perfusion. Initial fast distribution to liver and muscle due to high lipid content and large blood perfusion respectively, is followed by slow redistribution to other organs. Lipophilic POPs (OCP, PCB and PBDE) bioaccumulate in fatty tissues, whereas PFASs are distributed to serum proteins, and to proteins in liver, kidney, lungs and placenta (Fàbrega et al., 2014; Heuvel et al., 1991; Stanifer et al., 2018). PFASs bind to proteins like plasma albumin, and the protein content of the tissue influence their level of bioaccumulation in different tissues.

Metal bioaccumulation is influence by species, organ physiology and properties of the metal involved. Metals accumulate mainly in the liver, kidneys, skin, gills (fish) and muscle. In fish metal concentrations are highest in the liver and lowest in muscle (Jarić et al., 2011). Metallothionines and other proteins present in organs bind metals like Cu, Zn, Cd and Hg but not lead (Atli & Canli, 2003; Hamilton & Mehrle, 1986; Ploetz et al., 2007). The liver has higher levels of these proteins and thus bioaccumulates more metals (Atli & Canli, 2003; De Smet et al., 2001).

1.3.3.2 Biotransformation and Elimination

Pollutants may undergo biotransformation or remain unchanged before excretion in urine and feces. The liver is the main site of biotransformation and detoxification of compounds (Singh et al., 2016). Liver cytochrome P-450 enzymes (CYP1A) are involved in metabolism (biotransformation) of POPs (Sipes & Schnellmann, 1987; White et al., 1997). Lipophilic POPs are not soluble in water, and in order to excrete POPs in a water medium like urine, they must be transformed to a more water-soluble form. Hydroxylation and conjugation with glucuronic acid of lipophilic POPs makes them water-soluble, enabling excretion of these metabolites from the body via urine. PCBs with 5 or less chlorine atoms are less resistant to biotransformation and excretion than higher chlorinated congeners (Matthews et al., 1978). The opposite is true for PBDEs as less brominated congeners are more resistant to biotransformation and accumulate more than higher brominated ones (Berg, 2013; Roberts et al., 2011a; Watanabe & Sakai, 2003). PFASs are reported to be fully resistant to metabolization in animals and humans (Kudo, 2015) and the literature suggest that they are removed unmetabolized from the body mainly by glomerular filtration in the Kidneys and excreted in urine (Kudo, 2015).

Heavy metals bound to proteins in the blood are transport into tissues through protein channels (Leslie, 2012). Metallothioneins and other low molecular proteins prevent toxicity by sequestering metal ions, reducing the concentration of free ions in tissues/cells (Auclair & Gagné, 2014; Hamilton & Mehrle, 1986). Non-essential/toxic heavy metals (Hg, Cd, As & Pb) displace essential metal (Cu, Zn) from metallothioneins leading to an increase in intracellular Zn and Cu levels (Quig, 1998). High levels of toxic metals in the cell overwhelms metallothionein ability to bind them (Hamilton & Mehrle, 1986), leading to an increase of free toxic and essential metal ions in the cytosol. Methylation and conjugation with glutathione (GSH) of metals occurs and they are excreted in bile (conjugated form) and in urine (methylated form) (Cohen et al., 2006; Naranmandura et al., 2006).

1.4 Adverse effects of POPs and heavy metals

1.4.1 Humans

1.4.1.1 POPs

Several adverse effects in humans have been linked to exposure to POPs. Health effects of DDT in humans has been a subject of debate for many years (Conis, 2010; Kabasenche & Skinner, 2014). The exact effects on human health are not well established (M. Longnecker, P., 2005). However, studies have reported DDT association with neurological, carcinogenic, reproductive, immunological and developmental defects, endocrine disruption and premature birth in humans (Beard, 2006; Kabasenche & Skinner, 2014; M. P. Longnecker et al., 1997; Mansouri et al., 2017; Mrema et al., 2013; Rogan & Chen, 2005). Other OCP like HCB, Lindane have also be linked to cancer, immune suppression, endocrine disruption in humans (Beard, 2006). PCBs have also been reported to have carcinogenic, neurotoxic, cardiovascular, reproductive and endocrine disruptive effects (Grimm et al., 2015; Ljunggren et al., 2014; Vafeiadi et al., 2014). Similarly, PBDEs are also neurotoxic, carcinogenic and endocrine disruptors (Lyche et al., 2015; M. Siddiqi et al., 2003). In humans, PFASs are possible carcinogens, suppress immunity, and have genotoxic and cytotoxic effects (Coperchini et al., 2021; Neagu et al., 2021; Sunderland et al., 2019; Wielsøe et al., 2015).

1.4.1.2 Heavy metals

Heavy metal toxicity is mainly due to non-essential metals Hg, Pb, Cd, and As. The organic form of Hg (methylmercury) is more toxic than the metallic forms. Hg causes neural, renal, immune digestive and respiratory problems in humans (Bernhoft, 2012; Langford & Ferner, 1999; Yang et al., 2020). Mercury poisoning occurred in Minamata Bay, Japan (1956) and Iraq (1971) causing serious neural abnormalities in many individuals (Bernhoft, 2012; Skerfving & Copplestone, 1976; Yang et al., 2020). Pb is has neurotoxic, immunotoxic, carcinogenic and nephrotoxic in humans (Gidlow, 2015; Hou et al., 2013; Humans, 2006; Papanikolaou et al., 2005). It also causes reproductive (miscarriages) and heamatological (anaemia) problems (Gidlow, 2015; Papanikolaou et al., 2005). Cd causes cancers (beast, kidney, lung), liver and kidney damage, reproductive failure, osteoporosis and DNA damage (Bernard, 2008; Genchi et al., 2020; Sharma et al., 2015). Inorganic As is extremely toxic to man (J. C. Saha et al., 1999). Arsenic exposure in humans is

associated with cancer, diabetes, skin lesions, cardiovascular, respiratory and kidney disease (Kuo et al., 2017; Moon et al., 2012; Rasheed et al., 2016). Essential metals are rarely toxic. Toxicity only occurs in case of high levels of exposure. For instance, excessive levels of copper can result in liver and kidney damage, anemia, immunotoxicity, and developmental toxicity (Pohanka, 2019).

1.4.2 Fish

1.4.2.1 POPs

Effects of POPs on fish species range from outright mortalities to sublethal/subclinical. Organochlorines, DDT (DDT metabolites) and Lindane are linked to suppression of the immune system in fish and increased incidence of disease and mortality (Berg et al., 2016; Cuesta et al., 2008; Misumi et al., 2005). PCBs and PDBEs have been associated with immunosuppression, endocrine disruption, thyroid and reproductive dysfunction in fish (Baldigo et al., 2006; Dietz et al., 2019; T. B. Henry, 2015; Jørgensen et al., 2006; Yu et al., 2015). The effects of PFASs are still a subject of much research and debate. PFASs in fish have been linked to developmental deformities, growth suppression, oxidative stress, thyroid disruption, reproductive disruption, metabolic disturbances and immune toxicity (Han & Fang, 2010; J. W. Lee et al., 2020; Oakes et al., 2005).

1.4.2.2 Heavy metals

Toxicity of heavy metals in fish is also mainly due to non-essential metals. Metals cause reduced body condition, oxidative stress, genotoxicity, and pathological changes in several organs. Hg is a highly toxic metal in fish causing tissue damage, reproductive dysfunction, immunotoxicity, neurotoxicity, hepatotoxicity and teratogenic effects (Scheuhammer et al., 2016; Sweet & Zelikoff, 2001; Y. Wang et al., 2015; N. Zheng et al., 2019). Pb is also highly toxic causing oxidative stress, neurotoxicity, immunotoxicity, hypocalcemia hepatotoxicity, renal toxicity and heamatological changes (Kim & Kang, 2015; J.-W. Lee et al., 2019; Rogers et al., 2003). Cd also causes neurotoxicity, immunotoxicity, and oxidative stress (Green & Planchart, 2018; Jin et al., 2015; J.-L. Zheng et al., 2017). As affects reproduction, growth survival, and cause immune suppression in fish (Boyle et al., 2008; Datta et al., 2009; Gonzalez et al., 2010; Tuulaikhuu et al., 2016). Al toxicity is mainly due to gill toxicity and resulting hypoxia in fish (Gensemer & Playle, 1999;

Poléo, 1995; Poléo et al., 2017). Like humans, essential metals are rarely toxic except in high levels or chronic exposure. Cu can result in liver, kidney and gill damage and interfere with Na-K ATPase (Malhotra et al., 2020; Pohanka, 2019; Shaw & Handy, 2006). Excessive Zn levels cause damage in kidney tubules and hyperplasia in gills of fish (Kaya et al., 2017) and affects osmoregulation (calcium uptake) in gills (Bielmyer et al., 2012). The effects of individual metals may vary in different species and environmental conditions.

1.5 Risk assessment of POPs and Heavy metals

Risk assessment is the calculation or estimation of the risk to a given target organism, system, or (sub)population, including the identification of current uncertainties (Gaps in knowledge), following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system (IPCS, 2004). Risk assessment involves hazard identification (is the compound bioactive or inert), hazard characterization (identification of the dose, which cause harm), exposure assessment (identify the exposure levels for a population) and risk characterization whether (identify whether the exposure of the population under investigation exceed the harmful level) (Figure 9). A hazard is an inherent property (Bioactive: Have the potential to interfere with biological functions) of an agent or situation having the potential to cause adverse effects when an organism, system, or (sub)population exposed to that agent. Risk is defined as the probability of an adverse effect in an organism, system or (sub)-population caused under specified circumstances (exposure dose and – time) by exposure to an agent (IPCS, 2004, 2009). The first step in risk assessment is hazard identification, which involves identification of known or potential adverse health effects in an organism, system or population, associated with a particular agent (chemical, physical or biological). This is followed by Hazard characterization, which is the qualitative (adverse effects) and quantitative (harmful dose) description of the inherent properties of an agent or situation having the potential to cause adverse health effects (IPCS, 2004, 2009). A dose-response assessment is usually performed. The third step is exposure assessment which is a qualitative and/or quantitative evaluation of the degree of intake of an agent likely to occur in an organism, system or population. Lastly risk characterization is done. The last step uses the first 3 steps, in

doing a qualitative or/and quantitative evaluation of the probability of occurrence of known and potential adverse effects, of an agent in an organism, system or population (IPCS, 2004, 2009).
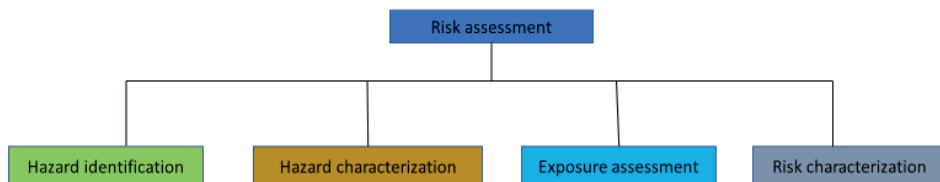
Figure 9: Components of risk assessment.

1.5.1 Human health risks assessment

Maximum limits (metals) and maximum residual limits (POPs) have been set for contaminant levels in food by WHO and EU (European Comimission EC, 2006; FAO/WHO, 2002). These are levels that must not be exceeded if food items (e.g., fish) are regarded as safe for human consumption. Non-carcinogenic risks due to exposure to pollutants, can be determined using Estimated daily intake (EDI), Target hazard quotient (THQ) and Hazard index (HI). These calculations estimate the possibility of an individual developing non-carcinogenic adverse health effects, due to a lifetime of exposure to a pollutant(s). EDI is an estimation of the daily intake of a pollutant by an individual (N. Saha et al., 2016). EDI is compared to established Tolerable daily intakes (TDI). TDI is established based on the dose causing no harm in dose-response studies (No Observed Adverse Effect Level; NOAEL) multiplied with safety factors for individual variability within species and variability between species. TDI is defined as the level of daily intake of a pollutant which will not cause any adverse health effects during a lifetime (Mwakalapa et al., 2019). EDI levels above TDI pose a risk and vice versa. Target hazard quotient is the ratio between the Estimated daily intake (EDI) and the oral reference dose (RfD, mg/kg bw/day) (N. Saha et al., 2016). RfD is an estimate of a daily oral exposure to a toxic substance that is likely to be without an appreciable risk of harmful effects during a lifetime (Mwakalapa et al., 2019; Varol et al., 2017). The Hazard index also refereed to Total Target hazard quotient (TTHQ) is the sum of individual

metal's THQs. Values of THQ and HI >1 pose a risk of developing non-carcinogenic effects in one's lifetime (Copat et al., 2018; N. Saha et al., 2016).

Carcinogenic risks (CR) are estimated as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to a potential carcinogen (Ahmed et al., 2016). USEPA acceptable lifetime cancer risk levels range from10^{-4} (1 in 10,000) to 10^{-6} (1 in 1,000,000) chance of an individual developing cancer (Varol et al., 2017). Cancer risk values greater than 10^{-4} are unacceptable and those less than 10^{-6} do not pose a risk of cancer in an individual's lifetime.

Toxic equivalency factors (TEFs) are used to calculate toxic equivalency values (TEQs) in biological tissues and food based on levels of dioxins and dioxin-like PCBs (EPA, March 30, 2022) with the purpose of making a health risk assessment. WHO has assigned a TEF for each of the dioxins (TCDDs/TCDFs) and dioxin-like PCBs (DL-PCBs) (Van den Berg et al., 2006), and 2,3,7,8-TCDD has a TEF a reference value of 1. The maximum for fish liver, set by the European Commission Regulation (EU) No 1259/2011 of 2 December 2011 is 6,5 pg/g TEQ wet weight.

1.5.2 Fish health risk assessment

To protect human and wildlife from hazardous substances, the European Union through its Water Framework Directive (WFD) (Directive, 2000/60/EC) has set Environmental Quality Standard (EQS) (European Comimission EC, 2000) for EU priority contaminants. These are aimed at protecting and improving the aquatic environment, through reduction of hazardous substance emission and improving the aquatic environment (European Comimission EC, 2000; Fliedner et al., 2016). 45 priority substances and pollutants are listed in the water framework directive, 10 of which relate to EQS in biota (fish, crustaceans and molluscs) (Europian Comimission EC, 2013). Substances monitored in fish under the WFD are, Brominated diphenyl ethers, Hexachlorobenzene, Hexachlorobutadiene, mercury, Dicofol, Perfluorooctane sulfonic acid and its derivatives, Dioxin and dioxin-like compounds, Hexabromocyclododecane and Heptachlor and Heptachlor epoxide.

1.6 Monitoring and control of pollution in African waters

Most African countries are signatories to international conventions such as the Stockholm Convention and Convention on long-range transboundary transfer which aim to monitor and control the spread of POPs and heavy metals in the environment. The past 2 decades have seen an increase in the awareness and monitoring of pollutants on the continent. This has led to several reports being produced on the occurrence and level of heavy metals and persistent organic pollutants in air, water, soil and biota (Fayiga et al., 2017; Olisah et al., 2020; Taiwo, 2019; Yabe et al., 2010). Research has also focused on the health effects of pollutants on humans and animals exposed to them (Simukoko et al., 2021). Despite the increased research, Africa still lags behind other parts of the world mainly due to lack of technical expertise and infrastructure needed for regular monitoring of pollutants (K'Oreje et al., 2020). The ongoing industrialization of Africa and increased trade with industrialised countries increases the risk of environment pollution. These developments call for more research and monitoring in order to control environmental pollution. To help address this need, our research aimed at providing additional information on occurrence and levels of heavy metals and POPs in wild and farmed marine and freshwater fish from Tanzania and Zambia. We also carried out risk assessment for human and fish health due to exposure to POPs and heavy metals.

2. Knowledge gaps and Aims

The aim of the study was to increase knowledge on the occurrence and levels of heavy metals and persistent organic pollutants in marine and freshwater fish from Tanzanian coastal waters and Zambian fresh waters. The study also aimed to assesses the potential health risks the analysed contaminants may pose to human and fish health. The environmental impact of introduction of fish farms on natural/wild fish stocks was also assessed in the study.

Specific objectives

- Determine occurrence and levels of persistent organic pollutants (POPs) in wild and farmed tilapia (*Oreochromis niloticus*) from lake Kariba Zambia.

- Determine and compare levels of heavy metals in wild and farmed tilapia (*Oreochromis niloticus*) from Lake Kariba Zambia.

- Determine levels of heavy metals in farmed and wild milkfish (*Chanos chanos*) and wild mullet (*Mugil cephalous*) along the coasts of Tanzania.

- Assess the health risks to humans associated with consumption of contaminated wild and farmed fish from the study areas.

- Assess the health risks that contaminants pose to fish in the study areas.

- To assess the influence of fish farming on levels and composition of contaminants in wild fish.

3. Materials and Methods

3.1 Study area

Lake Kariba is a man-made lake located on the southern border of Zambia with Zimbabwe (-17° S 28° E). It was built for the purpose of hydroelectric generation. The lake is 320 Km long with an area of 5400 Km², and an average depth of 29 meters. The water flows from west to east. The climate is sub-tropical with annual rainfall between 400 to 700 mm and temperature between 13 and 40°C. Wild and farmed *O. niloticu*s were collected from five locations (Sites 1, 2 & 3, and farms 1 & 2) along the lake (Figure 1). Sites 1 and 2 are in Sinazongwe and Gweembe districts respectively, over 100 km from site 3 with population of 98, 246 and 50,136 respectively (Zambia Central Statistical Office, 2012). Commercial fishing of Kapenta (*Limnothrissa miodon*), crop, livestock, and wildlife farming are practiced in site 1 and 2. In addition, coal mining is done in site 1. Site 3 is located in Siavonga district, on the eastern end of the lake with crop, livestock, crocodile and commercial fish farming, commercial fishing of kapenta and hospitality industry being the main economic activities. The area also has a feed processing plant. Farms 1 and 2 are also located in Siavonga district practice cage tilapia farming.
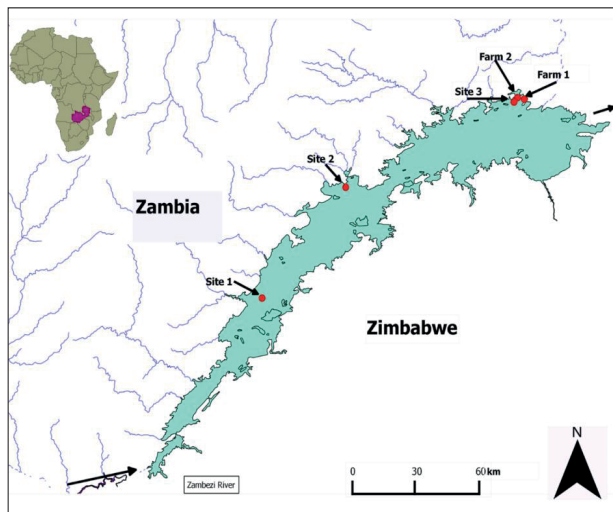
Fig. 10: Map of lake Kariba showing the 5 locations (sites1-3 and farms 1 and 2) where tilapia were collected on the Zambian side (Courtesy of Eliezer Brown Mwakalapa 2019)

Wild and farmed milk fish (*Chanos chanos*) and Mullet (*Mugil cephalus*) samples were from Tanzania mainland (Mtwara district) and Zanzibar islands of Unguja and Pemba. Mtwara district is located in southern Tanzania. Fish farming in ponds is practiced in this area. The main economic activities are agriculture, cashew nut production, oil and gas industry and cement factory. In Unguja and Pemba islands of Zanzibar, economic activities include tourism, crop and animal agriculture, seaweed production and salt extraction industry. Pond aquaculture is also practiced on both islands.

3.2 Species

Three species of fish. These were *Oreochromis niloticus* (Nile tilapia), Mullet (*Mugil cephalus*) and Milkfish (*Chanos chanos*) were included in the study. Nile tilapia is a fresh water omnivorous fish native to river Nile, with a worldwide distribution (FAO, 2005-2019). It belongs to the cichlidae family and order cichliformes. Tilapia feeds on zooplankton and phytoplankton and higher plants (like algae) and thrives in tropical and subtropical climates with environmental temperatures of 9–42ºC, living in shallow waters. It is found both as wild and farmed fish. It's fast growth and resistance against harsh conditions makes it favourable for aquaculture. Mullet (*Mugil cephalus*) is a marine fish, in the order mugiliformes and family mugilidae. It is found in tropical to subtropical coastal waters. It is a diurnal feeder, consuming mainly zooplankton, dead plant matter, and detritus. Mullet also graze on epiphytes and epifauna from seagrasses as well as ingest surface scum containing microalgae at the air-water interface (FAO, 2006-2021). It can survive in temperature and salinity from 8 to 24°C, and 0 ppt to 75 ppt respectively (Camara et al., 2019). Milkfish (*Chanos chanos*) is the only species in the Family Chanidae and belongs to the order Gonorychiformes. Its distribution is in low latitude tropics or the subtropical northern hemisphere, where temperatures are greater than 20 °C (FAO, 2007-2021). They are omnivorous feeding on copepods, diatoms, detritus and zooplankton.

3.3 Sample collection

Wild and farmed tilapia were collected from lake Kariba between June 2016 to July 2017. Physicochemical parameters (pH, temperature, conductivity and total dissolved solutes) were measured. Live wild tilapia was bought from fishermen as they pulled in their catch from the water,

place in ice wate and dissect once back at the shore. Dip netting was used to catch farmed tilapia then dissected at the shore. Fish length and weight were recorded. Stainless steel forceps and scalpel blades were used to dissected muscle tissue of approximately 10g. The tissue was placed in clean 15 ml Eppendorf tubes, then transported on ice in a cooler box to the University of Zambia, Veterinary Medicine School and stored at -20°C. Later samples were transported on ice to the Laboratory of Environment Toxicology at the Norwegian University of Life Science (NMBU) in Oslo, Norway and stored at - 20°C until analyses. Frozen liver and muscle samples were received from Tanzania, then analysed at Central Veterinary Research Institute (CVRI).

3.4 Analytical procedure

Persistent organic pollutants (Paper I) were analysed at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences in Oslo, Norway. The laboratory is accredited for testing chemicals in biological samples by the Norwegian accreditation according to the requirement of the NS-EN ISO/IEC 17025 (TEST 137). The samples were analysed for organoclorinated pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) such as, polybrominated diphenyl ethers (PBDEs) and HBCDD and Perfluoroalkyl substances (PFAS). Before analyses the samples were thawn at room temperature and protected from light during the analyses. Heavy metal analysis for paper II was done at the Laboratory for Soil and Water analysis, Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life Sciences (NMBU), Campus Ås, Norway. For paper III heavy metals (except for Hg analysed at MINA) were analysed at the laboratory of the Central Veterinary Research Institute in Zambia (CVRI). The laboratory was accredited by the Southern African Development Community Accreditation Services (SADCAS).

A

- Weighed 1-5g of liver tissue
- Internal standards added
- Liquid to liquid extraction using cyclohexane and acetone (3:2)
- Homogenization, Sonification & Centrifugation
- Lipid extraction by 97.5% H_2SO_4
- Gravimetric lipid determination by evaporation
- Centrifugation
- Extraction of analytes by evaporation
- Transfer to GC Vials
- OCPs and PCBs analysis by HRGC BFRs analysis by HRGC-LRMS

B

- Weighed 0.5-1g liver
- Internal standards added
- Liquid to liquid extraction in Cyclohexane nd acetone (3:2)
- Homogenization, Sonification & Centrifugation
- Lipid removal by active carbon (EnvCarb)
- Centrifugation
- Extraction of analysis (Evaporation)
- Transfer to GC vials
- PFASs analysis by LC-MS/MS

Fig. 11**:** Schematic overview for analysis of (A) OCPs, PCBs and BFRs and (B) PFASs analyses. Adopted from Mwakalapa (2019) with slight modification.

A

- 200 mg of muscle
- Add standards and 5ml Ultrapure HNO_3
- Digested at 260°C in an UltraCLAVE
- Add 1 ml HCL and diluted to 50 ml with distilled H_2O
- Analysis with Agilent 8800 ICP-MS

B

- 1 g liver and 5 g muscle
- Acid digestion in 20 ml 96% HNO_3
- Boil for 10 -20 min on Hot plate in Bio safety hood to half the volume
- Add 10 ml H2SO4 and boil till black colour
- Add HNO_3 boil till clear solution
- Filter, dilute to 25 ml distilled H_2O
- Analysis with 400 AAS, Perkin Elmer

Fig. 12**:** Schematic representation of Heavy metal analysis (A) MINA and (B) CVRI. Adopted from Mwakalapa 2019 (Mwakalapa et al., 2019) with modifications.

3.5 Ethical considerations

The study was approved by the University of Zambia, School of Veterinary Medicine research committee. Permission from local district fisheries and Veterinary officers was obtained before sampling in the areas. Managers at the fish farms gave permission for samples to be collected after being informed of the purpose of the study. Wild tilapia was bought from fishermen at an agreed price after educating them of the benefits of the research. Fish were humanely euthanised, by first stanning on the head before dissection. Permits to export and import fish samples to Norway were obtained from Ministry of livestock and fisheries in Zambia and Norwegian Food Safety Authority.

3.6 Statistical analysis

Data were organised in spread sheets (MS Excel, 2016). JMP 14 statistical software was used for further analysis. Readings below the limit of detected (<LOD) were treated as zero during analysis as the concentrations of POPs in general were very low. Since the data were not normally distributed, non-parametric tests Wilcoxon/Kruskal-Wallis test (rank sum) for differences among locations and Wilcoxon pair test for differences between locations were used. Spearman rank correlation was used to assess the correlation between variables. The difference between locations were considered statistically significant when $p<0.05$.

4. Summary of papers,

Paper -I

First findings of per- and polyfluoroalkyl substances (PFASs) in wild tilapia (Oreochromis niloticus) from Lake Kariba, Zambia, and higher levels of persistent organic pollutants (POPs) in wild versus farmed fish - implications for fish health. Simukoko CK, Mwakalapa EB, Muzandu K, Mutoloki S, Polder A, Lyche JL. Manuscript.

To assess the occurrence and level of persistent pollutants in wild and farmed tilapia (*Oreochromis niloticus*) on Lake Kariba in Zambia, fish liver samples were collected in 2017. The impact of fish farms on levels of POPs in near wild fish was also examined. Fish liver tissue was used to measure Organochlorine pesticides (OCPs), Polychlorinated biphenyls (PCB), Brominated flame retardants (BFR) and Perfluorinated flame retardants (PFRs).

Lipid percentages were highest in farmed fish and in wild fish nearby the farms. POP levels were highest in wild tilapia. DDT was the most abundant OCP in all sites and DDT levels were highest in wild tilapia. Low p,p'-DDE/p,p'-DDT ratio in wild tilapia from site 3, may indicate recent exposure to DDT. DDT use is still permitted by WHO for vector in countries where malaria is endemic, such as in Zambia. Other OCPs, such as HCB, HCH and chlordanes were also higher in wild than farmed tilapia. Mirex and heptachlor were below detection limit in all sites. PCBs were higher in wild tilapia, with congeners PCB-118, -138, -153 and -180 being the dominant ones. Similarly, PBDEs were also higher in the wild tilapia. Congeners BDE-47, -99, -154 and -209 were the dominant PBDEs. PFASs compounds PFOS, PFDA and PFNA were only detected in wild fish. The findings of the study showed that wild tilapia had higher levels of POPs than farmed tilapia. Farmed fish feed on highly nutritious commercial feed and grows to market weight (250 - 350g) much faster than wild fish. Furthermore, wild fish near fish farms have lower exposure to POPs as they have access of spillage of commercial feed from the farms. It can be concluded that fish farming produces fish with lower levels of POPs. Of the POPs examined, only PBDE concentrations were above the EQS$_{biota}$ limits which may pose health risk to both wild and farmed fish.

Paper -II

Assessment of heavy metals in wild and farmed tilapia (*Oreochromis niloticus*) on lake Kariba, Zambia - implications for human and fish health. Simukoko CK, Mwakalapa EB, Bwalya P, Muzandu K, Berg V, Mutoloki S, Polder A, Lyche JL. October 2021 Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment-DOI: 10.1080/19440049.2021.1975830.

The aim of the study was to assess and compare levels of heavy metals (Mg, Fe, Zn, Al, Cu, Se, Co, Mo, As, Cr, V, Ni, Hg, Pb, Li, Cd, and Ag) in farmed and wild tilapia on lake Kariba Zambia. The influence of fish farming on metals levels in wild fish was also examined. Risk assessment of metal levels in tilapia to human and fish health was also determined. Tilapia muscle tissue (n=176) was collected in July 2016 and examined for heavy metals using ICP-MS.

Levels of all the metals were below the maximum limits (MLs) set by WHO/EU. Essential metals (Cu, Fe, Zn and Mo) were significantly higher levels in farmed tilapia, while non-essential metals (Al, As, V, Hg, Pb, were higher in wild tilapia. Due to their importance in biological functions, essential metals added as trace elements to fish feed. This explains the higher levels observed in farmed fish. Due to a lower plan of nutrition, wild fish take longer to reach market weight (250-350g). This exposes them to heavy metals in the lake for a longer time, thus accounting for the higher levels of non-essential metals observed. Wild tilapia near the fish farms also had higher levels of essential metals than those far from the farms. This is due to feed and waste spillage rich in trace elements from the fish farms, that is accessed by the wild tilapia nearby. Human health risk assessment showed that estimated weekly intakes (EWI) for all metals were less than the Provisional tolerable weekly intakes (PTWI). The Target hazard quotients (THQ) and Hazard Index (HI) were also <1, indicating no non-carcinogenic health risks to humans from a lifetime of fish consumption. Total cancer risk (CR) was less than 1×10^{-4}, indicating less than 1 in 10,000 chance of cancer risk from a lifetime of fish consumption. Assessment of risks to fish health showed that Hg levels (0.021 mg/kg) in wild tilapia at site 1 was higher than the Environmental quality standard (EQS=0.020 mg/kg) set by EU. This indicates possible adverse effects to fish at

this location. Except for Hg, findings in this study show that the levels of metals in fish from lake Kariba, were safe for human consumption and had no adverse effects on fish.

Paper - III

Heavy metals in farmed and wild milkfish (*Chanos chanos*) and wild mullet (*Mugil cephalous*) along the coasts of Tanzania and associated health risk for humans and fish.
Mwakalapa EB, Simukoko CK, Mmochi AJ, Mdegela RH, Berg V, Müller MHB, Lyche JL, Polder A. Chemosphere 224 (2019) 176-186.

The study was conducted in 2016, to assess levels of heavy metals (Pb, Hg, Cd, As, Al, Fe, Zn, Cu, Ni, Co and Cr) in muscle and liver of farmed and wild milkfish (*Chanos chanos*) and wild mullet (*Mugil cephalous*) along the coast of Tanzania. All metals except Hg were analysed using Atomic Absorption Spectrometer (AAS) at Central Veterinary Research Institute (CVRI) in Lusaka, Zambia. Hg was analysed using ICP-MS at the Laboratory for Soil and Water analysis, Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life Sciences (NMBU), Campus Ås, Norway.

High concentrations of Pb were detected in muscle (upto 1.44 mg/kg) and liver (upto 47.37 mg/kg) tissue of wild and farmed milkfish and wild mullet from all sites. The high levels of Pb could be due to industrial activities and late phasing out of leaded gasoline in Tanzania. Liver tissue had significantly higher levels of Pb and Cu than muscle at all sites. Wild fish had higher levels of Pb than farmed fish. Metal levels also varied from one location to another, due to different geological conditions, fish management practices and economic activities. Pb was the only metal that exceeded the maximum limit (0.3 mg/kg ww) set by FAO/WHO in muscle tissue for human safety. Estimated weekly intakes (EWI) for all metals were less than the Provisional tolerable weekly intakes (PTWI). Target hazard quotients (THQ) and Hazard Index (HI) were both <1, indicating no non-carcinogenic health risks to humans. The median Hg levels in fish muscle were below the European Union set Environmental Quality Standards (EQS) of 0.02 mg/kg ww indicating no risk to fish health.

5. General Discussion

5.1 Methodology

5.1.1 Location and species selection

Research for papers I and II was carried out on lake Kariba in Zambia. This location provided access to both wild and farmed tilapia fish, making it suitable for comparison of levels of contaminants in both groups. It also enables us to assess the effect of fish farming on contaminant levels in wild tilapia close to the fish cages. Paper III research was conduct on the coastline of Tanzania, which also provided access to both farmed and wild milkfish and mullet fish species. Tilapia, milkfish and mullet fish species were selected because they were found as both farmed and wild species, and they are economically important species.

5.1.2 Analytical methods

Persistent organic pollutant (OCPs, PCBs, BFRs, PFASs) levels in wild and farmed tilapia in Paper I were measured at the environmental toxicology laboratory at NMBU. The laboratory is accredited for testing chemicals in biological samples by the Norwegian accreditation according to the requirement of the NS-EN ISO/IEC 17025 (TEST 137). OCPs, PCBs and BFRs which are fat soluble were extracted using organic solvents acetone and cyclohexane (Polder et al., 2008) which have good extraction yield. GC-MS was used for separation and detection of OCPs, PCBs and BFRs. GC-MS has high specificity and sensitivity making it suitable for such analysis. BDE-209 is not stable at high temperatures in the GC injector and column (de Boer & Cofino, 2002). It is also sensitive to UV light degradation and behaves different from chlorinated and lower brominated compounds (de Boer & Cofino, 2002). To avoid thermal decomposition of BDE-209 shorter GC column and thermally inert injection port were used (Beser et al., 2014). PFASs are water soluble and were extracted using methanol, while HPLC-MS was used for separation and detection. HPLC-MS has high specificity and sensitivity making it suitable for this analysis. All series (OCPs, PCBs, BFRs and PFASs) were run with blanks, standards and certified reference (CRM) samples for quality control. In addition, the laboratory participated in regularly in international ring test for confirmation of analytical quality.

Heavy metal Analysis in Paper II was done at the Laboratory for Soil and Water analysis, Faculty of Environmental Sciences and Natural Resource Management (MINA), (NMBU), Campus Ås, Norway. After digestion of nitric acid (HNO_3), ultrapure concentrated HCl was added in order to prevent loss of Hg. Metal analysis was then done using an Agilent 8800 ICP-MS, a method with high sensitivity and specificity. Fish Protein Certified Reference Material for Trace Metals (CRM Dorm-3, National Research Council Canada, Institute for National Measurement Standards, M-12, Ottawa, Ontario, Canada K1A 0R6) and fish muscle (ERM-BB422, European Commission – Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), 2440 Geel, Belgium) were used to validate the procedure. For paper III heavy metals were analysed using Atomic Absorption Spectrometer (AAS) at Central Veterinary Research Institute (CVRI) in Lusaka, Zambia. ULTRASPEC multi-element aqueous CRM certified reference material (De bruyn spectroscopic solutions) was used to verify the procedure. Recoveries were above 80%.

5.1.3 Statistical analysis

Data was organized in Microsoft excel before being transferred to JMP statistical and Stata SE/16 software for analysis. The data was not normally distributed, therefore nonparametric tests were used for statistical analysis. Wilcoxon/Kruskal-Wallis test (rank sum) was used to examine differences among locations, while Wilcoxon pair test for differences between locations. Spearman rank correlation was used to assess the correlation between variables. *p<0.05* values were considered significant. Non detectable readings (below limit of detection) were assigned a value of ½ LOD. This is one of the suggested method of dealing with non-detectable readings which are common in environmental studies (Baccarelli et al., 2005).

5.2 General findings

5.2.1 Persistent organic pollutants (POPs) in wild and farmed fish on Lake Kariba

Organochlorine pesticides (OCP) (DDTs, HCB, HCH and Chlordane) were detected in all fish liver samples (paper I). Mirex was not detected in any of the tested fish. The wide distribution of OCPs in all sites (DDT 100%, HCB 100%, HCH 76% and Chlordane 73%), showed past/ongoing exposure to OCPs in the area. Wild fish had higher levels of OCPs than farmed ones. Wild fish take longer to reach market weight, increases their exposure time to contaminants and therefore

bioaccumulate more contaminants (Van Ael et al., 2013). Contaminant levels in feed are closely regulated, and this ensures that exposure of farmed fish to contaminants is reduced (Glencross et al., 2020). DDT and its metabolites accounted for most of the OCPs detected in all locations. The continued use of DDT in malaria vector control may be the main source of DDT pollution. Due to its effectiveness and affordability, DDT use in malaria vector control is still permitted in endemic areas like sub-Saharan Africa (Stockholm Convention, 2009; WHO, 2011). The low ratio of p,p'-DDE/p,p'-DDT (5.8) observed at Sites 3 may indicate recent use of DDT (Bo Strandberg & Hites, 2001; P. Ssebugere et al., 2009). HCB was also detected in 100% of the analysed samples, however, in low levels. HCB observed could be due to its release into the environment caused by anthropogenic activities along the lake and/or precipitation of long range transported HCB (Santonen et al., 2017). HCH were also widely used as technical mixture with α-HCH, β-HCH γ-HCH and other isomers present, or as γ-HCH (Lindane). γ-HCH (Lindane) was the dominant isomer in all locations. Lindane is the only isomer that shows strong insecticidal properties (U.S. EPA, 2010; UNEP, 2007a), and thus the main component of technical HCH mixture. This may account for the dominance of lindane observed in the study. Due to lack of information on the use of γ-HCH in Zambia(UNEP, 2007b), the low levels observed are probably due to past use of lindane insecticides. Among the Chlordanes, trans-Nonaklor was the most abundant at all sites, contributing (34-63%) to ∑CHLs. Chlordane based insecticides are still permitted for use in termite control in the construction and agricultural industries (ECZ, 2007; Wood et al., 2018). Products on the Zambian market like Antkil, Termidan, Termicide, Chlordasol and Kontakil contain 30% to 60% chlordane (ECZ, 2007).

PCBs were detected in all locations, with congeners PCB-118, -153, -138 and -180 being the dominant ones (paper I). PCBs -138 and -153 are not strongly bound to sediments like higher chlorinated congeners, making them available to fish (Squadrone. et al., 2013). Their higher resistance to metabolism and elimination from fish organism than the lower congeners such as PCB -28, -52, and -101 (Squadrone. et al., 2013) also contributes to their occurrence in higher levels in fish tissues than other congeners. Levels of PCBs were low in all locations, with the highest concentration of $\sum_{17}$PCBs (3.2 ng/g lw) at site 1. The use of PCB containing transformers, hydraulic lubricant, plastics, and paints is the possible source of these compounds in lake Kariba

(Pius et al., 2019). Despite being banned in 1980 in industrialised countries (Pius et al., 2019), transformers and capacitors containing PCBs are still in use in the Southern African (Bouwman, 2003). Wild tilapia from locations with higher human activity (sites 1 and 3) showed significantly higher levels of PCBs than wild tilapia away from human settlements (site 2) and farmed tilapia. Anthropogenic activities such as industries, mining, agriculture and waste from human settlements are sources of POPs (Bouwman, 2003; Covaci et al., 2008; L. Henry & Kishimba, 2006; Lyche et al., 2015; Santonen et al., 2017). The larger populations in site 1 and 3 may therefore account for the higher levels of PCBs in wild fish from these locations.

Levels of PBDEs were also higher in wild than farmed tilapia (paper I). As mentioned earlier, the longer time that wild tilapia takes to reach market weight (250g) increases their exposure time to contaminants (Van Ael et al., 2013), while lower levels of contaminants in feed consumed by farmed fish reduces their exposure to contaminants (Glencross et al., 2020). Wild tilapia near the fish farms (site 3) also had lower levels of PBDEs than those away from the farms, probably due to escaped farmed fish and access to feed spillage from the farms. PBDEs are not locally manufactured, their presence in the fish is probably due to leakage from imported commercial products (thermoplastics for computer and TV housing, textiles, foams, furniture, electronics, building materials) (Covaci et al., 2008; Lyche et al., 2015). Poor waste disposal from households and industries as well as open sewage disposal into the lake is also a possible source of contamination (Polder et al., 2014). The dominant congeners were BDE-47 and BDE-209 in all locations. This finding was similar to reports by Polder (Polder et al., 2014) and Mwakalapa. et al. (2018) in Tanzania. Lower brominated congeners like BDE 47 and 100 accumulate more in aquatic organisms (Luo et al., 2019), due to their resistance to microbial degradation in the environment (Asante et al., 2013; Mwakalapa. et al., 2018; Patrick Ssebugere et al., 2014). Higher brominated congeners like BDE 154 and 209 accumulate more in terrestrial organism (Luo et al., 2019). In aquatic organisms BDE-209 is debrominated to lower congeners (Stapleton et al., 2004) which lowers its concentration in fish (Asante et al., 2013). The levels of BDE-209 found in this study could indicate an ongoing exposure. However, we could not identify a possible source of the compound.

PFASs were only detected in wild fish from sites 1 and 2 (paper I). Mining and coal thermal power plants use PFASs in their operations (Glüge et al., 2020). Therefore, coal mining and coal thermal power plant in site 1, maybe the source of the PFASs. PFOS, PFDA and PFNA were the only PFASs detected. Due to their water repellant properties, PFASs are used in various consumer products such as impregnated outdoor textiles, shoes, food containers, kitchen ware and firefighting foam. Their wide use has resulted in a wide distribution in the environment (Patrick Ssebugere et al., 2020). The findings in this study is the first report of presence of PFASs in fish from Zambia. To our knowledge, there are only two studies on PFASs in South African fish. Verhaert et al., (2017) found levels of PFOS, and PFNA in fish muscle (0.15 to 2.7, and <LOQ to 0.14 ng/g ww) from Olifants River basin comparable to our findings (0.19 to 0.64 ng/g ww), while Groffen et al., (2018) found PFOSs in fish liver from Vaal River in much higher levels (<LOQ to 289ng/g ww). Compared to a study in trout from the isolated large and deep inland Lake Femund, Norway, PFASs level in our study were 10 times lower (3.96 ng/g ww) (Lycke et al., 2018). The long chained (>6C) PFASs found in this study are regulated under Stockholm Convention and are therefore expected to decline in the future due to their restricted use.

5.2.2 Heavy metals in wild and farmed tilapia on Lake Kariba

The study revealed significant difference in metal concentrations based on location and type of rearing system (farmed and wild tilapia). Essential metals (Cu, Zn, Fe, Co and Mo) were higher in farmed and wild fish (site 3) near the farms, than in wild fish (sites 1 and 2) far from the farms (paper II). Higher levels of Cu, Zn, Fe, Co and Mo observed in farmed fish may be due to commercial feed containing trace elements (Burridge et al., 2010; Sapkota et al., 2008). Essential metals are necessary for various biological functions and are therefore added as trace elements to fish feed (Fallah et al., 2011; Yildiz, 2008). Feed spillage from the fish farms is accessed by the wild tilapia nearby, thereby exposing them to higher levels of essential metals resulting in the higher levels in fish feeding around the cages (Ballester-Molto et al., 2017; Basaran et al., 2010). Non-essential metals (Al, Ni, Cd, Hg) were higher in wild tilapia sampled far from the fish farms than from other locations. The higher Hg observed could possibly be due to coal mining in the area. Coal mining has been reported to release Hg into the environment (J. Liu et al., 2014; Plessl et al., 2019). Furthermore, wild tilapia can live up to nine years compared to farmed tilapia, which

is harvested within 6 months of cages-rearing on the lake. The wild tilapia can therefore accumulate higher levels of heavy metals with long biological half-lives such as Hg and Cd over a longer lifespan compared to farmed tilapia. Positive associations between Hg and Cd concentrations in tilapia and age as well as size are documented in other studies (Hamada et al., 2018). Other heavy metals such as like Pb, As, Li and V showed no significant difference between wild and farmed tilapia.

5.2.3 Heavy metals in wild and farmed fish along the cost of Tanzania

Paper III presents the first report of heavy metals (Cu, Pb, Fe, Zn, Co, Cr, Cd, Ni, Hg, Al, As), in liver and muscle of farmed and wild fish (milkfish and mullet) from coastal areas of Tanzania. Distribution and levels of heavy metals were influenced by location and rearing system (farmed or wild). Pb levels were high in both farmed and wild fish from all the sampled locations, exceeding the ML set by WHO/FAO for fish muscle. The wide distribution of Pb in the Tanzanian coastal areas could be due to regional emissions from recent rapid growing industrial activities in the region (Echegoyen et al., 2014). The late phase-out of leaded gasoline is another possible source of Pb. Regional emissions of Pb into the coastal waters may account for the significantly higher concentrations of Pb in wild milkfish and mullet muscle (from Zanzibar islands) compared to the farmed fish. This finding suggests that wild fish from the Indian Ocean coastline is more exposed to Pb than farmed fish. Low concentration of Cd was found, with the highest levels in muscles of wild mullet from Pemba Island, Zanzibar. Use of mineral fertilizers in the intensive clove production at Pemba could be the source of Cd in this area. The presence of low levels of Hg in all fish muscles samples indicated a wide distribution in the region. Other metals like Fe, Cu and Co showed geographic variations. The observed difference may be caused by differences in local geogenic sources and different feeding regime (Zhou et al., 2020).

5.2.4 Comparison of contaminants in fish from Kariba and Tanzanian coastal areas

Differences were observed in levels of heavy metals in fish muscle from Lake Kariba and Tanzania coastal areas. Levels of Pb, Cd, Ni were higher in fish from Tanzania, than those from Lake Kariba. The higher levels of Cd in Tanzanian coastal areas could be attributed to the use of chemical fertilizers in intensive clove production, while the Pb levels may be due to the higher industrial activities in this area (Mwakalapa et al., 2019) compared to lake Kariba. On the other hand, fish

from Kariba had higher levels of Fe, Zn, Cu and Cr, while levels of Hg and Co were similar in both locations. Essential metals (Fe, Cu, Zn and Cr) were higher in lake Kariba due to commercial aquaculture being practiced on the lake (Simukoko et al., 2021). Essential metals are a vital for normal biological functions, and are therefore added commercial feed used in the fish farms (Yildiz, 2008). Commercial feed is therefore a source of the higher levels of essential metals seen in wild and farmed fish from lake Kariba (Ballester-Molto et al., 2017; Burridge et al., 2010). Rock weathering is another source of heavy metal pollution in water bodies (Kapoor & Singh, 2021; Zhou et al., 2020). Therefore, different metal composition of rocks also influences the level and composition of metals found in fish at each location.

5.2.4 Species and environmental influences on bioaccumulation of metals

Bioavailability of metals to aquatic species is dependent on conditions of the aquatic environment like salinity, pH, temperature and organic matter/sediments (Dutton & Fisher, 2011; Moiseenko & Gashkina, 2020; Väänänen et al., 2018). Tilapia is a freshwater fish, while milkfish and mullet are marine fish. Salinity in marine waters has an average of 35 ppt while that of fresh water is below 0.5 ppt (Painter, 2020). salinity influence the bioavailability and bioaccumulation of certain metals in the aqueous environment to aquatic species like fish (Dutton & Fisher, 2011; Moiseenko & Gashkina, 2020). For instance, increasing salinity reduces the uptake and bioaccumulation of Cd in fish, while the opposite is true for As (Dutton & Fisher, 2011). Therefore, the different salinity levels in the study areas could also contribute to the difference observed in levels of heavy metals in the current study. Bioaccumulation of heavy metals is also species dependent (Anna Jakimska et al., 2011). Therefore, species differences may account for differences observed.

5.2.4 Comparison of levels of contaminants with other studies/regions

The level of contamination in fish in the 2 study areas were generally lower than in more industrialised regions and comparable to similar areas. $\sum$DDTs (the dominant OCP)) in wild tilapia from lake Kariba were 3 times higher than those from Brazil and Finland (Botaro et al., 2011; Koistinen et al., 2008), which may be explained by the continued use of DDT in malaria vector control in sub-Saharan Africa (WHO, 2011). DDT in fish from Tanzania, South Africa and China (L. Henry & Kishimba, 2006; Y.-X. Sun et al., 2014; Verhaert et al., 2017) was 5 times higher

than those from Kariba, which suggest similar continued use of DDT in these countries. PCBs and PBDEs were 8 times higher in fish from more industrialised countries like Finland, China and South Africa (Koistinen et al., 2008; Y.-X. Sun et al., 2014; Wepener et al., 2012) than the less industrialised lake Kariba area. Past production of chlorophenol formulations, PCB atmospheric deposition, and effluents containing PCBs and PBDEs from industry and municipalities are responsible for higher levels observed in more industrialised regions (Koistinen et al., 2008). Heavy metals from lake Kariba were lower than those from more urban and industrialised areas in Africa and Asia (Ibrahim et al., 2020; Leung et al., 2014; Mapenzi et al., 2020; Taweel et al., 2011). Use of fertilizers, pesticides, rock weathering and mining and manufacturing are the main sources of heavy metals in the environment (Zhou et al., 2020). Therefore, the type of activities in an area has influence on the level of contamination. Pb levels from Tanzania were higher than most findings in Africa and Asia (Qin et al., 2015; Simukoko et al., 2021; Varol et al., 2017) but considerably lower than findings from Kanya and China (Leung et al., 2014; Nyingi et al., 2016). The rest of the metals in fish from Tanzania were also lower than in more urbanized and industrialised areas.

5.2.4 Influence of aquaculture on levels and composition of contaminants in wild fish stock

 Aquaculture has been reported to cause pollution of natural waters and fish (Basaran et al., 2010; Sapkota et al., 2008). In the current study, it was observed that wild fish near the fish farms had similar levels and composition of heavy metals as farmed fish (Simukoko et al., 2021). This was not the case with wild fish collected away from the fish farms. Feed and waste spillage from the fish farms is accessed by the wild fish nearby, leading to the higher risk of exposure of compounds in the commercial fish feed (Ballester-Molto et al., 2017; Basaran et al., 2010; Simukoko et al., 2021). Escaped farmed fish also become part of the wild fish populations leading to interbreeding and spreading diseases and parasites (Li et al., 2011).

5.2.4 Risk assessment for fish and human health

Contaminants can cause adverse health effects to human and fish, when they exceed the safe or recommended limits. To protect human health from consumption of contaminated fish (food and water), WHO/FAO, USEPA and other organization have set maximum limits (for heavy metals)

and maximum residual limits (for POPs) for contaminants in the fish. These limits should not be exceeded if food is to be safe for human consumption. The EU has also set environmental quality standards (EQS) for the purpose of protecting animal and human health. Levels above the set EQS indicate possible harm to animal health.

5.2.4.1 Human health risk assessment

The concentrations of metals in the muscle of wild and farmed tilapia from Lake Kariba (Paper II) were below the maximum limits set by WHO/FAO (FAO/WHO 2002) and EU (EC EC 2006). The EWI was also much lower than the PTWI, while the THQs and HI were both less than 1 (<1). This indicates that the fish from Lake Kariba can be consumed regularly without any significant health risk from both single metal exposure and cumulative effect of exposure to multiple metals. HBV_{Se} from all sites were positive, indicating protecting effects of selenium against mercury in the fish (Ralston et al. 2016; Yabanli and Tay 2021). Mercury has been shown to inhibit selenium-dependent enzymes (selenoenzymes), which protect against potential oxidative brain damage and is involved in other vital functions such as foetal brain development, growth, thyroid hormone metabolism and calcium regulation (Squadrone et al. 2015; Ralston et al. 2016, 2019). Selenium sequesters mercury, forming an insoluble selenium-mercury (HgSe) compound, that is excreted from the body (Ralston et al. 2016; Yabanli and Tay 2021). The Total cancer risk (TCR) due exposure to As, Cd, Cr, Ni and Pb in the fish was less than $1 \times 10^{x-4}$ (Paper II), indicating that the risk of humans developing cancer during their lifetime, as a result of consuming wild and farmed tilapia from Lake Kariba was less than 1:10,000 (Varol et al. 2017).

All the analysed fish from the coastal areas of Tanzania, had Pb levels that exceeded the maximum limit (0.3 mg/kg ww) set by FAO/WHO in muscle tissue. However, the estimated weekly intakes (EWI) for all metals were less than the provisional tolerable weekly intakes (PTWI), while the THQ and HI were both <1, indicating no non-carcinogenic health risks to humans. Despite the THQ and HI being low, the Pb levels found in this study are of concern. Pb is highly toxic to humans, causing neurological, renal, haematological, and carcinogenic effects (Bosch et al., 2016). Children are reported to be more vulnerable (WHO, 2015), due to the development of vulnerable organs and tissues and their poorly developed excretory systems. Children may also be exposed to

additional Pb from old paint, toys and dust on the floor. Due to these other putative sources and the high Pb levels (above maximum limit) in fish, Pb could pose a danger to the health of children.

POPs are lipophilic; thus, liver tissues were used to measure their levels in fish during this study. In 2011, the EU set an MRL for Σ non-dioxin like (NDL) PCBs (PCB-28, -52, -101, -138, -153, and -180) in fish muscle at 75 ng/g ww (EUROPEAN COMMISSION EC, 2011). The results from this study cannot be directly used to assess human health risk, since they were measured in liver and not fish muscle. However, since liver has a higher lipid content (range 3-19 %) (present study) than muscle (0.4-4 %) (Polder et al., 2014), one can assume that POP levels in wild and farmed tilapia liver from the present study are much higher than in the muscle. The highest sum of ΣNDL-PCBs in the present study (sum of PCB 118, 138, 153, 180) was 0.2 ng/g ww in liver, and thus far below the EU MRL.

5.2.4.2 Fish health risk assessment

Chemical pollution of water poses a threat to the aquatic environment and may induce acute and chronic toxicity in aquatic organisms (European Comimission EC, 2006). Contaminants can adversely affect fish health if they exceed certain levels. In order to protect animals and humans from contaminants, the European Framework Directive was developed in 2008 and identified 33 priority hazardous substances for which Environmental Quality Standards (EQS) were set for water and biota (Europian Comimission EC, 2013). Heavy metals have been shown to cause adverse effects on fish health, such as alteration in condition indices, biochemical disorders, and histopathology when certain levels are exceeded (Javed. & Usmani., 2017). An exposure level of 5 mg/kg of Pb in *Clarias batrachus* (Cat fish) for 150 days has been shown to inhibit gonadal growth, reduce cholesterol and lipid content in brain, testis and ovaries, while increasing both in the liver (Katti & Sathyanesan, 1983). At 1 mg/kg, Pb exposure inhibits blood ALA-D activity in tilapia (Dos Santos et al., 2016). The high Pb levels in liver from wild milkfish (47.3 mg/kg ww) and wild mullet (6.26 mg/kg ww) from Tanzanian coastal waters may therefore pose a health risk to the fish. 22% of the fish exceeded the threshold EQSbiota (0.02 mg/kg ww) for Hg and this may also affect fish health. Hg levels in Tilapia from Lake Kariba were below the EQSbiota, except for wild tilapia at site 1 (mean 0.021 mg/ kg ww) which was slightly higher than the EQSbiota. This

may have an effect of the health of the fish in this location. The levels of PBDEs from both wild and farm tilapia on lake Kariba exceeded the European standard (EQS) for these contaminants in fish and may harm fish health. Follow up studies are needed to ensure that international regulations result in decrease of these and other contaminants that threaten the aquatic environment.

5.3 Strength and weakness

The study location enabled comparison of contaminant composition in wild and farmed fish from the same lake. The location also provided an opportunity to observe the effects of commercial fish farming on wild fish attracted to open-cage fish farms.

Analysis of the fish samples for POPs and heavy metals were done at accredited laboratories at Norwegian University of Life Sciences (NMBU) and Central Veterinary Research Institute (CVRI) in Zambia. The use of accredited analytical methods ensures a high degree of accuracy of the results obtained. The ability of the laboratories to analyse a wide range of contaminants (POPs and heavy metals), made it possible to present results on previously known and unknown contaminants in the study areas.

Two countries were involved in the study (Zambia and Tanzania). This provided an opportunity to compare contaminants in the 2 countries and how differences in local conditions such as industrial activities, marine types (freshwater vs marine water), management systems and fish species may affect levels of contamination in fish. Collaboration between researchers in the 2 countries will ensure better knowledge sharing and a coordinated approach to the control of contaminants in the region.

The involvement of researchers from Norway and several African countries in the project, ensured knowledge and skills transfer. The building of capacity in local researchers in the participating countries will result in better management of fish resources and control of environmental pollution in the region.

The study did not measure contaminants in feed, sediments, air, and water and it was therefore not possible to point out the source of contaminants observed in the fish. Pooled samples were analysed

due to financial constraints, which made it impossible to trace levels of contaminants to individual samples.

No survey was administered to the public in the study area to get information on the current industrial and domestic activities in the areas. Our reliance on published reports of activities in the study areas may not be an accurate representation of current activities and therefore possible sources of contamination. Information on local activities could have provided data on which contaminants needed to be measured in addition to the ones that are reported in the current study.

6. Conclusion

- The study showed that wild tilapia fish had higher levels of persistent organic pollutants (POPs) and non-essential heavy metals (As, Pb, Hg, Cd) than farmed tilapia from lake Kariba. The longer time required for wild tilapia to attain market weight increases their exposure to contaminants leading to higher levels. Due to availability of a high nutrient diet, farmed fish attain market weight earlier, thus having a shorter exposure time to contaminants in the aquatic environment.

- Essential metals (Cu, Zn, Fe, Mo) were higher in farmed tilapia from lake Kariba than in wild ones. The addition of essential minerals to commercial feed that is used in the fish farms, increasing the availability of the metals to farmed tilapia causing higher levels. It was also observed that wild tilapia near the fish farms also had higher levels of essential metals than wild tilapia at distance from the farms. Nutrient rick spillage from the farms increases the exposure levels to essential metals for wild tilapia near the farms. This finding demonstrates that fish farming activities on the lake can affect wild tilapia in the vicinity of the farms and therefore need to be monitored and regulated.

- Levels of both non-essential and essential metals among wild and farmed milk fish and mullet from Tanzanian coastal areas varied from one location to another. This suggested different sources of metals from one location to another.

- The dominant POPs in both fish from Zambia and Tanzania were DDT and its metabolites. Continued use of DDT in malaria vector control is likely to be the reason for this finding. Other abundant POPs found were PCBs (PCB-153, PCB-180, PCB-170 and PCB-138), PBDE (BDE-47, BDE-99, BDE-100, BDE-154 and BDE-209). PFASs (PFDA, PFNA and PFOS) were only found in wild tilapia from lake Kariba. This is the first report of PFASs in fish from Zambia and this calls for more research in this field to establish sources and how widespread the contaminant is in the country.

- Human health risk assessment for heavy metals showed that only Pb from fish muscle in Tanzania exceeded the maximum limit set by FAO/WHO. Despite this, all the calculated weekly intake, target hazard quotient, hazard index and cancer risks were below the

recommended levels, indicating no danger of non-carcinogenic and carcinogenic risk from fish consumption.

- Fish health risk assessment showed that Hg in some fish from Zambia and Tanzania exceeded the EQS set by the EU and could therefore pose a risk to fish in these areas. PDBEs in liver from tilapia in Zambia also exceed the EQS, this may also pose a health risk to the fish in lake Kariba.

7. Future Perspectives

- Future research is needed to find and eliminate possible sources of contaminants in wild and farmed fish. This should include testing of water, sediments, feed, air samples and discharges into the water bodies and air samples. Information gained from such monitoring activities will help in coming up with policies and regulations to control pollution of water bodies.

- Increase information dissemination on POPs and heavy metal contaminants to the public through seminars, workshops, publications, media and other available channels. Increased knowledge by the public will help in monitoring of activities in their localities and also bring about behavioral changes in the community.

- Regulations need to be enhanced to have routine monitoring of levels of POPs and heavy metals in fish and other products of animal origin. Imports of fish from more industrialised nations should be regularly monitor for POPs and metal contamination, as these may increase exposure to the citizens.

- Continued training of local experts in the field of toxicology and fish health will provide the necessary skills in this area. Continued collaboration between regional and international institutions will greatly enhance local expertise in this field.

- The growing aquaculture industry needs to be well regulated to ensure that the practice does not cause pollution of natural water resources and aquatic species. Guideline should be set up that will help in achieving good practices in the industry, that ensure human and fish health are protected.

- Climate change is expected to reduce fish catches, fish growth, lower water levels and flow in lakes and rivers due to drought and increased precipitation. Levels of pollutants are also expected to increase as result of reduced water flow and increased precipitation. It is therefore vital that research in climate change and its effects on fish stock, pollution and hydrology are carried out and remedial measures put in place.

8. References

Abbassy, M. M. S. (2018). Distribution pattern of persistent organic pollutants in aquatic ecosystem at the Rosetta Nile branch estuary into the Mediterranean Sea, North of Delta, Egypt. *Marine Pollution Bulletin, 131*, 115-121. doi:https://doi.org/10.1016/j.marpolbul.2018.03.049

Adeleke, B., Robertson-Andersson, D., Moodley, G., & Taylor, S. (2020). Aquaculture in Africa: A Comparative Review of Egypt, Nigeria, and Uganda Vis-À-Vis South Africa. *Reviews in Fisheries Science & Aquaculture*, 1-31. doi:10.1080/23308249.2020.1795615

Adriano, D. C. (2001). In *Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals* (2nd ed., pp. 91-131). New York: Springer Verlag.

Affum, A. O., Acquaah, S. O., Osae, S. D., & Kwaansa-Ansah, E. E. (2018). Distribution and risk assessment of banned and other current-use pesticides in surface and groundwaters consumed in an agricultural catchment dominated by cocoa crops in the Ankobra Basin, Ghana. *Sci Total Environ, 633*, 630-640. doi:10.1016/j.scitotenv.2018.03.129

Ahmed, K., Baki, M. A., Kundu, G. K., Saiful, I., Monirul, I., & Muzammel, H. (2016). Human health risks from heavy metals in fish of Buriganga river, Bangladesh. *SpringerPlus, 5*(1), 1697. doi:10.1186/s40064-016-3357-0

Aktar, M. W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol, 2*(1), 1-12. doi:10.2478/v10102-009-0001-7

Aktar, M. W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol, 2*(1), 1-12. doi:10.2478/v10102-009-0001-7

Anh, H. Q., Watanabe, I., Minh, T. B., & Takahashi, S. (2021). Unintentionally produced polychlorinated biphenyls in pigments: An updated review on their formation, emission sources, contamination status, and toxic effects. *Science of The Total Environment, 755*, 142504. doi:https://doi.org/10.1016/j.scitotenv.2020.142504

Anna Jakimska, Piotr Konieczka, Krzysztof Skóra, & Namieśnik, J. (2011). Bioaccumulation of Metals in Tissues of Marine Animals, Part I: the Role and Impact of Heavy Metals on Organisms. *20*(5), 1117-1125.

Arisekar, U., Shakila, R. J., Shalini, R., & Jeyasekaran, G. (2020). Human health risk assessment of heavy metals in aquatic sediments and freshwater fish caught from Thamirabarani River, the Western Ghats of South Tamil Nadu. *Marine Pollution Bulletin, 159*, 111496. doi:https://doi.org/10.1016/j.marpolbul.2020.111496

Asante, K. A., Takahashi, S., Itai, T., Isobe, T., Devanathan, G., Muto, M., . . . Tanabe, S. (2013). Occurrence of halogenated contaminants in inland and coastal fish from Ghana: Levels, dietary exposure assessment and human health implications. *Ecotoxicology and Environmental Safety, 94*, 123-130. doi:https://doi.org/10.1016/j.ecoenv.2013.05.008

Atli, G., & Canli, M. (2003). Natural Occurrence of Metallothionein-Like Proteins in the Liver of Fish Oreochromis niloticus and Effects of Cadmium, Lead, Copper, Zinc, and Iron Exposures on Their Profiles. *Bulletin of Environmental Contamination and Toxicology, 70*(3), 0619-0627. doi:10.1007/s00128-003-0030-4

Auclair, J., & Gagné, F. (2014). Chapter 5 - Metal Metabolism and Detoxification. In F. Gagné (Ed.), *Biochemical Ecotoxicology* (pp. 75-101). Oxford: Academic Press.

Azab, M. M., Darwish, A. A., Mahmoud, H. A., & Sdeek, F. A. (2013). Residue levels of organochlorine pesticides in some ecosystem components of Manzala Lake. *Environmental Monitoring and Assessment, 185*(12), 10257-10268. doi:10.1007/s10661-013-3330-0

Baccarelli, A., Pfeiffer, R., Consonni, D., Pesatori, A. C., Bonzini, M., Patterson, D. G., . . . Landi, M. T. (2005). Handling of dioxin measurement data in the presence of non-detectable values: Overview of available methods and their application in the Seveso chloracne study. *Chemosphere, 60*(7), 898-906. doi:https://doi.org/10.1016/j.chemosphere.2005.01.055

Baines, C., Lerebours, A., Thomas, F., Fort, J., Kreitsberg, R., Gentes, S., . . . Sepp, T. (2021). Linking pollution and cancer in aquatic environments: A review. *Environment International, 149*, 106391. doi:https://doi.org/10.1016/j.envint.2021.106391

Baldantoni, D., Bellino, A., Lofrano, G., Libralato, G., Pucci, L., & Carotenuto, M. (2018). Biomonitoring of nutrient and toxic element concentrations in the Sarno River through aquatic plants. *Ecotoxicology and Environmental Safety, 148*, 520-527. doi:https://doi.org/10.1016/j.ecoenv.2017.10.063

Baldigo, B. P., Sloan, R. J., Smith, S. B., Denslow, N. D., Blazer, V. S., & Gross, T. S. (2006). Polychlorinated biphenyls, mercury, and potential endocrine disruption in fish from the Hudson River, New York, USA. *Aquatic Sciences, 68*(2), 206-228. doi:10.1007/s00027-006-0831-8

Ballester-Molto, M., Sanchez-Jerez, P., & Aguado-Gimenez, F. (2017). Consumption of particulate wastes derived from cage fish farming by aggregated wild fish. An experimental approach. *Mar Environ Res, 130*, 166-173. doi:10.1016/j.marenvres.2017.07.014

Banda., S. F., & Mundia., A. Y. (2009). An assessment of the demand for DDT (A case study for Zambia) An assessment of the demand for DDT (A case study for Zambia).

. Current World Environment, 4, 293-298.

Basaran, A. K., Aksu, M., & Egemen, O. (2010). Impacts of the fish farms on the water column nutrient concentrations and accumulation of heavy metals in the sediments in the eastern Aegean Sea (Turkey). *Environmental Monitoring and Assessment, 162*(1), 439-451. doi:10.1007/s10661-009-0808-x

Beard, J. (2006). DDT and human health. *Science of The Total Environment, 355*(1), 78-89. doi:https://doi.org/10.1016/j.scitotenv.2005.02.022

Béné, C., Barange, M., Subasinghe, R., Pinstrup-Andersen, P., Merino, G., Hemre, G.-I., & Williams, M. (2015). Feeding 9 billion by 2050 – Putting fish back on the menu. *Food Security, 7*(2), 261-274. doi:10.1007/s12571-015-0427-z

Berg, V. (2013). *Effects of natural mixtures of persistent organic pollutants (POPs) in wild fish, a fish model and cell cultures.* (Philosophiae Doctor (PhD) PhD), Norwegian School of Veterinary Science, Oslo. (160)

Berg, V., Kraugerud, M., Nourizadeh-Lillabadi, R., Olsvik, P. A., Skåre, J. U., Alestrøm, P., . . . Lyche, J. L. (2016). Endocrine effects of real-life mixtures of persistent organic pollutants (POP) in experimental models and wild fish. *Journal of Toxicology and Environmental Health, Part A, 79*(13-15), 538-548. doi:10.1080/15287394.2016.1171980

Bernard, A. (2008). Cadmium & its adverse effects on human health. *The Indian journal of medical research, 128*(4), 557-564.

Bernhoft, R. A. (2012). Mercury Toxicity and Treatment: A Review of the Literature. *Journal of Environmental and Public Health, 2012*, 460508. doi:10.1155/2012/460508

Beser, M. I., Beltrán, J., & Yusà, V. (2014). Design of experiment approach for the optimization of polybrominated diphenyl ethers determination in fine airborne particulate matter by microwave-assisted extraction and gas chromatography coupled to tandem mass spectrometry. *Journal of Chromatography A, 1323*, 1-10. doi:https://doi.org/10.1016/j.chroma.2013.10.081

Bielmyer, G. K., Bullington, J. B., Decarlo, C. A., Chalk, S. J., & Smith, K. (2012). The effects of salinity on acute toxicity of zinc to two euryhaline species of fish, Fundulus heteroclitus and Kryptolebias marmoratus. *Integr Comp Biol, 52*(6), 753-760. doi:10.1093/icb/ics045

Birnbaum, L. S., & Grandjean, P. (2015). Alternatives to PFASs: Perspectives on the Science. *123*(5), A104-A105. doi:doi:10.1289/ehp.1509944

Bo Strandberg, & Hites, R. A. (2001). Concentration of organochlorine pesticides in wine corks. *Chemosphere 44*, 729-735.

Bondad-Reantaso, M. G., Subasinghe, R. P., Arthur, J. R., Ogawa, K., Chinabut, S., Adlard, R., . . . Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinary parasitology, 132*(3-4), 249-272. doi:10.1016/j.vetpar.2005.07.005

Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E., & Hoffman, L. C. (2016). Heavy metals in marine fish meat and consumer health: a review. *96*(1), 32-48. doi:https://doi.org/10.1002/jsfa.7360

Botaro, D., Torres, J. P., Malm, O., Rebelo, M. F., Henkelmann, B., & Schramm, K. W. (2011). Organochlorine pesticides residues in feed and muscle of farmed Nile tilapia from Brazilian fish farms. *Food Chem Toxicol, 49*(9), 2125-2130. doi:10.1016/j.fct.2011.05.027

Bouwman, H. (2003). POPs in Southern Africa. In H. Fiedler (Ed.), *The Handbook of Environmental Chemistry Vol.3,Part O Persistent Organic Pollutants* (Vol. 3, pp. 298-317): Springer-Verlag Berlin Heidelberg.

Boyle, D., Brix, K. V., Amlund, H., Lundebye, A.-K., Hogstrand, C., & Bury, N. R. (2008). Natural Arsenic Contaminated Diets Perturb Reproduction in Fish. *Environmental Science & Technology, 42*(14), 5354-5360. doi:10.1021/es800230w

Burridge, L., Weis, J. S., Cabello, F., Pizarro, J., & Bostick, K. (2010). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture, 306*(1), 7-23. doi:https://doi.org/10.1016/j.aquaculture.2010.05.020

Camara, K. D., Carpenter, K. E., Djiman, R., Nunoo, F., Sagna, A., Sidibé, A., . . . Sparks, J. S. (2019). Mugil cephalus. The IUCN Red List of Threatened Species 2019: e.T135567A127923853. (Publication no. https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T135567A127923853.en).

Carolin, C. F., Kumar, P. S., Saravanan, A., Joshiba, G. J., & Naushad, M. (2017). Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *Journal of Environmental Chemical Engineering, 5*(3), 2782-2799. doi:https://doi.org/10.1016/j.jece.2017.05.029

Chain, E. P. o. C. i. t. F. (2011). Scientific Opinion on Tetrabromobisphenol A (TBBPA) and its derivatives in food. *9*(12), 2477. doi:https://doi.org/10.2903/j.efsa.2011.2477

Chan, C. Y., Tran, N., Pethiyagoda, S., Crissman, C. C., Sulser, T. B., & Phillips, M. J. (2019). Prospects and challenges of fish for food security in Africa. *Global Food Security, 20*, 17-25. doi:10.1016/j.gfs.2018.12.002

Cohen, S. M., Arnold, L. L., Eldan, M., Lewis, A. S., & Beck, B. D. (2006). Methylated Arsenicals: The Implications of Metabolism and Carcinogenicity Studies in Rodents to Human Risk

Assessment. *Critical Reviews in Toxicology, 36*(2), 99-133. doi:10.1080/10408440500534230

Conis, E. (2010). Debating the health effects of DDT: Thomas Jukes, Charles Wurster, and the fate of an environmental pollutant. *Public Health Rep, 125*(2), 337-342. doi:10.1177/003335491012500224

Copat, C., Grasso, A., Fiore, M., Cristaldi, A., Zuccarello, P., Signorelli, S. S., . . . Ferrante, M. (2018). Trace elements in seafood from the Mediterranean sea: An exposure risk assessment. *Food and Chemical Toxicology, 115*, 13-19. doi:https://doi.org/10.1016/j.fct.2018.03.001

Coperchini, F., Croce, L., Ricci, G., Magri, F., Rotondi, M., Imbriani, M., & Chiovato, L. (2021). Thyroid Disrupting Effects of Old and New Generation PFAS. *11*(1077). doi:10.3389/fendo.2020.612320

Cordner, A., De La Rosa, V. Y., Schaider, L. A., Rudel, R. A., Richter, L., & Brown, P. (2019). Guideline levels for PFOA and PFOS in drinking water: the role of scientific uncertainty, risk assessment decisions, and social factors. *Journal of Exposure Science & Environmental Epidemiology, 29*(2), 157-171. doi:10.1038/s41370-018-0099-9

Covaci, A., Voorspoels, S., Roosens, L., Jacobs, W., Blust, R., & Neels, H. (2008). Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in human liver and adipose tissue samples from Belgium. *Chemosphere, 73*(2), 170-175. doi:https://doi.org/10.1016/j.chemosphere.2008.02.059

Cristale, J., Hurtado, A., Gómez-Canela, C., & Lacorte, S. (2016). Occurrence and sources of brominated and organophosphorus flame retardants in dust from different indoor environments in Barcelona, Spain. *Environmental Research, 149*, 66-76. doi:https://doi.org/10.1016/j.envres.2016.05.001

Cuesta, A., Meseguer, J., & Ángeles Esteban, M. (2008). Effects of the organochlorines p,p′-DDE and lindane on gilthead seabream leucocyte immune parameters and gene expression. *Fish & Shellfish Immunology, 25*(5), 682-688. doi:https://doi.org/10.1016/j.fsi.2008.02.006

Datta, S., Ghosh, D., Saha, D. R., Bhattacharaya, S., & Mazumder, S. (2009). Chronic exposure to low concentration of arsenic is immunotoxic to fish: Role of head kidney macrophages as biomarkers of arsenic toxicity to Clarias batrachus. *Aquatic Toxicology, 92*(2), 86-94. doi:https://doi.org/10.1016/j.aquatox.2009.01.002

David, O. C. (2006). Polychlorinated Biphenyls (PCBs): Routes of Exposure and Effects on Human Health %J Reviews on Environmental Health. *21*(1), 1-24. doi:doi:10.1515/REVEH.2006.21.1.1

de Boer, J., & Cofino, W. P. (2002). First world-wide interlaboratory study on polybrominated diphenylethers (PBDEs). *Chemosphere, 46*(5), 625-633. doi:https://doi.org/10.1016/S0045-6535(01)00226-0

De Smet, H., De Wachter, B., Lobinski, R., & Blust, R. (2001). Dynamics of (Cd,Zn)-metallothioneins in gills, liver and kidney of common carp Cyprinus carpio during cadmium exposure. *Aquatic Toxicology, 52*(3), 269-281. doi:https://doi.org/10.1016/S0166-445X(00)00136-3

Defarge, N., Spiroux de Vendômois, J., & Séralini, G. E. (2018). Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides. *Toxicology Reports, 5*, 156-163. doi:https://doi.org/10.1016/j.toxrep.2017.12.025

Dietz, R., Letcher, R. J., Desforges, J.-P., Eulaers, I., Sonne, C., Wilson, S., . . . Víkingsson, G. (2019). Current state of knowledge on biological effects from contaminants on arctic wildlife and fish. *Science of The Total Environment, 696*, 133792. doi:https://doi.org/10.1016/j.scitotenv.2019.133792

Domingo, J. L., & Nadal, M. (2019). Human exposure to per- and polyfluoroalkyl substances (PFAS) through drinking water: A review of the recent scientific literature. *Environmental Research, 177*, 108648. doi:https://doi.org/10.1016/j.envres.2019.108648

Dos Santos, C. R., Cavalcante, A. L. M., Hauser-Davis, R. A., Lopes, R. M., & Da Costa Mattos, R. D. C. O. (2016). Effects of sub-lethal and chronic lead concentrations on blood and liver ALA-D activity and hematological parameters in Nile tilapia. *Ecotoxicology and Environmental Safety, 129*, 250-256. doi:https://doi.org/10.1016/j.ecoenv.2016.03.028

Dutton, J., & Fisher, N. S. (2011). Salinity effects on the bioavailability of aqueous metals for the estuarine killifish Fundulus heteroclitus. *30*(9), 2107-2114. doi:https://doi.org/10.1002/etc.600

EC, E. C. (2000). *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. OJ L 327 (22 December 2000).* Retrieved from https://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF

EC, E. C. (2006). *COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.* Retrieved from IOP Publishing PhysicsWep: https://www.fsai.ie/uploadedFiles/Consol_Reg1881_2006.pdf

COMMISSION REGULATION (EU) No 1259/2011-amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuf, (2011).

DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, (2013).

Echegoyen, Y., Boyle, E. A., Lee, J.-M., Gamo, T., Obata, H., & Norisuye, K. (2014). Recent distribution of lead in the Indian Ocean reflects the impact of regional emissions. *Proceedings of the National Academy of Sciences, 111*(43), 15328. doi:10.1073/pnas.1417370111

ECZ, E. c. o. Z. (2007). *National Implementation Plan (NIP) for the management of PersistentnOrganic Pollutants (POPs) in Zambia April 2007.* Retrieved from https://wedocs.unep.org/rest/bitstreams/16208/retrieve

Erickson, M. D., & Kaley, R. G. (2011). Applications of polychlorinated biphenyls. *Environmental Science and Pollution Research, 18*(2), 135-151. doi:10.1007/s11356-010-0392-1

Espana, A., Victor, Andres, Mallavarapu, M., & Naidu, R. (2015). Treatment technologies for aqueous perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA): A critical

review with an emphasis on field testing. *Environmental Technology & Innovation, 4*, 168-181. doi:https://doi.org/10.1016/j.eti.2015.06.001

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J. L., & Nadal, M. (2014). PBPK modeling for PFOS and PFOA: validation with human experimental data. *Toxicol Lett, 230*(2), 244-251. doi:10.1016/j.toxlet.2014.01.007

Fallah, A. A., Saei-Dehkordi, S. S., Nematollahi, A., & Jafari, T. (2011). Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (Oncorhynchus mykiss) using ICP-OES technique. *Microchemical Journal, 98*(2), 275-279. doi:https://doi.org/10.1016/j.microc.2011.02.007

FAO. (2005-2019). Cultured Aquatic Species Information Programme. Oreochromis niloticus. Cultured Aquatic Species Information Programme. Text by Rakocy, J. E. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 18 February 2005. [Cited 23 October 2019]. Retrieved 23/10/2019 www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en

FAO. (2006-2021). Cultured Aquatic Species Information Programme. Mugil cephalus. Cultured Aquatic Species Information Programme. Text by Saleh, M.A. In: FAO Fisheries Division [online]. Rome. Updated 7 April 2006.[Cited 9 February 2021] .

FAO. (2007). THE STATE OF WORLD FISHERIES AND AQUACULTURE 2006. *FAO (Fisheries and Aquaculture Department, Rome, 2007)*, 1-180.

FAO. (2007-2021). Cultured Aquatic Species Information Programme. Chanos chanos. Cultured Aquatic Species Information Programme. Text by Nelson, A.L.; Marygrace C.Q. In: FAO Fisheries Division [online]. Rome. Updated 23 November 2007. . Retrieved Cited 9 February 2021

FAO. (2018). *THE STATE OF WORLD FISHERIES AND AQUACULTURE - MEETING THE SUSTAINABLE DEVELOPMENT GOALS. Rome.*

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. doi:doi.org/10.4060/ca9229en

FAO/WHO. (2002). *Codex Alimentarius, Schedule 1 of the proposed draft Codex general standards for contaminants and toxins in food. Joint FAO/WHO Food Standards Programme, Codex Committee, Rotterdam*. Retrieved from PhysicsWep: http://www.fao.org/input/download/report/28/Al03_12e.pdf.

Fayiga, A. O., Ipinmoroti, M. O., & Chirenje, T. (2017). Environmental pollution in Africa. *Environment, Development and Sustainability, 20*(1), 41-73. doi:10.1007/s10668-016-9894-4

Fernandez, P., & Grimalt, J. (2003). On the Global Distribution of Persistent Organic Pollutants. *CHIMIA International Journal for Chemistry, 57*, 514-521. doi:10.2533/000942903777679000

Fiedler, H., & Sadia, M. (2021). Regional occurrence of perfluoroalkane substances in human milk for the global monitoring plan under the Stockholm Convention on Persistent Organic Pollutants during 2016–2019. *Chemosphere, 277*, 130287. doi:https://doi.org/10.1016/j.chemosphere.2021.130287

Fiedler, H., van der Veen, I., & de Boer, J. (2020). Global interlaboratory assessments of perfluoroalkyl substances under the Stockholm Convention on persistent organic pollutants. *TrAC Trends in Analytical Chemistry, 124*, 115459. doi:https://doi.org/10.1016/j.trac.2019.03.023

Fliedner, A., Lohmann, N., Rüdel, H., Teubner, D., Wellmitz, J., & Koschorreck, J. (2016). Current levels and trends of selected EU Water Framework Directive priority substances in freshwater fish from the German environmental specimen bank. *Environmental Pollution, 216*, 866-876. doi:https://doi.org/10.1016/j.envpol.2016.06.060

Geiger, S. D., Yao, P., Vaughn, M. G., & Qian, Z. (2021). PFAS exposure and overweight/obesity among children in a nationally representative sample. *Chemosphere, 268*, 128852. doi:https://doi.org/10.1016/j.chemosphere.2020.128852

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The Effects of Cadmium Toxicity. *Int J Environ Res Public Health, 17*(11). doi:10.3390/ijerph17113782

Genschick, S., Kaminski, A. M., Kefi, A. S., & Cole, S. M. (2017). Aquaculture in Zambia- An overview and evaluation of the sector's responsiveness to the needs of the poor. Penang, Malaysia: CGIAR Research Program on Fish Agri-Food Systems and Lusaka, Zambia: Department of Fisheries. Working Paper: FISH-2017-08.

Gensemer, R. W., & Playle, R. C. (1999). The Bioavailability and Toxicity of Aluminum in Aquatic Environments. *Critical Reviews in Environmental Science and Technology, 29*(4), 315-450. doi:10.1080/10643389991259245

Gidlow, D. A. (2015). Lead toxicity. *Occupational medicine (Oxford, England), 65*(5), 348-356. doi:10.1093/occmed/kqv018

Gimeno-García, E., Andreu, V., & Boluda, R. (1996). Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. *Environ Pollut, 92*(1), 19-25. doi:10.1016/0269-7491(95)00090-9

Glencross, B. D., Baily, J., Berntssen, M. H. G., Hardy, R., MacKenzie, S., & Tocher, D. R. (2020). Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. *12*(2), 703-758. doi:https://doi.org/10.1111/raq.12347

Glüge, J., Scheringer, M., Cousins, I. T., DeWitt, J. C., Goldenman, G., Herzke, D., . . . Wang, Z. (2020). An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environmental Science: Processes & Impacts, 22*(12), 2345-2373. doi:10.1039/D0EM00291G

Gonzalez, H. O., Hu, J., Gaworecki, K. M., Roling, J. A., Baldwin, W. S., Gardea-Torresdey, J. L., & Bain, L. J. (2010). Dose-responsive gene expression changes in juvenile and adult mummichogs (Fundulus heteroclitus) after arsenic exposure. *Marine Environmental Research, 70*(2), 133-141. doi:https://doi.org/10.1016/j.marenvres.2010.04.003

Green, A. J., & Planchart, A. (2018). The neurological toxicity of heavy metals: A fish perspective. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 208*, 12-19. doi:https://doi.org/10.1016/j.cbpc.2017.11.008

Grimm, F. A., Hu, D., Kania-Korwel, I., Lehmler, H.-J., Ludewig, G., Hornbuckle, K. C., . . . Robertson, L. W. (2015). Metabolism and metabolites of polychlorinated biphenyls. *Critical Reviews in Toxicology, 45*(3), 245-272. doi:10.3109/10408444.2014.999365

Groffen, T., Wepener, V., Malherbe, W., & Bervoets, L. (2018). Distribution of perfluorinated compounds (PFASs) in the aquatic environment of the industrially polluted Vaal River, South Africa. *Science of The Total Environment, 627*, 1334-1344. doi:https://doi.org/10.1016/j.scitotenv.2018.02.023

Gupta, P. K. (2004). Pesticide exposure--Indian scene. *Toxicology, 198*(1-3), 83-90. doi:10.1016/j.tox.2004.01.021

Hamilton, S. J., & Mehrle, P. M. (1986). Metallothionein in Fish: Review of Its Importance in Assessing Stress from Metal Contaminants. *115*(4), 596-609. doi:https://doi.org/10.1577/1548-8659(1986)115<596:MIF>2.0.CO;2

Han, J., & Fang, Z. (2010). Estrogenic effects, reproductive impairment and developmental toxicity in ovoviparous swordtail fish (Xiphophorus helleri) exposed to perfluorooctane sulfonate (PFOS). *Aquatic Toxicology, 99*(2), 281-290. doi:https://doi.org/10.1016/j.aquatox.2010.05.010

Hap, N., UN, S., & NASIELSKI, J. (2016). *A review of socioeconomic studies in the fisheries sector in Cambodia* Retrieved from Phnom Penh, Cambodia. 14 pp.:

Henry, L., & Kishimba, M. A. (2006). Pesticide residues in Nile tilapia (Oreochromis niloticus) and Nile perch (Lates niloticus) from Southern Lake Victoria, Tanzania. *Environ Pollut, 140*(2), 348-354. doi:10.1016/j.envpol.2005.06.029

Henry, T. B. (2015). Ecotoxicology of polychlorinated biphenyls in fish--a critical review. *Crit Rev Toxicol, 45*(8), 643-661. doi:10.3109/10408444.2015.1038498

Hepburn, E., Madden, C., Szabo, D., Coggan, T. L., Clarke, B., & Currell, M. (2019). Contamination of groundwater with per- and polyfluoroalkyl substances (PFAS) from legacy landfills in an urban re-development precinct. *Environmental Pollution, 248*, 101-113. doi:https://doi.org/10.1016/j.envpol.2019.02.018

Heuvel, J. P. V., Kuslikis, B. I., Van Rafelghem, M. J., & Peterson, R. E. (1991). Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *6*(2), 83-92. doi:https://doi.org/10.1002/jbt.2570060202

Hou, S., Yuan, L., Jin, P., Ding, B., Qin, N., Li, L., . . . Deng, Y. (2013). A clinical study of the effects of lead poisoning on the intelligence and neurobehavioral abilities of children. *Theoretical Biology and Medical Modelling, 10*(1), 13. doi:10.1186/1742-4682-10-13

Hu, G., Bakhtavar, E., Hewage, K., Mohseni, M., & Sadiq, R. (2019). Heavy metals risk assessment in drinking water: An integrated probabilistic-fuzzy approach. *J Environ Manage, 250*, 109514. doi:10.1016/j.jenvman.2019.109514

Humans, I. W. G. o. t. E. o. C. R. t. (2006). Inorganic and organic lead compounds. *IARC Monogr Eval Carcinog Risks Hum, 87*, 1-471.

Ibrahim, M., Sayed, El-Sherif, A., Shaban, Abdel-Ghafour, S., Abouzied, S., Abdel-Rahman, Rabea, M., Hassan, El-Lahamy, A., Adel, . . . Abo–Zeid, S., Kamel. (2020). Effect of location and grilling process on heavy metals concentration in muscles of different fish species, Egypt %J Egyptian Journal of Aquatic Biology and Fisheries. *24*(6), 15-24. doi:10.21608/ejabf.2020.109083

IPCS. (2004). *The International Programme on Chemical Safety. Risk Assessment Terminology*. Retrieved from http://www.inchem.org/documents/harmproj/harmproj/harmproj1.pdf.

IPCS. (2009). *Principles and methods for the risk assessment of chemicals in food* Retrieved from Stuttgart, Germany: https://apps.who.int/iris/bitstream/handle/10665/44065/WHO_EHC_240_13_eng_Annex 1.pdf?sequence=13

Jarić, I., Višnjić-Jeftić, Ž., Cvijanović, G., Gačić, Z., Jovanović, L., Skorić, S., & Lenhardt, M. (2011). Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES. *Microchemical Journal, 98*(1), 77-81. doi:https://doi.org/10.1016/j.microc.2010.11.008

Javed, M., & Usmani, N. (2017). An Overview of the Adverse Effects of Heavy Metal Contamination on Fish Health. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 89*(2), 389-403. doi:10.1007/s40011-017-0875-7

Javed., M., & Usmani., N. (2017). An Overview of the Adverse Effects of Heavy Metal Contamination on Fish Health

The National Academy of Sciences, India. doi:10.1007/s40011-017-0875-7

Jayaraj, R., Megha, P., & Sreedev, P. (2016). Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdiscip Toxicol, 9*(3-4), 90-100. doi:10.1515/intox-2016-0012

Jensen, A. (2015). Hexabromocyclododecane (HBCDD) - A Hazardous Flame Retardant Used in Polystyrene Building Materials. In (pp. 25-31).

Jin, Y., Liu, Z., Liu, F., Ye, Y., Peng, T., & Fu, Z. (2015). Embryonic exposure to cadmium (II) and chromium (VI) induce behavioral alterations, oxidative stress and immunotoxicity in zebrafish (Danio rerio). *Neurotoxicology and Teratology, 48*, 9-17. doi:https://doi.org/10.1016/j.ntt.2015.01.002

Jørgensen, E. H., Vijayan, M. M., Killie, J. E., Aluru, N., Aas-Hansen, Ø., & Maule, A. (2006). Toxicokinetics and effects of PCBs in Arctic fish: a review of studies on Arctic charr. *J Toxicol Environ Health A, 69*(1-2), 37-52. doi:10.1080/15287390500259053

Jurgens, M. D., Crosse, J., Hamilton, P. B., Johnson, A. C., & Jones, K. C. (2016). The long shadow of our chemical past - High DDT concentrations in fish near a former agrochemicals factory in England. *Chemosphere, 162*, 333-344. doi:10.1016/j.chemosphere.2016.07.078

K'Oreje, K. O., Okoth, M., Van Langenhove, H., & Demeestere, K. (2020). Occurrence and treatment of contaminants of emerging concern in the African aquatic environment: Literature review and a look ahead. *Journal of Environmental Management, 254*, 109752. doi:https://doi.org/10.1016/j.jenvman.2019.109752

Kabasenche, W. P., & Skinner, M. K. (2014). DDT, epigenetic harm, and transgenerational environmental justice. *Environmental Health, 13*(1), 62. doi:10.1186/1476-069X-13-62

Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J. F., Hansen, K. J., . . . Giesy, J. P. (2001). Accumulation of Perfluorooctane Sulfonate in Marine Mammals. *Environmental Science & Technology, 35*(8), 1593-1598. doi:10.1021/es001873w

Kapoor, D., & Singh, M. P. (2021). 10 - Heavy metal contamination in water and its possible sources. In V. Kumar, A. Sharma, & A. Cerdà (Eds.), *Heavy Metals in the Environment* (pp. 179-189): Elsevier.

Katti, S. R., & Sathyanesan, A. C. (1983). Lead nitrate induced changes in lipid and cholesterol levels in the freshwater fish Clarias batrachus. *Toxicology Letters, 19*(1), 93-96. doi:https://doi.org/10.1016/0378-4274(83)90267-9

Kaya, H., Duysak, M., Akbulut, M., Yılmaz, S., Gürkan, M., Arslan, Z., . . . Ateş, M. (2017). Effects of subchronic exposure to zinc nanoparticles on tissue accumulation, serum biochemistry, and histopathological changes in tilapia (Oreochromis niloticus). *Environ Toxicol, 32*(4), 1213-1225. doi:10.1002/tox.22318

Kefeni, Okonkwo, O., Olukunle, O., & Botha, B. (2011). Brominated flame retardants: Sources, distribution, exposure pathways, and toxicity. *Environmental Reviews, 19*. doi:10.1139/a11-010

Kim, J.-H., & Kang, J.-C. (2015). The lead accumulation and hematological findings in juvenile rock fish Sebastes schlegelii exposed to the dietary lead (II) concentrations. *Ecotoxicology and Environmental Safety, 115*, 33-39. doi:https://doi.org/10.1016/j.ecoenv.2015.02.009

Koistinen, J., Kiviranta, H., Ruokojärvi, P., Parmanne, R., Verta, M., Hallikainen, A., & Vartiainen, T. (2008). Organohalogen pollutants in herring from the northern Baltic Sea: Concentrations, congener profiles and explanatory factors. *Environmental Pollution, 154*(2), 172-183. doi:https://doi.org/10.1016/j.envpol.2007.10.019

Konradsen, F., van der Hoek, W., Cole, D. C., Hutchinson, G., Daisley, H., Singh, S., & Eddleston, M. (2003). Reducing acute poisoning in developing countries—options for restricting the

availability of pesticides. *Toxicology, 192*(2), 249-261. doi:https://doi.org/10.1016/S0300-483X(03)00339-1

Kudo, N. (2015). Metabolism and Pharmacokinetics. In J. C. DeWitt (Ed.), *Toxicological Effects of Perfl uoroalkyl and Polyfl uoroalkyl Substances* (pp. 151-176). Springer International Publishing Switzerland Humana Press.

Kuo, C.-C., Moon, K. A., Wang, S.-L., Silbergeld, E., & Navas-Acien, A. (2017). The Association of Arsenic Metabolism with Cancer, Cardiovascular Disease, and Diabetes: A Systematic Review of the Epidemiological Evidence. *Environmental health perspectives, 125*(8), 087001-087001. doi:10.1289/EHP577

Lallas, P. L. (2001). The Stockholm Convention on Persistent Organic Pollutants. *The American Journal of International Law, 95*(3), 692-708. doi:10.2307/2668517

Langford, N. J., & Ferner, R. E. (1999). Toxicity of mercury. *Journal of Human Hypertension, 13*(10), 651-656. doi:10.1038/sj.jhh.1000896

Lee, J.-W., Choi, H., Hwang, U.-K., Kang, J.-C., Kang, Y. J., Kim, K. I., & Kim, J.-H. (2019). Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental Toxicology and Pharmacology, 68*, 101-108. doi:https://doi.org/10.1016/j.etap.2019.03.010

Lee, J. W., Choi, K., Park, K., Seong, C., Yu, S. D., & Kim, P. (2020). Adverse effects of perfluoroalkyl acids on fish and other aquatic organisms: A review. *Science of The Total Environment, 707*, 135334. doi:https://doi.org/10.1016/j.scitotenv.2019.135334

Leslie, E. M. (2012). Arsenic–glutathione conjugate transport by the human multidrug resistance proteins (MRPs/ABCCs). *Journal of Inorganic Biochemistry, 108*, 141-149. doi:https://doi.org/10.1016/j.jinorgbio.2011.11.009

Letcher, R. J., Bustnes, J. O., Dietz, R., Jenssen, B. M., Jorgensen, E. H., Sonne, C., . . . Gabrielsen, G. W. (2010). Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Sci Total Environ, 408*(15), 2995-3043. doi:10.1016/j.scitotenv.2009.10.038

Leung, H. M., Leung, A. O. W., Wang, H. S., Ma, K. K., Liang, Y., Ho, K. C., . . . Yung, K. K. L. (2014). Assessment of heavy metals/metalloid (As, Pb, Cd, Ni, Zn, Cr, Cu, Mn) concentrations in edible fish species tissue in the Pearl River Delta (PRD), China. *Marine Pollution Bulletin, 78*(1), 235-245. doi:https://doi.org/10.1016/j.marpolbul.2013.10.028

Li, X., Li, J., Wang, Y., Fu, L., Fu, Y., Li, B., & Jiao, B. (2011). Aquaculture Industry in China: Current State, Challenges, and Outlook. *Reviews in Fisheries Science, 19*(3), 187-200. doi:10.1080/10641262.2011.573597

Liu, J., Xu, X., Yu, S., Cheng, H., Hong, Y., & Feng, X. (2014). Mercury pollution in fish from South China Sea: Levels, species-specific accumulation, and possible sources. *Environmental Research, 131*, 160-164. doi:https://doi.org/10.1016/j.envres.2014.03.004

Liu, Q., Tian, S., Jia, R., & Liu, X. (2017). Pollution characteristics and ecological risk assessment of HCHs and DDTs in estuary wetland sediments from the Bohai Bay, North China. *Environmental Science and Pollution Research, 24*(35), 26967-26973. doi:10.1007/s11356-015-5882-8

Ljunggren, S. A., Helmfrid, I., Salihovic, S., van Bavel, B., Wingren, G., Lindahl, M., & Karlsson, H. (2014). Persistent organic pollutants distribution in lipoprotein fractions in relation to cardiovascular disease and cancer. *Environment International, 65*, 93-99. doi:https://doi.org/10.1016/j.envint.2013.12.017

Longnecker, M., P. (2005). REVIEW OF HUMAN DATA ON EFFECTS OF DDT, AND SOME NEW RESULTS. *16*(5), S34.

Longnecker, M. P., Rogan, W. J., & Lucier, G. (1997). THE HUMAN HEALTH EFFECTS OF DDT (DICHLORODIPHENYLTRICHLOROETHANE) AND PCBS (POLYCHLORINATED BIPHENYLS) AND AN OVERVIEW OF ORGANOCHLORINES IN PUBLIC HEALTH. *18*(1), 211-244. doi:10.1146/annurev.publhealth.18.1.211

Luo, Y.-L., Luo, X.-J., Ye, M.-X., Lin, L., Zeng, Y.-H., & Mai, B.-X. (2019). Species-specific debromination of polybromodiphenyl ethers determined by deiodinase activity in fish. *Environmental Pollution, 246*, 710-716. doi:https://doi.org/10.1016/j.envpol.2018.12.089

Lyche, J. L., Rosseland, C., Berge, G., & Polder, A. (2015). Human health risk associated with brominated flame-retardants (BFRs). *Environment International, 74*, 170-180. doi:https://doi.org/10.1016/j.envint.2014.09.006

MALF, Ministry, of, Agriculture, Livestock, and, & Fishery. (2016). *"Tanzania fisheries sector; challenges and opportunities"*. Retrieved from https://www.wiomsa.org/download/the-tanzanian-fisheries-sectors-challengesand-opportunities/

Malhotra, N., Ger, T.-R., Uapipatanakul, B., Huang, J.-C., Chen, K. H.-C., & Hsiao, C.-D. (2020). Review of Copper and Copper Nanoparticle Toxicity in Fish. *10*(6), 1126.

Mansour, S. A., & Sidky, M. M. (2002). Ecotoxicological Studies. 3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food Chemistry, 78*(1), 15-22. doi:https://doi.org/10.1016/S0308-8146(01)00197-2

Mansouri, A., Cregut, M., Abbes, C., Durand, M.-J., Landoulsi, A., & Thouand, G. (2017). The Environmental Issues of DDT Pollution and Bioremediation: a Multidisciplinary Review. *Applied Biochemistry and Biotechnology, 181*(1), 309-339. doi:10.1007/s12010-016-2214-5

Mapenzi, L. L., Shimba, M. J., Moto, E. A., Maghembe, R. S., & Mmochi, A. J. (2020). Heavy metals bio-accumulation in tilapia and catfish species in Lake Rukwa ecosystem Tanzania. *Journal of Geochemical Exploration, 208*, 106413. doi:https://doi.org/10.1016/j.gexplo.2019.106413

Marengo, M., Durieux, E. D. H., Ternengo, S., Lejeune, P., Degrange, E., Pasqualini, V., & Gobert, S. (2018). Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health. *Ecotoxicology and Environmental Safety, 158*, 204-212. doi:https://doi.org/10.1016/j.ecoenv.2018.04.034

Matthews, H., Fries, G., Gardner, A., Garthoff, L., Goldstein, J., Ku, Y., & Moore, J. (1978). Metabolism and Biochemical Toxicity of PCBs and PBBs. *Environmental health perspectives, 24*, 147-155. doi:10.2307/3428807

Maurya, P. K., Malik, D. S., Yadav, K. K., Kumar, A., Kumar, S., & Kamyab, H. (2019). Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. *Toxicology Reports, 6*, 472-481. doi:https://doi.org/10.1016/j.toxrep.2019.05.012

Misumi, I., Vella, A. T., Leong, J.-A. C., Nakanishi, T., & Schreck, C. B. (2005). p,p′-DDE depresses the immune competence of chinook salmon (Oncorhynchus tshawytscha) leukocytes. *Fish & Shellfish Immunology, 19*(2), 97-114. doi:https://doi.org/10.1016/j.fsi.2004.11.005

Mohamed Shaalan, Magdy El-Mahdy, Mona Saleh, & El-Matbouli, M. (2018). Aquaculture in Egypt: Insights on the Current Trends and Future Perspectives for Sustainable Development. *Reviews in Fisheries Science & Aquaculture, 26*(1), 99-110. doi:10.1080/23308249.2017.1358696

Moiseenko, T. I., & Gashkina, N. A. (2020). Distribution and bioaccumulation of heavy metals (Hg, Cd and Pb) in fish: influence of the aquatic environment and climate. *Environmental Research Letters, 15*(11), 115013. doi:10.1088/1748-9326/abbf7c

Moon, K., Guallar, E., & Navas-Acien, A. (2012). Arsenic Exposure and Cardiovascular Disease:An Updated Systematic Review. *Current Atherosclerosis Reports, 14*(6), 542-555. doi:10.1007/s11883-012-0280-x

Morato, T., Watson, R., Pitcher, T. J., & Pauly, D. (2006). Fishing down the deep. *7*(1), 24-34. doi:https://doi.org/10.1111/j.1467-2979.2006.00205.x

Mosha, S. S., & Daudi, J. M. (2020). A Review of Aquaculture Production in Tanzania; Recent Status, Challenges and

Opportunities, and Its Impact in Poverty Alleviation. *Sumerianz Journal of Agriculture and Veterinary*(38), 107-115. doi:10.47752/sjav.38.107.115

Mrema, E. J., Rubino, F. M., Brambilla, G., Moretto, A., Tsatsakis, A. M., & Colosio, C. (2013). Persistent organochlorinated pesticides and mechanisms of their toxicity. *Toxicology, 307*, 74-88. doi:10.1016/j.tox.2012.11.015

Müller, M., Polder, A., Brynildsrud, O., Karimi, M., Lie, E., Manyilizu, W., . . . Lyche, J. (2017). Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in human breast milk and associated health risks to nursing infants in Northern Tanzania. *Environmental research, 154*, 425-434. doi:10.1016/j.envres.2017.01.031

Mwakalapa, E. B., Simukoko, C. K., Mmochi, A. J., Mdegela, R. H., Berg, V., Bjorge Muller, M. H., . . . Polder, A. (2019). Heavy metals in farmed and wild milkfish (Chanos chanos) and wild mullet (Mugil cephalus) along the coasts of Tanzania and associated health risk for humans and fish. *Chemosphere, 224*, 176-186. doi:10.1016/j.chemosphere.2019.02.063

Mwakalapa., E. B., Mmochi., A. J., Müller., M. H. B., Mdegela., R. H., Lyche., J. L., & Polder., A. (2018). Occurrence and levels of persistent organic pollutants (POPs) in farmed and wild marine fish from Tanzania. A pilot study. *Chemosphere 191*, 438-449. doi:org/10.1016/j.chemosphere.2017.09.121

Naranmandura, H., Suzuki, N., & Suzuki, K. T. (2006). Trivalent Arsenicals Are Bound to Proteins during Reductive Methylation. *Chemical Research in Toxicology, 19*(8), 1010-1018. doi:10.1021/tx060053f

Nazir, R., Khan, M., Masab, M., Rehman, H. U., Rauf, N. U., Shahab, S., . . . Shaheen, Z. (2015). Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. *Journal of Pharmaceutical Sciences and Research, 7*, 89-97.

Neagu, M., Constantin, C., Bardi, G., & Duraes, L. (2021). Adverse Outcome Pathway in Immunotoxicity of Perfluoroalkyls. *Current Opinion in Toxicology*. doi:https://doi.org/10.1016/j.cotox.2021.02.001

Nie, X. P., Lan, C. Y., An, T. C., Wong, M. H., & Li, K. B. (2006). Distributions and Congener Patterns of PCBs in Fish from Major Aquaculture Areas in the Pearl River Delta, South China. *Human and Ecological Risk Assessment: An International Journal, 12*(2), 363-373. doi:10.1080/10807030500537908

Nie, Z., Yang, Z., Fang, Y., Yang, Y., Tang, Z., Wang, X., . . . Huang, Q. (2015). Environmental risks of HBCDD from construction and demolition waste: a contemporary and future issue.

Environmental science and pollution research international, 22. doi:10.1007/s11356-015-5487-2

Nyingi, B., Gitahi, K. J., Kiptoo, M., & Jackson, K. (2016). Heavy metas concentrations in water and selected fish species (tilapia, catfish and lungfish) from Lake Baringo, Kenya. *Int. J. Sci. Environ., 5*, 4288-4295.

Oakes, K. D., Sibley, P. K., Martin, J. W., Maclean, D. D., Solomon, K. R., Mabury, S. A., & Van Der Kraak, G. J. (2005). Short-term exposures of fish to perfluorooctane sulfonate: Acute effects on fatty acyl-CoA oxidase activity, oxidative stress, and circulating sex steroids. *24*(5), 1172-1181. doi:https://doi.org/10.1897/04-419.1

Olisah, C., Okoh, O. O., & Okoh, A. I. (2020). Occurrence of organochlorine pesticide residues in biological and environmental matrices in Africa: A two-decade review. *Heliyon, 6*(3), e03518. doi:https://doi.org/10.1016/j.heliyon.2020.e03518

Painter, S. C. (2020). The biogeochemistry and oceanography of the East African Coastal Current. *Progress in Oceanography, 186*, 102374. doi:https://doi.org/10.1016/j.pocean.2020.102374

Panseri, S., Chiesa, L., Ghisleni, G., Marano, G., Boracchi, P., Ranghieri, V., . . . Tecilla, M. (2019). Persistent organic pollutants in fish: biomonitoring and cocktail effect with implications for food safety. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 36*(4), 601-611. doi:10.1080/19440049.2019.1579926

Papanikolaou, N., Hatzidaki, E., Belivanis, S., Tzanakakis, G., & Tsatsakis, A. (2005). Lead toxicity update. A brief Review. *Medical science monitor : international medical journal of experimental and clinical research, 11*, RA329-336.

Pelch, K. E., Reade, A., Wolffe, T. A. M., & Kwiatkowski, C. F. (2019). PFAS health effects database: Protocol for a systematic evidence map. *Environment International, 130*, 104851. doi:https://doi.org/10.1016/j.envint.2019.05.045

Pius, C., Sichilongo, K., Mswela, P., Koosaletse, , & Dikinya, O. (2019). Monitoring polychlorinated dibenzo-p-dioxins/dibenzofurans and dioxin-like polychlorinated biphenyls in Africa since the implementation of the Stockholm Convention—an overview.

Environmental Science and Pollution Research, 26, 101–113. doi:https://doi.org/10.1007/s11356-018-3629-z

Plessl, C., Gilbert, B. M., Sigmund, M. F., Theiner, S., Avenant-Oldewage, A., Keppler, B. K., & Jirsa, F. (2019). Mercury, silver, selenium and other trace elements in three cyprinid fish species from the Vaal Dam, South Africa, including implications for fish consumers. *Science of The Total Environment, 659*, 1158-1167. doi:https://doi.org/10.1016/j.scitotenv.2018.12.442

Ploetz, D. M., Fitts, B. E., & Rice, T. M. (2007). Differential Accumulation of Heavy Metals in Muscle and Liver of a Marine Fish, (King Mackerel, Scomberomorus cavalla Cuvier) from the Northern Gulf of Mexico, USA. *Bulletin of Environmental Contamination and Toxicology, 78*(2), 134-137. doi:10.1007/s00128-007-9028-7

Pohanka, M. (2019). Copper and copper nanoparticles toxicity and their impact on basic functions in the body. *Bratislavske lekarske listy, 120*(6), 397-409. doi:10.4149/bll_2019_065

Polder, A., Muller, M. B., Lyche, J. L., Mdegela, R. H., Nonga, H. E., Mabiki, F. P., . . . Lie, E. (2014). Levels and patterns of persistent organic pollutants (POPs) in tilapia (Oreochromis sp.) from four different lakes in Tanzania: geographical differences and implications for human health. *Sci Total Environ, 488-489*, 252-260. doi:10.1016/j.scitotenv.2014.04.085

Polder, A., Thomsen, C., Lindström, G., Løken, K., & Skaare, J. (2008). Levels and temporal trends of chlorinated pesticides, polychlorinated biphenyls and brominated flame retardants in individual human breast milk samples from Northern and Southern Norway. *Chemosphere, 73*, 14-23. doi:10.1016/j.chemosphere.2008.06.002

Poléo, A. B. S. (1995). Aluminium polymerization — a mechanism of acute toxicity of aqueous aluminium to fish. *Aquatic Toxicology, 31*(4), 347-356. doi:https://doi.org/10.1016/0166-445X(94)00083-3

Poléo, A. B. S., Schjolden, J., Sørensen, J., & Nilsson, G. E. (2017). The high tolerance to aluminium in crucian carp (Carassius carassius) is associated with its ability to avoid hypoxia. *PloS one, 12*(6), e0179519-e0179519. doi:10.1371/journal.pone.0179519

Post, G., Gleason, J., & Cooper, K. (2017). Key scientific issues in developing drinking water guidelines for perfluoroalkyl acids: Contaminants of emerging concern. *PLoS biology, 15*, e2002855. doi:10.1371/journal.pbio.2002855

Pritchard, J. B. (1993). Aquatic toxicology: past, present, and prospects. *100*, 249-257. doi:doi:10.1289/ehp.93100249

Qin, D., Jiang, H., Bai, S., Tang, S., & Mou, Z. (2015). Determination of 28 trace elements in three farmed cyprinid fish species from Northeast China. *Food Control, 50*, 1-8. doi:https://doi.org/10.1016/j.foodcont.2014.08.016

Quig, D. (1998). Cysteine metabolism and metal toxicity. *Alternative Medicine Review, 3*(4), 262-270.

Rasheed, H., Slack, R., & Kay, P. (2016). Human health risk assessment for arsenic: A critical review. *Critical Reviews in Environmental Science and Technology, 46*(19-20), 1529-1583. doi:10.1080/10643389.2016.1245551

Rhind, S. M. (2002). Endocrine disrupting compounds and farm animals: their properties, actions and routes of exposure. *Domestic Animal Endocrinology, 23*(1), 179-187. doi:https://doi.org/10.1016/S0739-7240(02)00155-8

Rico, A., Satapornvanit, K., Haque, M. M., Min, J., Nguyen, P. T., Telfer, T. C., & van den Brink, P. J. (2012). Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review. *4*(2), 75-93. doi:https://doi.org/10.1111/j.1753-5131.2012.01062.x

Roberts, S. C., Noyes, P. D., Gallagher, E. P., & Stapleton, H. M. (2011a). Species-specific differences and structure-activity relationships in the debromination of PBDE congeners in three fish species. *Environmental science & technology, 45*(5), 1999-2005. doi:10.1021/es103934x

Roberts, S. C., Noyes, P. D., Gallagher, E. P., & Stapleton, H. M. (2011b). Species-Specific Differences and Structure−Activity Relationships in the Debromination of PBDE Congeners in Three Fish Species. *Environmental Science & Technology, 45*(5), 1999-2005. doi:10.1021/es103934x

Rogan, W. J., & Chen, A. (2005). Health risks and benefits of bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT). *Lancet (London, England), 366*(9487), 763-773. doi:10.1016/s0140-6736(05)67182-6

Rogers, J. T., Richards, J. G., & Wood, C. M. (2003). Ionoregulatory disruption as the acute toxic mechanism for lead in the rainbow trout (Oncorhynchus mykiss). *Aquatic Toxicology, 64*(2), 215-234. doi:https://doi.org/10.1016/S0166-445X(03)00053-5

Saha, J. C., Dikshit, A. K., Bandyopadhyay, M., & Saha, K. C. (1999). A Review of Arsenic Poisoning and its Effects on Human Health. *Critical Reviews in Environmental Science and Technology, 29*(3), 281-313. doi:10.1080/10643389991259227

Saha, N., Mollah, M. Z. I., Alam, M. F., & Safiur Rahman, M. (2016). Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. *Food Control, 70*, 110-118. doi:https://doi.org/10.1016/j.foodcont.2016.05.040

Santonen, T., Nielsen, G., Papameletiou, D., & Klein, C. (2017). *SCOEL/REC/188 Hexachlorobenzene - Recommendation from the Scientific Committee on Occupational Exposure Limits*.

Sapkota, A., Sapkota, A. R., Kucharski, M., Burke, J., McKenzie, S., Walker, P., & Lawrence, R. (2008). Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environ Int, 34*(8), 1215-1226. doi:10.1016/j.envint.2008.04.009

Scheuhammer, A., Basu, N., Depew, D., Burgess, N., Champoux, L., Wayland, M., . . . Chan, L. (2016). Health effects of mercury in fish and wildlife in Canada. In (pp. 669-692).

Sharma, H., Rawal, N., & Mathew, B. (2015). The characteristics, toxicity and effects of cadmium. *International Journal of nanotechnology and nanoscience, 3*, 1-9.

Shaw, B. J., & Handy, R. D. (2006). Dietary copper exposure and recovery in Nile tilapia, Oreochromis niloticus. *Aquat Toxicol, 76*(2), 111-121. doi:10.1016/j.aquatox.2005.10.002

Siddiqi, M., Laessig, R., & Reed, K. (2003). Polybrominated Diphenyl Ethers (PBDEs): New Pollutants-Old Diseases. *Clin Med Res, 1*, 281-290. doi:10.3121/cmr.1.4.281

Siddiqi, M. A., Laessig, R. H., & Reed, K. D. (2003). Polybrominated diphenyl ethers (PBDEs): new pollutants-old diseases. *Clin Med Res, 1*(4), 281-290. doi:10.3121/cmr.1.4.281

Simukoko, C. K., Mwakalapa, E. B., Bwalya, P., Muzandu, K., Berg, V., Mutoloki, S., . . . Lyche, J. L. (2021). Assessment of heavy metals in wild and farmed tilapia (Oreochromis niloticus) on Lake Kariba, Zambia: implications for human and fish health. *Food Additives & Contaminants: Part A*, 1-18. doi:10.1080/19440049.2021.1975830

Singh, D., Cho, W., & Upadhyay, G. (2016). Drug-Induced Liver Toxicity and Prevention by Herbal Antioxidants: An Overview. *Frontiers in Physiology, 6*. doi:10.3389/fphys.2015.00363

Sipes, I. G., & Schnellmann, R. G. (1987). Biotransformation of PCBs: Metabolic Pathways and Mechanisms. . In S. S (Ed.), *Polychlorinated Biphenyls (PCBs): Mammalian and Environmental Toxicology.* (Vol. 1, pp. 97-110). Berlin, Heidelberg.: Springer.

Skerfving, S. B., & Copplestone, J. F. (1976). Poisoning caused by the consumption of organomercury-dressed seed in Iraq. *Bull World Health Organ, 54*(1), 101-112.

Squadrone., S., Favaro., L., Prearo., M., Vivaldi., B., Brizio., P., & Abete., M. C. (2013). NDL-PCBs in muscle of the European catfish (Silurus glanis): An alert from Italian rivers. *Chemosphere 93*, 521–525. doi:http://dx.doi.org/10.1016/j.chemosphere.2013.06.037

Ssebugere, P., Kiremire, B. T., Kishimba, M., Wandiga, S. O., Nyanzi, S. A., & Wasswa, J. (2009). DDT and metabolites in fish from Lake Edward, Uganda. *Chemosphere, 76*(2), 212-215. doi:10.1016/j.chemosphere.2009.03.049

Ssebugere, P., Sillanpää, M., Matovu, H., Wang, Z., Schramm, K.-W., Omwoma, S., . . . Odongo, S. (2020). Environmental levels and human body burdens of per- and poly-fluoroalkyl substances in Africa: A critical review. *The Science of The Total Environment, 739*. doi:10.1016/j.scitotenv.2020.139913

Ssebugere, P., Sillanpää, M., Wang, P., Li, Y., Kiremire, B. T., Kasozi, G. N., . . . Jiang, G. (2014). Polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polybrominated diphenyl ethers in sediments and fish species from the Murchison Bay of Lake Victoria,

Uganda. *Science of The Total Environment, 500-501*, 1-10. doi:https://doi.org/10.1016/j.scitotenv.2014.08.076

Stalling, D. L., & Mayer, F. L. (1972). Toxicities of PCBs to Fish and Environmental Residues. *Environmental Health Perspectives, 1*, 159-164. doi:doi:10.1289/ehp.7201159

Stanifer, J. W., Stapleton, H. M., Souma, T., Wittmer, A., Zhao, X., & Boulware, L. E. (2018). Perfluorinated Chemicals as Emerging Environmental Threats to Kidney Health: A Scoping Review. *Clin J Am Soc Nephrol, 13*(10), 1479-1492. doi:10.2215/CJN.04670418

Stapleton, H. M., Letcher, R. J., & Baker, J. E. (2004). Debromination of Polybrominated Diphenyl Ether Congeners BDE 99 and BDE 183 in the Intestinal Tract of the Common Carp (Cyprinus carpio). *Environmental Science & Technology, 38*(4), 1054-1061. doi:10.1021/es0348804

Steenland, K., Fletcher, T., & Savitz, D. A. (2010). Epidemiologic Evidence on the Health Effects of Perfluorooctanoic Acid (PFOA). *118*(8), 1100-1108. doi:doi:10.1289/ehp.0901827

Stockholm Convention. (2009). *Persistent Organic Pollutants (POPs) as amended in 2009*.

Stringer, R., & Johnston, P. (2002). PCBs (polychlorinated biphenyls). . In S. R. & J. P (Eds.), *In: Chlorine and the Environment.* (pp. 277-304): Springer, Dordrecht.

Sun, R., Luo, X., Li, Q. X., Wang, T., Zheng, X., Peng, P., & Mai, B. (2018). Legacy and emerging organohalogenated contaminants in wild edible aquatic organisms: Implications for bioaccumulation and human exposure. *Science of The Total Environment, 616-617*, 38-45. doi:https://doi.org/10.1016/j.scitotenv.2017.10.296

Sun, Y.-X., Hao, Q., Xu, X.-R., Luo, X.-J., Wang, S.-L., Zhang, Z.-W., & Mai, B.-X. (2014). Persistent organic pollutants in marine fish from Yongxing Island, South China Sea: Levels, composition profiles and human dietary exposure assessment. *Chemosphere, 98*, 84-90. doi:https://doi.org/10.1016/j.chemosphere.2013.10.008

Sunderland, E. M., Hu, X. C., Dassuncao, C., Tokranov, A. K., Wagner, C. C., & Allen, J. G. (2019). A review of the pathways of human exposure to poly- and perfluoroalkyl

substances (PFASs) and present understanding of health effects. *Journal of Exposure Science & Environmental Epidemiology, 29*(2), 131-147. doi:10.1038/s41370-018-0094-1

Sweet, L., I., & Zelikoff, J., T. . (2001). TOXICOLOGY AND IMMUNOTOXICOLOGY OF MERCURY: A COMPARATIVE REVIEW IN FISH AND HUMANS. *Journal of Toxicology and Environmental Health, Part B, 4*(2), 161-205. doi:10.1080/10937400117236

Taiwo, A. M. (2019). A review of environmental and health effects of organochlorine pesticide residues in Africa. *Chemosphere, 220*, 1126-1140. doi:https://doi.org/10.1016/j.chemosphere.2019.01.001

Taweel, A., Shuhaimi-Othman, M., & A.k, A. (2011). Heavy metals concentration in different organs of tilapia fish (Oreochromis niloticus) from selected areas of Bangi, Selangor, Malaysia. *African Journal of Biotechnology, 10(55)*.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Exp Suppl, 101*, 133-164. doi:10.1007/978-3-7643-8340-4_6

Thomaidi, V. S., Tsahouridou, A., Matsoukas, C., Stasinakis, A. S., Petreas, M., & Kalantzi, O. I. (2020). Risk assessment of PFASs in drinking water using a probabilistic risk quotient methodology. *Science of The Total Environment, 712*, 136485. doi:https://doi.org/10.1016/j.scitotenv.2019.136485

Tran, N., Chu, L., Chan, C. Y., Genschick, S., Phillips, M. J., & Kefi, A. S. (2019). Fish supply and demand for food security in Sub-Saharan Africa: An analysis of the Zambian fish sector. *Marine Policy, 99*, 343-350. doi:https://doi.org/10.1016/j.marpol.2018.11.009

Tuulaikhuu, B.-A., Bonet, B., & Guasch, H. (2016). Effects of low arsenic concentration exposure on freshwater fish in the presence of fluvial biofilms. *Science of The Total Environment, 544*, 467-475. doi:https://doi.org/10.1016/j.scitotenv.2015.11.126

U.S. EPA. (2010). . *Recommended Toxicity Equivalence Factors (TEFs) for Human Health Risk Assessments of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Dioxin-Like Compounds. Risk Assessment Forum, Washington, DC. EPA/600/R-10/005.*

UNECE. (2015). *UPDATED HANDBOOK FOR THE 1979 CONVENTION ON LONG-RANGE TRANSBOUNDARY AIR POLLUTION AND ITS PROTOCOLS*. Retrieved from CH-1211 Geneva 10, Switzerland:

UNEP. (2007a). *Stockholm Convention on Persistent Organic Pollutants Persistent Organic Pollutants Review Committee Third meeting Geneva, 19–23 November 2007*

Report of the Persistent Organic Pollutants Review Committee on the work of its third meeting. Retrieved from

UNEP. (2007b). Annex F Information. In.

Väänänen, K., Leppänen, M. T., Chen, X., & Akkanen, J. (2018). Metal bioavailability in ecological risk assessment of freshwater ecosystems: From science to environmental management. *Ecotoxicology and Environmental Safety, 147*, 430-446. doi:https://doi.org/10.1016/j.ecoenv.2017.08.064

Vafeiadi, M., Vrijheid, M., Fthenou, E., Chalkiadaki, G., Rantakokko, P., Kiviranta, H., . . . Kogevinas, M. (2014). Persistent organic pollutants exposure during pregnancy, maternal gestational weight gain, and birth outcomes in the mother–child cohort in Crete, Greece (RHEA study). *Environment International, 64*, 116-123. doi:https://doi.org/10.1016/j.envint.2013.12.015

Valdersnes, S., Nilsen, B. M., Breivik, J. F., Borge, A., & Maage, A. (2017). Geographical trends of PFAS in cod livers along the Norwegian coast. *PLOS ONE, 12*(5), e0177947. doi:10.1371/journal.pone.0177947

Vallack, H. W., Bakker, D. J., Brandt, I., Broström-Lundén, E., Brouwer, A., Bull, K. R., . . . Taalman, R. D. F. (1998). Controlling persistent organic pollutants–what next?1Correspondence to: Nicole G Weavers, ETAF, European Training and Assessment Foundation, PO Box 182, 6700 AD, Wageningen, The Netherlands. Tel.: +31-317-484924; Fax: +31-317-4849411. *Environmental Toxicology and Pharmacology, 6*(3), 143-175. doi:https://doi.org/10.1016/S1382-6689(98)00036-2

Van Ael, E., Covaci, A., Das, K., Lepoint, G., Blust, R., & Bervoets, L. (2013). Factors Influencing the Bioaccumulation of Persistent Organic Pollutants in Food Webs of the Scheldt Estuary. *Environmental Science & Technology, 47*(19), 11221-11231. doi:10.1021/es400307s

Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., . . . Peterson, R. E. (2006). The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci, 93*(2), 223-241. doi:10.1093/toxsci/kfl055

Varol, M., Kaya, G. K., & Alp, A. (2017). Heavy metal and arsenic concentrations in rainbow trout (Oncorhynchus mykiss) farmed in a dam reservoir on the Firat (Euphrates) River: Risk-based consumption advisories. *Science of The Total Environment, 599-600*, 1288-1296. doi:https://doi.org/10.1016/j.scitotenv.2017.05.052

Verhaert, V., Covaci, A., Bouillon, S., Abrantes, K., Musibono, D., Bervoets, L., . . . Blust, R. (2013). Baseline levels and trophic transfer of persistent organic pollutants in sediments and biota from the Congo River Basin (DR Congo). *Environment International, 59*, 290-302. doi:https://doi.org/10.1016/j.envint.2013.05.015

Verhaert, V., Newmark, N., D'Hollander, W., Covaci, A., Vlok, W., Wepener, V., . . . Bervoets, L. (2017). Persistent organic pollutants in the Olifants River Basin, South Africa: Bioaccumulation and trophic transfer through a subtropical aquatic food web. *Science of The Total Environment, 586*, 792-806. doi:https://doi.org/10.1016/j.scitotenv.2017.02.057

Vicente-Zurdo, D., Gómez-Gómez, B., Pérez-Corona, M. T., & Madrid, Y. (2019). Impact of fish growing conditions and cooking methods on selenium species in swordfish and salmon fillets. *Journal of Food Composition and Analysis, 83*. doi:10.1016/j.jfca.2019.103275

Wang, Y., Wang, D., Lin, L., & Wang, M. (2015). Quantitative proteomic analysis reveals proteins involved in the neurotoxicity of marine medaka Oryzias melastigma chronically exposed to inorganic mercury. *Chemosphere, 119*, 1126-1133. doi:https://doi.org/10.1016/j.chemosphere.2014.09.053

Wang, Z., Cousins, I. T., Scheringer, M., & Hungerbuehler, K. (2015). Hazard assessment of fluorinated alternatives to long-chain perfluoroalkyl acids (PFAAs) and their precursors:

Status quo, ongoing challenges and possible solutions. *Environment International, 75*, 172-179. doi:https://doi.org/10.1016/j.envint.2014.11.013

Wania, F., & Mackay, D. (1993). Global Fractionation and Cold Condensation of Low Volatility Organochlorine Compounds in Polar Regions *Ambio, 22*.

Waseem, A., Arshad, J., Iqbal, F., Sajjad, A., Mehmood, Z., & Murtaza, G. (2014). Pollution status of Pakistan: a retrospective review on heavy metal contamination of water, soil, and vegetables. *Biomed Res Int, 2014*, 813206. doi:10.1155/2014/813206

Watanabe, I., & Sakai, S.-i. (2003). Environmental release and behavior of brominated flame retardants. *Environment International, 29*(6), 665-682. doi:https://doi.org/10.1016/S0160-4120(03)00123-5

Wepener, V., Smit, N., Covaci, A., Dyke, S., & Bervoets, L. (2012). Seasonal Bioaccumulation of Organohalogens in Tigerfish, Hydrocynus vittatus Castelnau, from Lake Pongolapoort, South Africa. *Bulletin of environmental contamination and toxicology, 88*, 277-282. doi:10.1007/s00128-011-0439-0

White, R. D., Shea, D., & Stegeman, J. J. (1997). Metabolism of the Aryl Hydrocarbon Receptor Agonist 3,3′,4,4′-Tetrachlorobiphenyl by the Marine Fish Scup (Stenotomus chrysops) In Vivo and <em>In Vitro</em&gt. *Drug Metabolism and Disposition, 25*(5), 564.

WHO. (2011). DDT IN INDOOR RESIDUAL SPRAYING: HUMAN HEALTH ASPECTS (Environmental Health Criteria 241). *WHO Library Cataloguing-in-Publication Data*.

WHO. (2015). *Lead Exposure in African Children: Conteporry Sources and Concern.South Africa.* Retrieved from https://apps.who.int/iris/bitstream/handle/10665/200168/9780869707876.pdf

Wielsøe, M., Long, M., Ghisari, M., & Bonefeld-Jørgensen, E. C. (2015). Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. *Chemosphere, 129*, 239-245. doi:https://doi.org/10.1016/j.chemosphere.2014.10.014

Wood, A., Phiri, N., Bateman, M., & Dougoud, J. (2018). *Study on crop protection where the 'Green Innovation Centres for the Agriculture and Food Sector' (GIAE) initiative is being implemented ZAMBIA*. Retrieved from https://www.cabi.org/wp-content/uploads/Country-report-Zambia.pdf

Wu, Z., Han, W., Yang, X., Li, Y., & Wang, Y. (2019). The occurrence of polybrominated diphenyl ether (PBDE) contamination in soil, water/sediment, and air. *Environmental Science and Pollution Research, 26*(23), 23219-23241. doi:10.1007/s11356-019-05768-w

Xiao, H., Zang, S., Guan, Y., Liu, S., Gao, Y., Sun, Q., . . . Pei, X. (2014). Assessment of potential risks associated with heavy metal contamination in sediment in Aobaopao Lake, China, determined from sediment cores. *Ecotoxicology, 23*(4), 527-537. doi:10.1007/s10646-014-1220-z

Xie, J., Bian, Z., Lin, T., Tao, L., Wu, Q., & Chu, M. (2020). Global occurrence, bioaccumulation factors and toxic effects of polychlorinated biphenyls in tuna: A review. *Emerging Contaminants, 6*, 388-395. doi:https://doi.org/10.1016/j.emcon.2020.11.003

Yabe, J., Ishizuka, M., & Umemura, T. (2010). Current levels of heavy metal pollution in Africa. *The Journal of veterinary medical science, 72*(10), 1257-1263. doi:10.1292/jvms.10-0058

Yang, L., Zhang, Y., Wang, F., Luo, Z., Guo, S., & Strähle, U. (2020). Toxicity of mercury: Molecular evidence. *Chemosphere, 245*, 125586. doi:https://doi.org/10.1016/j.chemosphere.2019.125586

Yildiz, M. (2008). Mineral composition in fillets of sea bass (Dicentrarchus labrax) and sea bream (Sparus aurata): a comparison of cultured and wild fish. *Journal of Applied Ichthyology, 24*(5), 589-594. doi:https://doi.org/10.1111/j.1439-0426.2008.01097.x

Yu, L., Han, Z., & Liu, C. (2015). A review on the effects of PBDEs on thyroid and reproduction systems in fish. *General and Comparative Endocrinology, 219*, 64-73. doi:https://doi.org/10.1016/j.ygcen.2014.12.010

Zambia Central Statistical Office. (2012). *Zambia 2010 Census of Population and Housing*. Lusaka, Zambia

Zeller, D., & Pauly, D. (2005). Good news, bad news: global fisheries discards are declining, but so are total catches. *6*(2), 156-159. doi:https://doi.org/10.1111/j.1467-2979.2005.00177.x

Zheng, J.-L., Yuan, S.-S., Wu, C.-W., Lv, Z.-M., & Zhu, A.-Y. (2017). Circadian time-dependent antioxidant and inflammatory responses to acute cadmium exposure in the brain of zebrafish. *Aquatic Toxicology, 182*, 113-119. doi:https://doi.org/10.1016/j.aquatox.2016.11.017

Zheng, J., Luo, X.-J., Yuan, J.-G., Wang, J., Wang, Y.-T., Chen, S.-J., . . . Yang, Z.-Y. (2011). Levels and sources of brominated flame retardants in human hair from urban, e-waste, and rural areas in South China. *Environmental Pollution, 159*(12), 3706-3713. doi:https://doi.org/10.1016/j.envpol.2011.07.009

Zheng, N., Wang, S., Dong, W., Hua, X., Li, Y., Song, X., . . . Li, Y. (2019). The Toxicological Effects of Mercury Exposure in Marine Fish. *Bulletin of Environmental Contamination and Toxicology, 102*(5), 714-720. doi:10.1007/s00128-019-02593-2

Zhou, Q., Yang, N., Li, Y., Ren, B., Ding, X., Bian, H., & Yao, X. (2020). Total concentrations and sources of heavy metal pollution in global river and lake water bodies from 1972 to 2017. *Global Ecology and Conservation, 22*, e00925. doi:https://doi.org/10.1016/j.gecco.2020.e00925

Appendix: Papers I-III

Paper I

1 **Title:** First findings of per- and polyfluoroalkyl substances (PFASs) in wild tilapia

2 *(Oreochromis niloticus)* from Lake Kariba, Zambia, and higher levels of persistent organic

3 pollutants (POPs) in wild versus farmed fish - implications for fish health.

4

5 Chalumba Kachusi Simukoko [a, b], Eliezer Brown Mwakalapa [c], Kaampwe Muzandu [b], Stephen

6 Mutoloki [d], Øystein Evensen [d], Erik Magnus Ræder [a], Mette Bjørge Müller [e], Anuschka Polder

7 [a*], Jan Ludvig Lyche [a]

8 [a] Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University

9 of Life Sciences, P. O. Box 5003 NMBU, 1432 Ås, Norway

10 [b] Department of Biomedical Sciences, School of Veterinary Medicine, University of Zambia,

11 P. O. Box 32379 Lusaka, Zambia

12 [c] Department of Natural Sciences, Mbeya University of Science and Technology, P. O. Box

13 131, Mbeya, Tanzania

14 [d] Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine,

15 Norwegian University of Life Sciences, P. O. Box 5003 NMBU, 1432 Ås, Norway

16 [e] Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine,

17 Norwegian University of Life Sciences, P. O. Box 5003 NMBU, 1432 Ås, Norway

18

19 Corresponding author: Anuschka Polder, Norwegian University of Life Sciences, Faculty of

20 Veterinary Medicine, Norwegian University of Life Sciences, P. O. Box 5003 NMBU, 1432

21 Ås, Norway. E-mail: anuschka.polder@nmbu.no

Abstract

The current study was carried out to investigate a wide variety of persistent organic pollutants (POPs) in wild and farmed tilapia *(Oreochromis niloticus)* in Lake Kariba, Zambia, and estimate possible implications for fish health. Concentrations of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyls (PBDEs), hexabromocyclododecane (HBCDD) and perfluoroalkyl substances (PFASs) were determined in liver samples of tilapia. PFASs compounds PFOS, PFDA and PFNA were only detected in wild fish, with highest median PFOS levels in site 1 (0.66 ng/g ww). Concentrations of POPs were in general highest in wild tilapia. The highest median $\sum$DDTs (93 and 81 ng/g lw) were found in wild tilapia from sites 1 and 2, respectively 165 km and 100 km west of the farms. Lower DDE/DDT ratios in sites 1 and 3 may indicate relatively recent exposure to DDT. The highest median of $\sum_{17}$PCBs (3.2 ng/g lw) and $\sum_{10}$PBDEs (8.1 ng/g lw) were found in wild tilapia from site 1 and 2, respectively. The dominating PCB congeners were PCB-118, -138, -153 and -180 and for PBDEs, BDE-47, -154, and -209. The $\sum_{10}$PBDE concentrations in wild and farmed fish were above EQS$_{biota}$ limits set by the EU. This may indicate a risk to the fish species and threaten biodiversity.

1. Introduction

Fish serves as a major source of proteins to most people in the world and is essential for food security and sustainability (FAO, 2020). The growing human population has led to increased demand for fish, leading to overexploitation of wild fisheries, and reduction of some fish stocks (FAO, 2005). To meet the future demand for increasing fish consumption in African countries,

49 aquaculture industry is rapidly growing in this continent (FAO, 2018). Nile tilapia
50 (*Oreochromis niloticus*) is a fast-growing species in tropical and subtropical climate and
51 therefore commonly used for commercial purposes. According to Genschick et al., 2017,
52 Zambia is the major producer of tilapia in the Southern African Development Community
53 (SADC) and the sixth-largest producer of farmed fish in Africa. Based on recent reports,
54 Zambia produced approximately 30,000 tons of fish in fish farms which is 27 percent of its
55 total fish production (WorldFish, 2022).

56 During commercial fish farming, organic substances, chemicals, and antibiotics may be
57 released in the water bodies and may disturb biodiversity and threaten freshwater ecosystems.
58 (Allan, 2005; Berg et al., 1992; Kishimba et al., 2004; Li et al., 2011; Mwakalapa et al., 2018;
59 Nonga et al., 2011; Polder et al., 2014; Simukoko et al., 2021; Ssebugere et al., 2014;
60 Subasinghe, 2005). Pollution of aquatic environments by persistent organic pollutants (POPs)
61 may also affect fish and human health (Barni et al., 2016; Burreau et al., 2004; Rodriguez-
62 Hernandez et al., 2017; VKM, 2014). POPs include compounds like organochlorine pesticides
63 (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) such as,
64 polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) and
65 Perfluoroalkyl substances (PFASs) (Stockholm Convention, 2021). Except for PFASs, which
66 are protein bound (EPA, 2019), POPs are lipophilic and can therefore accumulate in fatty
67 tissues of organisms including fish, and bioaccumulate in the food chain (Burreau et al., 2004;
68 Deribe et al., 2011; Letcher et al., 2010; Sharma et al., 2009; Squadrone et al., 2013, Ssebugere
69 et al., 2009). Their semi volatile nature coupled with long environmental half-lives results in
70 long-range transport and global distribution (Wania & Mackay, 1993). Anthropogenic
71 activities such as industries, mining, agriculture, and waste from human settlements are known
72 sources of POPs (Covaci et al., 2008; Henry & Kishimba, 2006; Lyche et al., 2015; Nieuwoudt
73 et al., 2009; Santonen et al., 2017). Despite the Stockholm Convention of 2001, protecting
74 human health and the environment from POPs (Stockholm Convention, 2021), and global
75 measures taken, POPs are still present in most parts of the world (Ashraf, 2017).

76 The growing aquaculture industry in Africa may be threatened by the presence of POPs and
77 other contaminants in the water and fish. To enable sustainable aquaculture development, it is
78 of key importance to gain knowledge on toxicological risk factors and the potential adverse
79 effects of pollutants and other environmental factors on fish health. The current study was

80 carried out to establish knowledge on the concentrations of a wide variety of POPs in wild and
81 farmed tilapia from Lake Kariba, Zambia, with emphasis on fish health.

82 **2. Materials and methods**

83 *Description of sampling area*

84 The man-made Lake Kariba is a natural border between Zambia and Zimbabwe (16° 28′ to 18°
85 04′S; 26° 40′ to 29° 03′ E). On the Zambian side it covers three districts (Sinazongwe, Gwembe
86 and Siavonga). Five different locations (sites 1, 2 and 3, and farms 1 and 2) (Figure 1) were
87 chosen for collecting of samples. Details and characteristics of the lake and sites and were
88 earlier described (Simukoko et al., 2022). In short, the water runoff is eastwards, with a
89 hydroelectric power plant located at the Kariba Gorge, east of farm 1 (not shown on the map).
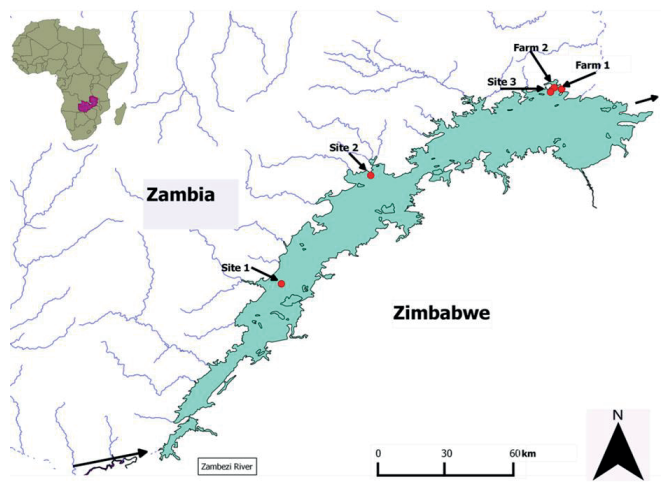
90

91 **Figure 1:** Map of lake Kariba, showing the 5 sampling locations (sites1-3 and farms 1 and 2).
92 (Courtesy of Eliezer Brown Mwakalapa 2019). Simukoko et al. (2022).

93 Site 1 is influenced by coal mining, while sites 2 and 3 are dominated by agricultural activities.
94 The fish farms are in site 3, supplied with a local fish feed processing plant. Sites 1 and 2 are
95 situated respectively 165 km and 100 km west of the farms.

96 *Ethical consideration and permission for the study*

4

97   The study proposal was approved by the University of Zambia, School of Veterinary Medicine
98   research committee. Local district fisheries and Veterinary officers were consulted and
99   involved in the relevant locations. Collection of fish from the fish farms was agreed on with
100   the farm managers. The permission to transport samples from Tanzania to Norway was granted
101   by The Ministry of Agriculture, Livestock and Fisheries and The Norwegian Food Safety
102   Authority.

103   *Sample collection*

104   A total of 142 wild and farmed tilapia samples were collected from June to July 2017.
105   Physicochemical parameters (pH, temperature, conductivity, and total dissolved solutes) were
106   measured in all sites (not shown). Live wild tilapia was bought from local fishermen as they
107   pulled in their catch from the water. The fish were placed in a container containing ice water
108   and transported to the shore for dissection. Farmed tilapias were sampled by dip netting and
109   placed in containers containing ice water. The length and sex of the fish were recorded (Table
110   1A, 1B). The size of the fish was sometimes considerably different within the study sites. Only
111   length was used in statistical analyses because fish weight measured in the field was considered
112   as not precise. As described earlier (Simukoko et al., 2021), the fish was dissected at the shore.
113   Liver tissue was removed and placed in clean 15 ml Eppendorf tubes. The samples were
114   transported to the University of Zambia, Veterinary Medicine School placed on ice in a cooler
115   box, where they were stored at -20ºC. The samples were later transported on ice to the
116   Laboratory of Environment Toxicology at the Norwegian University of Life Science (NMBU)
117   at Ås, Norway, and stored at -20ºC until analyses.

118   *Sample analysis of OCPs, PCBs and BFRs,*

119   Based on fish size, out of the 142 fish collected, 82 liver samples from male fish were selected
120   and pooled for analyses on POPs (Table 1A). Each homogenate contained two or three liver
121   samples.
122   Before analyses the samples were thawed at room temperature and protected from light during
123   the analyses. The samples were analysed for organochlorinated pesticides (OCPs):
124   hexachlorobenzene (HCB), α, β- and γ-hexachlorocyclohexanes (HCHs), heptachlor,
125   oxychlordane, trans-chlordane, cis-chlordane and trans-nonachlor (CHLs), mirex, bis-2,2-(4-
126   chlorophenyl)-1,1,1- trichloroethane (*p,p′*-DDT) and its metabolites *p,p′*-DDE, *p,p′*-DDD and
127   *o,p′*-DDT, polychlorinated biphenyls PCBs: PCB-101, -105, -110, -118, -128, -138, -141, -149,

128 -151, -153, -156, -170, -180, -183, -194, -206 and -209 ($\sum_{17}$PCBs), and brominated flame
129 retardants (BFRs): polybrominated diphenyl ethers PBDEs: BDE -47, -99, -100, -153, -154, -
130 183, -196, -202, -206 ($\sum_9$PBDEs) and BDE-209 ($\sum_{10}$PBDEs is $\sum_9$PBDEs + BDE-209), and
131 hexabromocyclododecane (HBCDD). Mirex, PCB -28, -52, -56, -66, -74, -87, -99, -114, -136,
132 -137, -157, and -187; BDE-28, -207, and -208 were analysed but were not detected above LOD.
133 Those compounds were not included in any data analyses further.
134
135 *Sample analysis of PFASs,*
136 PFAS compounds were analysed in individual liver samples (N=26) in other fish than used for
137 POP analyses, but from the same catch. The perfluoroalkyl substances analysed for were:
138 perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonamide (FOSA) and perfluorooctane
139 sulfonate (PFOS)* and 9 PFCAs: perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid
140 (PFHpA),   perfluorooctanoic   acid   (PFOA),   perfluorononanoic   acid   (PFNA)*,
141 perfluorodecanoic acid (PFDA)*, perfluoroundecanoic acid (PFUdA), perfluorododecanoic
142 acid (PFDoA), and perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid
143 (PFTeDA). Because of low sample amounts available, some female fish were included for
144 PFAS analyses (Table 1B). Compounds marked with * were included in $\sum_3$PFAS. Other PFAS
145 components were not detected in levels >LOD and were not used in any data analyses further.
146

Table 1A

Location and fish characteristics for analyses of OCPs, PCBs and BFRs; Sampling time, mean and range of individual length and number of pooled liver samples from wild (sites 1,2 & 3) and farmed tilapia (farm 1 & 2) from Lake Kariba, Zambia. Gender: all males.

Location	Sampling time 2017	Mean individual length (cm)	Range of individual length (cm)	No. of individual samples	No. of fish per pool	No. of pooled samples
Site 1 Sinazongwe	June	27	20-41	18	3	6
Site 2 Gwembe	June	29	24-37	16	2-3	6
Site 3 Siavonga	July	30	22-47	16	2-3	6
Farm 1 Siavonga	July	36	30-40	18	3	6
Farm 2 Siavonga	July	26	18-34	14	2-3	6

Table 1B

Location and fish characteristics for analyses of PFASs; Sampling time, mean and range of individual length and number of pooled liver samples from wild (sites 1,2 & 3) and farmed tilapia (farm 1 & 2) from Lake Kariba, Zambia.

Location	Sampling time 2017	Mean individual length (cm)	Range of individual length (cm)	N	Gender Female/male
Site 1 Sinazongwe	June	29	26-34	5	3/2
Site 2 Gwembe	June	36	32-42	5	2/3
Site 3 Siavonga	July	27	24-30	6	0/6
Farm 1 Siavonga	July	26	25-29	5	n.d.
Farm 2 Siavonga	July	28	26-30	5	1/4

n.d.= not documented

Chemical analyses of OCPs, PCB, BFRs

149 The analytical method for analysing of OCs was first described by Brevik (1978) and modified
150 by Polder et al. (2014), in which full details are described. In short, the method is based on
151 repeated fat extraction of the homogenised liver with acetone, cyclohexane, and water, using
152 an ultrasonic homogenizer and separation of the fat using centrifugation. Lipid determination
153 was done gravimetrically using 1 mL aliquot of the fat extract. The rest of the extract was
154 treated with 96% H_2SO_4 for cleaning of fat and the final extract was concentrated before GC
155 analyses. Before extraction, internal standards PCB -29, -112 and -207 (1000 µg/mL) (Ultra-
156 Scientific, RI, USA); 20 µL of BDE -77, -119, -181, and $^{13}C_{12}$-209, $^{13}C_{12}$-TBBP-A (500
157 µg/mL) (Cambridge Isotope Laboratories, Inc., MA, USA) were added in all the samples.
158 During the analyses, the samples were protected from light and amber GC-vials were used.

159 *Chemical analyses of PFASs*

160 The analytical method was described by Grønnestad et al. (2017). In short, internal standards
161 (Wellington laboratories, ON N1G 3M5, CA) were added to 0.5 g homogenized liver samples.
162 Extraction was performed twice with 5ml methanol and an ultrasonic probe sonicator followed
163 by centrifugation. The supernatants were combined and cleaned with approximately 0.2 g
164 graphitized carbon (EnviCarb). Finally, the samples were evaporated to near dryness and
165 dissolved in 500 µl Methanol / water 1:1. Analysis of the samples on HPLC-MS resulted in
166 substantial matrix effects, suggesting that further cleanup was necessary. An additional 0.2 g
167 EnviCarb was added to the samples followed by filtration with Spin-X centrifuge filters
168 (Corning). The EnviCarb and filters were washed with 500µl methanol, and the filtrates were
169 combined and concentrated to dryness and finally reconstituted in 200µl MeOH.

170 *Instrumental analysis*

171 *Separation and detection of the POPs*

172 OCPs, PCBs and BFRs were separated and detected using GC-MS methods as previously
173 described (Mwakalapa et al., 2018; Polder et al., 2014), on a HRGC (Agilent 6890 Series)
174 coupled to a MS detector (Agilent 5975C Agilent Technologies) which was operated in
175 negative chemical ionization (NCI) mode with selected ion monitoring (SIM). The OCPs and
176 PCBs (injection volume of 1 µL) were separated on a DB-5 MS column (J&W Scientific,
177 Agilent Technologies) (60 m, 0.25 mm i.d., 0.25 mm film thickness). BFRs (injection volume

178 of 2 µL) were separated on a DB-5 MS column (J&W Scientific, Agilent Technologies) (30 m,
179 0.25 mm i.d., 0.25 mm film thickness). The separation and identification of BDE-209 (injection
180 volume of 10 µL) were performed on a GC-5-MS (Agilent 6890 Series/5973Network)
181 configured with a programmable temperature vaporization (PTV) injector (Agilent
182 Technologies) equipped with a DB-5-MS column (10 m, 0.25 mm i.d., 0.10 mm film thickness)
183 (J&W Scientific, Agilent Technologies). For all components, five-to eight-point linear
184 calibration curves were used and calculations were done within the linear range for the
185 component. OCPs, PCBs and BFRs were monitored using negative chemical ionization (NCI)
186 in selected ion monitoring (SIM).

187 *Separation and detection of the PFASs*

188 Samples were analyzed on an Agilent 1200 HPLC-system coupled to an Agilent 6460 Triple
189 Quad Mass Spectrometer (Agilent Technologies). A Phenomenex C18 Luna Omega 3µm
190 100x4,6 mm (Phenomenex) was used as the analytical column and a 50 mm version of the
191 same column was installed between the pump and the injector to act as a delay-column to
192 reduce blank contamination. The injected amount was 20 µl.

193 *Quality Assurance (QA)/ Quality control (QC)*

194 The chemical analysis of the liver samples was conducted at the Laboratory of Environmental
195 Toxicology at the Norwegian University of Life Sciences in Oslo, Norway. The laboratory is
196 accredited for testing chemicals in biological samples by the Norwegian accreditation
197 according to the requirement of the NS-EN ISO/IEC 17025 (TEST 137).

198 *OCPs, PCBs, BFRs.* Every analytical series included one blind sample of non-spiked salmon
199 trout (*Salmo trutta*), two samples of spiked salmon trout for recovery, three procedural blanks
200 of solvents and the laboratory's own reference material of the blubber of a harp seal (*Pagophilus*
201 *groenlandicus*). The analytical quality was successfully approved by routinely analysing
202 different Certified Reference Materials (CRMs). Within the same period, the laboratory
203 successfully participated in round 1 and 2 of Quasimeme inter-laboratory studies for 2016 for
204 POPs in fish muscle, fish liver and shellfish tissue. The limits of detections (LOD) for
205 individual analytes were defined as 3 times the noise level of each analyte. The LODs (ng/g
206 wet weight, ww) ranged from 0.003 to 0.166 for OCPs, 0.003 to 0.101 for PCBs and 0.003 to

207 0.036 for BFRs. The relative recoveries were 82-137% for OCPs, 92-127% for PCBs, and 68-
208 120% for BFRs.

209 For PFASs, every analytical series included three blanks of solvent, two samples of spiked
210 Atlantic cod (*Gadus morhua*) for recoveries and one blind sample of non-spiked Atlantic cod.
211 LOD was calculated as 3 times the noise in the chromatogram. The LOD for PFAS ranged
212 between 0.093 ng/g ww to 0.706 ng/g ww. Matrix-matched calibration curves ranged from 0 –
213 50 ng/ml and were linear with $R^2>0,99$, except for PFTrDA. The analytical quality of the
214 method was assessed by including an inter-laboratory test (AMAP) in the analysis of samples.
215 The relative recoveries were 67-115%.

216 *Statistical data analysis*

217 Detection rate was defined as percentage of samples with a detectable value, i.e., above LOD.
218 The compounds with detection rate above 50% were reported with descriptive statistics. Levels
219 below LOD were replaced with 1/2 LOD. Compounds with a detection rate lower than 50%
220 were reported with range and the levels below LOD were replaced with a value of 0.0001 when
221 calculating the sum of the compound group for all results presented. Stata SE/16 (Stata Corp.,
222 College Station, TX, USA) was used for statistical analysis. Normality of the data was tested
223 using Shapiro-Wilk. If data from one of the locations failed the Shapiro-Wilk test, the data of
224 all locations were log-transformed. The nonparametric Kruskal-Wallis test were used as the
225 present data failed Shapiro-Wilk after being log-transformed. Dunn`s post-hoc test was applied
226 for pairwise comparisons between the locations, with and without Bonferroni corrections for
227 multiple comparison. Spearman rank correlation was used to assess the correlation between
228 variables. The statistical significance level was set at $p<0.05$.

229 **3. Results**

230 *Fish characteristics*

231 Fish weight and length correlated strongly for fish below 1 kg for both wild fish (r=0.93) and
232 farmed fish (r=0.92). Since fish weight above 1 kg was not specified, fish length was used as
233 indicator of fish size. Fish from farm 1 were significantly longer (mean 36 cm), (p<0.05) than
234 the other locations (Table 1A). The median liver lipid contents (%) of farmed fish from farm 1
235 (7.5 %) and 2 (8,8 %) were significantly higher than for wild fish from site 2 (4.9 %), and was
236 also significantly higher in farm 1 than in site 1 (4.1 %). In addition, there was a significant

10

237 difference in liver lipid content between the wild fish at site 2 (4.9 %) and 3 (7.2 %) (Fig.2).

238 Length of the individual fish for PFAS analyses were all in the same range (Table 1B).

239

240 *Levels of OCPs, PCBs, BFRs*

241 HCB and *p,p'*-DDE were the OCPs detected in 100% of the liver samples. Median

242 concentrations of HCB were significantly higher in wild fish from all sites compared to farmed

243 fish (both farms) (Table 2B, Fig. 2). Of the HCHs, γ-HCH (lindane) and α-HCH were detected

244 in 77% and 50 % of the samples, respectively. The highest median concentration of ∑HCHs

245 was 0.05 ng/g lw in site 3. The γ-HCH was the dominant HCH, contributing 56 %, 69 % and

246 65 % to ∑HCHs in wild fish from site 1, 2 and 3, and 79 % and 58 % in fish from farm 1 and

247 2 (Fig. 3). α-HCH contributed between 21-34 % to the ∑HCHs and β-HCH between 1-14%.

248 The median ∑HCHs was significantly higher in site 2 and 3 compared to farm 1 (Fig. 2). DDTs

249 were the most abundant OCPs in all the locations with the highest median concentrations of

250 ∑DDTs in wild fish from site 1 and 2 (93 and 81 ng/g lw) (Table 2B). *P,p'*-DDD and *p,p'*-

251 DDT were detected in 93 % and 73 %, while *o,p'*-DDD and *o,p'*-DDT were detected in 17 %

252 and 3% of the samples, respectively. *P,p'*-DDE and ∑DDTs were significantly higher in site 1

253 and 2 compared to farm 1 and 2, while site 3 was only significantly higher than farm 1 (Fig.2).

254 The contribution of *p,p'*-DDE to the ∑DDTs was highest in wild fish from 2 (61 %), lower in

255 wild fish from site 1 and 3 (48 % and 46 %) but lowest in farmed fish from farm 1 and 2 (30

256 % and 31 %), respectively (Fig. 3) . In farm 1 and 2, the contribution of *p,p'*-DDD to the

257 ∑DDTs was higher than that of *p,p'*-DDE with 68 % and 63 %, respectively (Fig. 3).

258
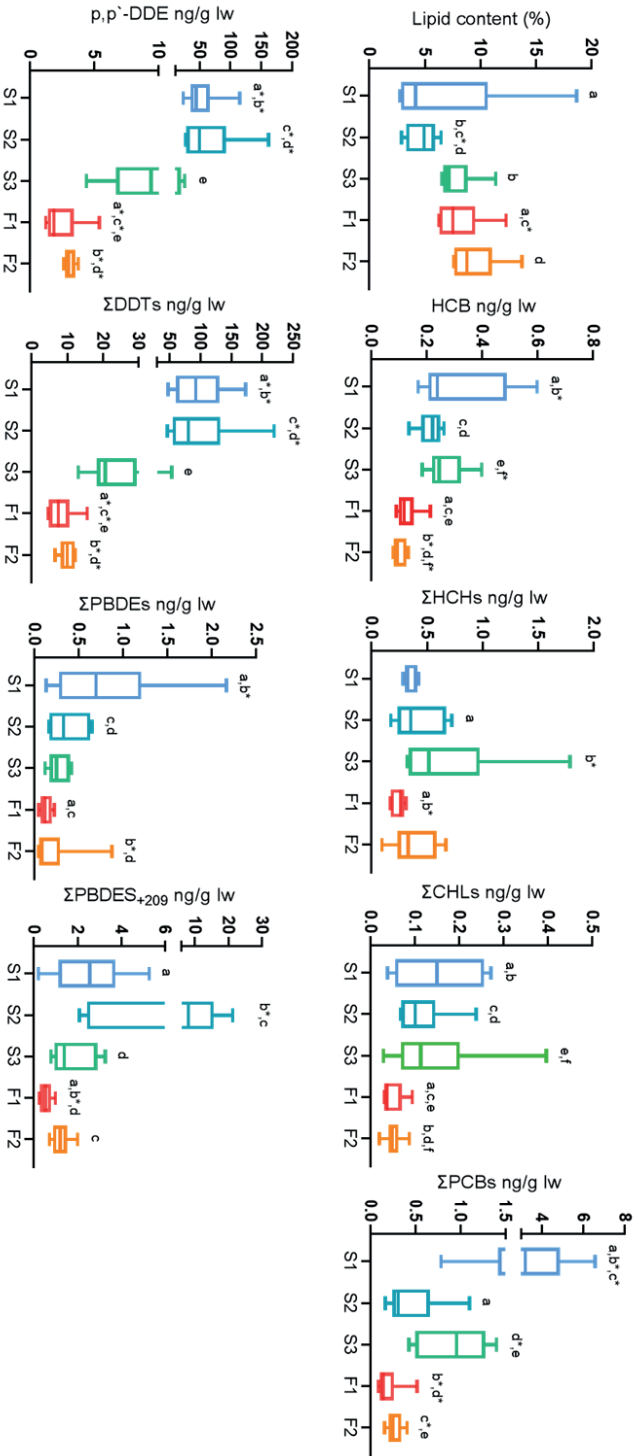
Figure 2: Liver lipid content and contaminant concentrations in livers from wild fish (site S1-3) and farmed fish (farm F1-2) in Lake Kariba, Zambia. Liver lipid content is given in % and liver concentrations are presented as ng/g lipid weight (lw). Box plots show median (line), IQR (box) and minimum to maximum (whiskers). Statistical differences were determined using Kruskal Wallis with Dunn´s post hoc test with and without Bonferroni's corrections for multiple testing. Letters (a-e) indicates statistical significant difference (p<0.05) between the sites and farms. Asterisk (*) indicates statistical significance (p<0.05) after Bonferroni's corrections for multiple testing.

259

12

Table 2A: Concentrations (ng/g wet weight (ww) of persistent organic pollutants (POPs) in wild (sites 1, 2 and 3) and farmed tilapia (farms 1 and 2) from Lake Kariba, Zambia.

Location		Lipid %	HCB	ΣHCH	ΣDDTS	ΣCHLs	Σ₁₇PCBs	Σ₁₀PBDEs
					Ww			
Site 1	Mean	**6.77**	**0.02**	**0.03**	**6.90**	**0.01**	**0.18**	**0.14**
	Median	4.17	0.01	0.01	4.20	0.01	0.15	0.16
	Min	2.77	0.01	0.01	1.38	<LOD	0.04	0.01
	Max	18.66	0.05	0.08	15.7	0.03	0.34	0.27
	N = 6							
Site 2	Mean	**4.73**	**0.01**	**0.02**	**4.67**	**0.01**	**0.02**	**0.36**
	Median	4.92	0.01	0.02	3.68	<LOD	0.02	0.31
	Min	2.91	0.01	0.01	1.36	<LOD	0.01	0.13
	Max	6.45	0.01	0.03	10.11	0.02	0.03	0.63
	N = 6							
Site 3	Mean	**7.82**	**0.02**	**0.05**	**2.16**	**0.01**	**0.07**	**0.14**
	Median	7.15	0.02	0.04	1.51	0.01	0.07	0.09
	Min	6.53	0.01	0.02	0.88	<LOD	0.03	0.06
	Max	11.37	0.03	0.12	6.10	0.05	0.16	0.32
	N = 6							
Farm 1	Mean	**8.09**	**0.01**	**0.02**	**0.63**	**<LOD**	**0.02**	**0.04**
	Median	7.51	0.01	0.02	0.56	<LOD	0.01	0.04
	Min	6.26	0.01	0.02	0.31	<LOD	0.01	0.03
	Max	12.31	0.02	0.02	1.07	0.01	0.04	0.32
	N = 6							
Farm 2	Mean	**9.44**	**0.01**	**0.03**	**0.94**	**<LOD**	**0.02**	**0.11**
	Median	8.77	0.01	0.03	0.91	<LOD	0.02	0.11
	Min	7.62	0.01	0.01	0.51	<LOD	0.02	0.07
	Max	13.72	0.02	0.06	1.35	0.01	0.03	0.17
	N = 6							

Σ-HCHs: Sum of α-, β- and γ-HCHs

Σ-CHLs: Sum of oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor and cis-nonachlor

Σ-DDTs: Sum of p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT and p,p'-DDT

Σ-₁₇PCBs: Sum of CB-101, -105, -110, -118, -128, -138, -141, -149, -151, -153, -156, -170, -180, -183, -194, -206 and -209

Σ-₁₀PBDEs: Sum of BDE-47, -99, -100, -153, -154, -183, -196, -202, -206 and -209

260
261

Table 2B: Concentrations (ng/g lipid weight (lw)) of persistent organic pollutants (POPs) in wild (sites 1, 2 and 3) and farmed tilapia (farms 1 and 2) from Lake Kariba, Zambia.

Location	n (6)	Lipid %	HCB	∑HCH	∑DDTS	∑CHLs	∑$_{17}$PCBs	∑$_{10}$PBDEs
					Lw			
Site 1	Mean	**6.77**	**0.32**	**0.35**	**97.95**	**0.15**	**3.29**	**2.55**
	Median	4.17	0.24	0.32	92.51	0.15	3.21	2.56
	Min	2.77	0.17	0.28	47.98	0.04	0.78	0.21
	Max	18.66	0.60	0.42	173.03	0.27	6.58	5.29
	N=6							
Site 2	Mean	**4.73**	**0.21**	**0.42**	**98.14**	**0.12**	**0.44**	**9.29**
	Median	4.92	0.22	0.35	80.72	0.10	0.31	8.09
	Min	2.91	0.14	0.17	46.65	0.07	0.16	2.08
	Max	6.45	0.26	0.72	219.22	0.24	1.10	21.24
	N=6							
Site 3	Mean	**7.82**	**0.27**	**0.70**	**24.94**	**0.15**	**0.92**	**1.77**
	Median	7.15	0.24	0.52	20.69	0.11	0.96	1.38
	Min	6.53	0.18	0.32	13.13	0.03	0.43	0.78
	Max	11.37	0.40	1.79	53.62	0.40	1.40	3.28
	N=6							
Farm 1	Mean	**8.09**	**0.13**	**0.24**	**8.20**	**0.05**	**0.19**	**0.55**
	Median	7.51	0.12	0.26	7.56	0.04	0.14	0.52
	Min	6.26	0.09	0.17	4.62	0.03	0.09	0.25
	Max	12.31	0.21	0.31	15.64	0.09	0.52	0.98
	N=6							
Farm 2	Mean	**9.44**	**0.10**	**0.38**	**9.96**	**0.05**	**0.26**	**1.24**
	Median	8.77	0.09	0.33	10.01	0.05	0.25	1.21
	Min	7.62	0.08	0.09	6.62	0.02	0.15	0.71
	Max	13.72	0.13	0.67	12.30	0.09	0.41	1.99
	N=6							

∑-HCHs: Sum of α-, β- and γ-HCHs

∑-CHLs: Sum of oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor and cis-nonachlor

∑-DDTs: Sum of p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT and p,p'-DDT

∑-$_{17}$PCBs: Sum of CB-101, -105, -110, -118, -128, -138, -141, -149, -151, -153, -156, -170, -180, -183, -194, -206 and -209

∑-$_{10}$PBDEs: Sum of BDE-47, -99, -100, -153, -154, -183, -196, -202, -206 and -209

262

263

264

The ratio of *p,p'*-DDE/*p,p'*-DDT was highest in wild fish from site 2 and farmed fish from farm 1 and lowest in wild fish from site 3. *Trans*-nonachlor was detected in 73%, and *cis*-nonachlor, *cis*-chlordane and *trans*-chlordane in only 17%, 13 % and 7% of the samples, respectively. *Trans*-Nonachlor contributed 66-87% to $\sum$CHLs. Median $\sum$CHLs were significantly higher in wild fish for all sites compared to farmed fish (both farms). Mirex and heptachlor were not detected in any of the samples.

PCBs were detected in all locations in low concentrations. PCBs-118, -138, -153 and -180 were the most abundant PCBs and found in 53 %, 60 %, 90 % and 77 % of the samples, contributing average 8 %, 14 %, 25 % and 16 % to $\sum_{17}$PCBs respectively (Fig. 3). The highest median concentration of $\sum_{17}$PCBs was found in site 1 at 3.2 ng/g lw <site 3<site 2<farm 2<farm 1 (Table 2B, Fig. S1). Median $\sum_{17}$PCBs was significantly higher in site 1 compared to site 2, farm 1 and 2. Site 3 was significantly higher compared farm 1 and farm 2 (Fig. 2).

PBDEs were detected in all samples, except one. BDE-47, -99, -154, and 209 were the most abundant BDEs detected in 70 %, 30 %, 57 % and 97 % of the samples. BDE-209 dominated the PBDE pattern and contributed average 84 % to $\sum_{10}$PBDEs (Fig. 3). The highest median concentration of $\sum_{10}$PBDEs (including BDE-209) was 8.1 ng/g lw in site 2. Median $\sum_{9}$PBDEs (excluding BDE-209) were significantly higher in site 1 and site 2 compared to farm 1 and 2, while the median $\sum_{9}$PBDEs was significantly higher in all sites compared to farm 1. Site 2 was also significantly higher than farm 2 (Fig. 2). HBCDD was only detected in one sample from farm 1, with a concentration of 0.27 ng/g lw.

285
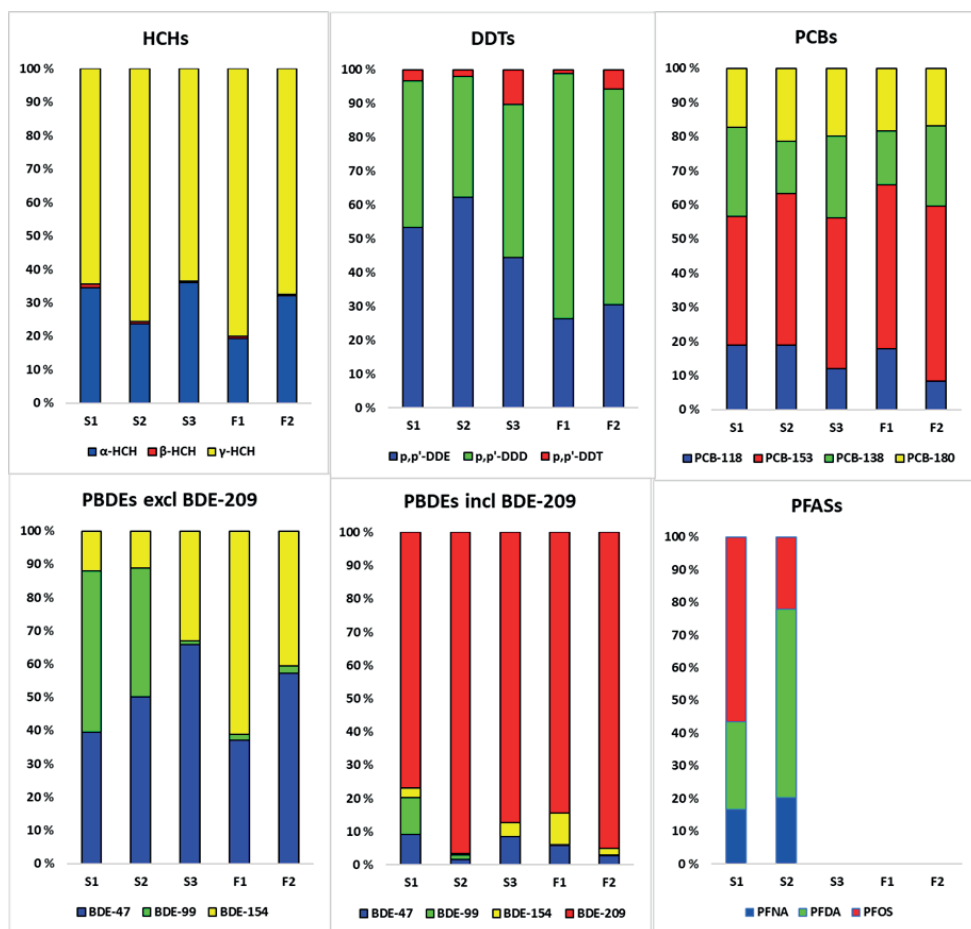
Figure 3. Relative contribution of individual compounds to ∑HCHs, ∑DDTs, ∑CHLs, ∑PCBs,

287 ∑PBDEs and PFASs

288 *Occurrence and levels of PFASs*

289 PFOS, PFDA and PFNA were the only PFASs detected in levels >LOD in individual wild fish

290 (Table 2B). PFOS and PFNA were detected in 100 % and 40 % in wild fish from site 1 and 2,

291 respectively, while PFNA was detected in 20 % in site 1 and 2. The highest median level of

292 PFOS (0.66 ng/g ww) was found in wild fish from site 1 while highest median level of PFDA

293 (0.37 ng/g ww) was found in site 2 (Table 3; Fig. S1). No PFASs were detected in site 3, farm

294 1 or farm 2.

295

Table 3: Mean concentrations (ng/g ww), median (in brackets) and range of PFAS compounds detected in wild and farmed tilapia from Lake Kariba, Zambia.

PFAS compound	Location				
	Site 1 (n=5)	Site 2 (n=5)	Site 3 (n=6)	Farm 1 (n=5)	Farm 2 (n=5)
PFNA	**0.185** (0.194) <LOD-0.274 1/5	**0.207** (0.129) <LOD-0.677 1/5	<LOD	<LOD	<LOD
PFDA	**0.301** (0.313) 0.221-0.374 5/5	**0.478** (0.367) <LOD-1.18 4/5	<LOD	<LOD	<LOD
PFOS	**0.643** (0.658) 0.46-0.859 5/5	**0.34** (<LOD) <LOD-0.866 2/5	<LOD	<LOD	<LOD

PFNA: perfluorononanoic acid
PFDA: perfluorodecanoic acid
PFOS: perfluorooctane sulfonate

296

Correlations

298 Spearman correlations coefficients for the main compounds HCB, $\sum$DDTs, $\sum_{17}$PCBs, $\sum_9$BDEs

299 and $\sum_{10}$BDE are presented in Table S2. In site 1, strong correlations were found between HCB

300 and $\sum_{10}$BDE (r=-0.83) and between $\sum_{17}$PCBs and $\sum_9$BDEs. In site 2, strong correlations were

301 found between HCB and $\sum_{17}$PCBs (r=0.77) and between $\sum_9$BDEs and $\sum_{10}$BDE (r=0.89). In

302 site 3, $\sum_{17}$PCBs and $\sum_9$BDEs strongly correlated (r=0.77). In farm 1, $\sum_{17}$PCBs and $\sum$DDTs

303 showed strong correlations (r=0.83), while in farm 2, no strong correlations were found.

304 *Compliance with reference levels*

305 Compared to recommended Environmental quality standards (EQS) from European

306 commission most of the POPs were below the limits for fish except for $\sum_{10}$PBDEs (mean 0.04

307 - 0.36 ng/g ww) which was higher than the EQS limits of 0.0085 ng/g set the EU (European

308 Commission, 2013; Jürgens et al., 2015).

309 **4. Discussion**

310 The main goal in fish farming industry is to obtain high quality fish for sale in short time. The

311 fish must fulfil criteria set by health authorities, as regards to nutritional value as well as to the

312 presence of environmental contaminants/pollutants (Saavedra et al., 2017; Skåre et al., 2014).

313 The compounds in fish feed are therefore specially put together for the purposes of fast growth,

314 high content of nutrients and low content of pollutants. Median fish liver lipid contents from

315 farm 1 and 2, and from wild fish from site 3, were all higher compared to wild fish from sites

316   1 and 2 (Table 2, Fig 2). This indicates good conditions for the farmed fish, but also for the

317   wild fish foraging around the fish farms. Other studies confirm that spillage of feed and organic

318   waste from the fish cages makes more nutrients available to wild fish (Ballester-Moltó et al.,

319   2017; Bustnes et al., 2010; Varol, 2019).

320   *Levels and congener profile of OCPs, PCBs and BFRs*

321   DDTs were the dominant OCPs in both wild and farmed fish liver tissues from Lake Kariba,

322   but wild fish had significantly ($p < 0.05$) higher levels of median ∑DDTs compared to the

323   farmed fish (Table 2, Fig. 2). The dominance of DDTs in OCP pattern was similar to findings

324   in other studies (Berg et al., 1992; Mwakalapa et al., 2018) (Table 4). The countries around

325   Lake Kariba (Zambia and Zimbabwe), have a history of DDT use for vector control in

326   combatting malaria and tsetse control operations in addition to agriculture (Berg et al., 1995).

327   Lake Kariba was filled with water in 1958-1963. Because the flooded areas were earlier treated

328   with DDT, the sediments of the Lake is still a reservoir of DDT residues. Due to long half-life

329   of DDT and different metabolism under anaerobic conditions, DDT and its metabolites

330   originating from the time before the Lake was filled, may still contribute to exposure of living

331   organisms in Lake Kariba today (Berg et al., 1995; Brevik, 1996). In addition, DDT may have

332   entered the Lake Kariba by run-off and atmospheric deposition (Banda & Mundia, 2009; Berg

333   et al., 1992; Ssebugere et al., 2009). Use of DDT was banned globally in the 1970s but is still

334   allowed for use in indoor residual spraying (IRS) and for production of insecticide-treated

335   mosquito nets (ITN) (WHO, 2011). Due to these campaigns, the levels of DDT are expected

336   to decrease in the environment of the Southern African region. Biodegradation of DDT results

337   mainly in the more persistent metabolites *p,p'*-DDE and *p,p'*-DDD. In the present study, the

338   contribution of *p,p'*-DDE to ∑DDTs was highest in wild fish from site 1 and 2 but decreased

339   eastwards in wild fish from site 3 (Fig. 3). In the farmed fish, *p,p'*-DDD was contributing most

340   to ∑DDTs (Table 2, Fig. 3). Higher ratio of *p,p'*-DDD/ p,p'-DDE is related to anaerobic

341   degradation in soil and sediments and uptake in plant roots (Buah-Kwofie et al., 2017; Chen et

342   al., 2007). Periods of drought, or increased water flow in the farming area may contribute to an

343   increased bioavailability of chemical pollutants stored in sediments below the fish cages.

344   However, this needs to be studied further. The ratios of *p,p'*-DDE/*p,p'*-DDT were lower in

345   several wild fish from site 1 and site 3, indicating relatively recent use of DDT in the area

346   (Table 4). In addition, one of the pooled samples from site 1 contained *o,p'*-DDT, strengthening

347   this observation. Levels of mean ∑DDTs in the wild tilapia were lower than those reported in

348 Lake Victoria (Henry & Kashimbi, 2006; Polder et al., 2014), but higher than from other areas

349 (Deribe et al., 2011; Gbeddy et al., 2015; Mdegela et al., 2009) (Table 4). Farmed tilapia in the

350 present study had similar levels of mean ∑DDTs to those reported earlier from Lake Kariba,

351 Zimbabwean side (Berg et al., 1992) (Table 4).

352 The second dominant OCP, HCB, was detected in low levels which were below the EQS set

353 by the EU (Table 4). Median levels of HCB were significantly higher in wild fish from all sites

354 compared to farmed fish (Table 2B, Fig. 2). This is in accordance with previous studies from

355 Lake Kariba (Berg et al., 1992). HCB was used as a fungicide, in rubber synthesis and wood

356 preservation among other uses, but is banned under the Stockholm Convention (Stockholm

357 Convention, 2021). The low levels observed may reflect a general background level related to

358 long range atmospheric transport from emission of industries at far distance (Polder et al.,

359 2014). Levels of HCB in the current study were in the same range as levels reported in tilapia

360 liver from Tanzania (Mwakalapa et al., 2018; Mdegela et al., 2009), but lower than in tilapia

361 muscle from Tanzania (Polder et al., 2014), and much lower than in brown trout in Norway

362 (Lyche et al., 2018), (Table 4).

363 Although levels of HCHs generally were low in all study sites, the median ∑HCHs was

364 significantly higher in wild fish from site 2 and 3 compared to farm 1 ($p < 0.05$) (Fig. 2). Lindane

365 (γ-HCH) was used as an insecticide on fruit and vegetable crops, for seed treatment and

366 treatment of lice and scabies, mainly in West-European and Asian countries (Vijgen et al.,

367 2006). Pure lindane is contaminated with α-HCH and β-HCH of which β-HCH is the most

368 persistent isomer. Due to regulations, levels of lindane are decreasing. The patterns of HCHs

369 found in this study may thus reflect historic use of the technical mixture combined with

370 relatively recent use of γ-HCH. The general HCH levels in tilapia from Lake Kariba were in

371 the same range as those reported earlier by Berg et al. (1992) in Lake Kariba, Mwakalapa et al.

372 (2018) and Polder et al. (2014) in Tanzania, but much lower than findings in South Africa by

373 Verhaert et al. (2017) and in Ghana by Gbeddy et al. (2015) (Table 4).

374 The ∑CHL levels were very low, but nevertheless significantly higher in wild than in farmed

375 fish (Fig. 2). Chlordanes are banned compounds and are no longer used as insecticides

376 (Stockholm Convention 2021) and their levels are expected to decrease. Mean levels of ∑CHL

377 were less than those reported by Polder et al. (2014) and Mdegela et al. (2009) in Tanzania and

Table 4: Comparison of mean concentrations (ng/g lw) of persistent organic pollutants in tilapia and other fish species from various countries.

Country	Location	Sample	Lipid%	HCB	∑HCH	p,p'-DDE	p,p'-DDT	∑DDTs	DDE/DDTs	∑CHLs	∑PCBs	∑PBDEs	∑HBCDD	References
Zambia	Wild S1 *Oreochromis niloticus*	Liver	6.77	0.32	0.35	52.3	6.88	97.9	17	0.15	3.29	2.55	<LOD	Current study
Zambia	Wild S2 *Oreochromis niloticus*	Muscle	4.73	0.21	0.42	64.4	2.0	98.1	60	0.12	0.44	9.29	<LOD	Current study
Zambia	Wild S3 *Oreochromis niloticus*	Muscle	7.82	0.27	0.70	12.2	2.37	24.9	5.8	0.14	0.92	1.77	<LOD	Current study
Zambia	Farm F1 *Oreochromis niloticus*	Liver	8.09	0.13	0.24	2.41	0.07	8.20	32	0.05	0.19	0.55	0.05*	Current study
Zambia	Farm F2 *Oreochromis niloticus*	Muscle	9.44	0.10	0.38	3.07	0.57	9.96	20	0.05	0.26	1.24	<LOD	Current study
Zimbabwe	Caged *Oreochromis spp*	Muscle		<LOD	0.3	4.2	1.5	6.9						(Berg et al., 1992)
Zimbabwe	Wild *Oreochromis spp*	Muscle		0.5	0.4	26	2.4	31.9						(Berg et al., 1992)
Zimbabwe	Pond *Tilapia rendalli*	Muscle		0.1	0.4	4.1	1.7	6.7						(Berg et al., 1992)
Zimbabwe	Wild *Tilapia rendalli*	Muscle		0.2	0.6	14.7	4.3	21.1						(Berg et al., 1992)
Tanzania	Pond *Chanos chanos*	Liver	7.98	0.22	0.05	13.5	3.2	16.60			1.47	1.64	<LOD	(Mwakalapa et al., 2018)
Tanzania	Indian ocean *Chanos chanos*	Liver	7.81	0.20	0.10	103	2.50	177	41		1.30	1.30	<LOD	(Mwakalapa et al., 2018)
Tanzania	Indian ocean *Mugil Cephalus*	Liver	4.85	0.20	0.04	60.7	2.2	73.3	35	0.90	0.20	1.10	5.20	(Mwakalapa et al., 2018)
Tanzania (LT)	*Oreochromis niloticus*	Muscle	3.30	1.20	1.10	116	41.8	273	2.8	0.5	0.90	4.10	<LOD	(Polder et al., 2014)
Tanzania (LV)	*Oreochromis niloticus*	Muscle	0.4	2.5	<LOD	27.1	1.4	34.7	9.4		17.2	34.4	<LOD	(Polder et al., 2014)
Tanzania (LN)	*Oreochromis niloticus*	Muscle	1.9	2.1	1.2	7.1	4.4	13.2	1.7		0.6	6.5	<LOD	(Polder et al., 2014)
Tanzania (LB)	*Oreochromis niloticus*	Muscle	4.1	0.9	<LOD	28.9	2.1	42.7	3.7		0.1	1.5	2.4	(Polder et al., 2014)
Tanzania	*Oreochromis niloticus*	Muscle	1.50					500						(Henry & Kishimba, 2006)
Tanzania	*Oreochromis urolepis*	Muscle	8.00	2.50		25.0	21.3	46.3		2.50	2.50			(Mdgela et al., 2009)
DR Congo	*Distichodus fasciolatus*	Muscle	1.2	0.02	0.04	0.04		0.14			0.89	0.2		(Verhaert et al., 2013)
South Africa	*Oreochromis mossambicus*	Muscle	0.98	117.5	34.7	4,162	937	5,537		10.2	532	5.8	<LOD	(Verhaert et al., 2017)
South Africa	*Hydrocynus vittatus*	Muscle	3.83		12.9			520		2.9	<LOD	<LOD	5.8	(Wepener et al., 2012)
Ethiopia	*Oreochromis niloticus*	Muscle						6.90			0.05	5.6		(Deribe et al., 2011)
Uganda	*Oreochromis niloticus*	Muscle												(Ssebugere et al., 2014)
Ghana	*Oreochromis niloticus*	Muscle	2.8			41.6				4.02	54	7.1		(Gbeddy, et al., 2015)
Ghana	*Oreochromis niloticus*	Muscle	1.9		0.15	0.15	0.53	10.7					0.75	(Asante et al., 2013)
Norway	Femunden *Salmo trutta*	Muscle	3	18.1							108	28	15	(Lycke et al., 2018)

<LOD: lower than limit of detection

* Mean of six, of which only one sample positive with 0.27 ng/g lw

LT-Lake Tanganyika, LV- Lake Victoria, LN-Lake Nyasa and LB-Lake Babati.

379   Gbeddy et al. (2015) in Ghana. Mirex and Heptachlor are also banned substances and were
380   below detection limit in all samples (Table S 1.1).

381   PCBs were detected in very low levels in wild and farmed tilapia and those levels were in the
382   same range as in other East African countries (Deribe et al., 2011; Kidd et al., 2004; Mwakalapa
383   et al., 2018), but lower than in tilapia from Tanzania and Ghana, and other fish from South
384   Africa and Norway (Polder et al., 2014; Wepener et al., 2012; Asante et al., 2013; Lyche et al.,
385   2018) (Table 4). Occurrence and levels of PCB (17.2 ng/g lw) in wild tilapia from Lake
386   Tanganyika was suggested to be related to human activities and small local industries (Polder
387   et al., 2014). Wild tilapia from site 1 showed the highest median $\sum_{17}$PCBs (3.2 ng/g lw) (Table
388   2B). Emission from a coal mine in the area may be a possible source. Other possible sources
389   of PCBs in countries with limited historic use of PCBs are waste burning, transportation,
390   household heating, discharges from cities, sewage processing, e-waste burning, hospital waste
391   incineration, and transformer oil (Pius et al., 2019). In the present study only PCB-118, -138, -
392   153 and -180 were detected in more than 50% of the samples. These congeners are the most
393   persistent PCBs and contributed more than 60 % to $\sum_{17}$PCBs in the present study. The
394   dominance of PCB-153, PCB-180 and PCB-138 in the PCB pattern was similar to findings in
395   other studies (Asante et al., 2013; Hayward et al., 2007; Mwakalapa et al., 2018; Polder et al.,
396   2014). In the present study, PCB-118 was detected in 72 % of the wild tilapia, but only in 25
397   % of the farmed tilapia (data not shown). PCB-118 is a mono-*ortho* substituted PCB and has a
398   toxic equivalent factor of 0.00003 (Van den Berg et al., 2006). In the study by Polder et al.
399   (2014) PCB-118 was only detected in one tilapia sample from Lake Victoria and one from
400   Lake Babati in Tanzania. It seems thus, that the environment in Lake Kariba is exposed to a
401   different historic PCB mixture than in other studies in the region.

402   PBDEs were used as flame retardants in thermoplastics (computer and TV housing), textiles,
403   foams, furniture, electronics, building materials and interiors of cars, busses, and airplanes
404   (Covaci et al., 2008; Lyche et al., 2015). In general, low PBDE levels were detected in the
405   present study. BDE-47, -99 and -154 were detected in more than 50 %, whereas BDE-209 was
406   detected in 97 % of the samples in all areas. Tetra-BDE (BDE- 47) and penta-BDE (BDE-99)
407   are the most abundant, toxic, and bioaccumulative PBDE congeners (Ssebugere et al., 2014;
408   Asante et al., 2013; Mwakalapa et al., 2018). Due to differences in metabolism, lower
409   brominated congeners such as BDE 47 and 100 accumulate more in aquatic organisms, while
410   higher brominated congeners like BDE 154 and 209 accumulate more in terrestrial organism
411   (Luo et al., 2019). Deca-BDE, a mixture of nona-, octa and deca BDEs were used as a

21

412 replacement for the tetra and penta- BDE mixtures after they got banned (Stockholm
413 Convention, 2021). However, deca-BDE debrominates to lower brominated and more
414 persistent BDE congeners, such as BDE-47 (Stapleton et al., 2004). Half-life of BDE-209 may
415 vary in different compartments. Luo et al. (2013) found that the dose dependent half-life of
416 BDE-209 in the muscle of rice fish was from 17 to 19.4 days. Recent studies showed that there
417 is a significant amount of gaseous BDE-209 in the global atmosphere, which is subject to long-
418 range atmospheric transport (LRAT) (Li et al., 2017). The occurrence of BDE-209 in nearly
419 100 % of the fish samples from Lake Kariba may therefore be explained by precipitation of
420 atmospheric transported BDE-209, although the high median BDE-209 levels in site 2 (7.8
421 ng/g lw) may suggest additional exposure from a local source. Median levels of $\sum_{10}$PBDEs
422 were significantly higher in wild fish from site 1 and 2, compared to the farmed fish,
423 strengthening the hypothesis that wild fish has been exposed to historic precipitation of
424 atmospheric transported BDE-209. $\sum_{10}$PBDE levels in wild tilapia sites 1 and 2 were from four
425 to thirteen-fold lower than in tilapia muscle from Lake Victoria but comparable to findings in
426 the other lakes in Tanzania (Polder et al., 2014). They were in the same range as in tilapia from
427 Ghana (Asante et al., 2013) and African tigerfish (*Hydrocynus vittatus*) from South Africa
428 (Wepener et al., 2012), and tilapia in Uganda (Ssebugere et al., 2014), higher than in Milkfish
429 (*Chanos chanos*) from Tanzania (Mwakalapa et al., 2018) but much lower than in trout (*Salmo*
430 *trutta*) in Norway (Lyche et al., 2018) (Table 4). A global deca-BDE ban was adopted under
431 the UN Stockholm Convention in 2017 and BDE-209 levels and its debromination products
432 are thus expected to decrease in the environment.

433

434 *Levels and pattern of PFAS*
435 This is the first time PFASs are detected in fish from Zambia. PFASs is a large group of
436 perfluorinated substances produced since the 1950 and because of their water repellant
437 properties used in various consumer products such as impregnated outdoor textiles, shoes, food
438 containers, kitchen ware and firefighting foam. They are very persistent to degradation and
439 may cause adverse health effects in living species. In contrast to the lipophilic POPs, PFASs
440 bind to proteins and are more soluble in water (Groffen et al., 2018; Gronnestad et al., 2017;
441 Lam et al. 2014; Mudumbi et al., 2014). In the present study PFASs were only detected in wild
442 tilapia in site 1 and 2, and not in site 3 and in farmed fish. PFDA and PFOS were both detected
443 in levels >LOD in 100 % of the tilapia from site 1, and in 80 % and 40 % in site 2, respectively
444 (Fig. 3). PFNA was only found in one sample from site 1 and 2. The occurrence of PFASs in

445   the wild fish from Sinazongwe (site 1), Gwembe (site 2) may be related to mining industries in

446   the area. There are to our knowledge only two studies on PFASs in South African fish. Verhaert

447   et al., (2017) found comparable concentrations of PFOS, and PFNA in muscle tissue ranging

448   from 0.15 to 2.7, and <LOQ to 0.14 ng/g ww in muscle of fish from the Olifants River basin,

449   while Groffen et al. (2018) found PFOS in fish liver from Vaal River in much higher levels

450   similar or higher than in USA, Europe and Asia. than in European studies. PFOS levels in trout

451   from the isolated, large and deep inland Lake Femund, Norway, were 3.96 ng/g ww, thus 10

452   times higher than in tilapia from the present study (Lycke et al., 2018). The PFASs found in

453   this study were long chained (>6C), of which PFOS are regulated under Stockholm Convention

454   since 2009. These compounds are therefore expected to decline in the future.

455

456   *Possible implications for fish and human health*

457   In the present study, levels of PBDEs exceeded the European standard (EQS) for these

458   contaminants in fish and may harm fish health (Table 5). Follow up studies are needed to ensure

459   that international regulations result in decrease of these and other contaminants that threaten

460   the aquatic environment.

461

462   Dioxin-like (DL) PCB-118 TEQ levels (pg/g ww) were below EQS for DL-PCPs (Table 5).

463   Liver tissues were used in this study because lipophilic POPs would show highest levels in the

464   lipid rich liver. Results from this study can, therefore, not directly be used in a risk assessment

465   for humans since humans consume fish muscle. However, the percentage of lipid in tilapia liver

466   (range 3-19 %) (present study) is higher than in its muscle, (0.4-4 %) (Polder et al., 2014).

467   Therefore, one can assume that POP levels in wild and farmed tilapia liver from the present

468   study are much higher than in the muscle tissue. In 2011, the EU set an MRL for Σ non-dioxin

469   like (NDL) PCBs (PCB-28, -52, -101, -138, -153, and -180) in fish filet to 75 ng/g ww. The

470   highest sum of ΣNDL-PCBs in the present study (sum of PCB 118, 138, 153, 180) was 0.2

471   ng/g ww in liver, and thus far below this EU MRL. POP levels varied sometimes much between

472   samples from the same area. This may have consequences when calculating risk in future

473   studies. Therefore, analyzing of individual samples is recommended to get a better view on the

474   variation in the specified areas.

Table 5: Environmental quality standards (EQS) for contaminants in fish and median levels (expressed as ng/g wet weight (ww), (dioxin-like PCBs, DL-PCBs as pg/g TEQs ww) in wild and farmed (grouped and individual) tilapia from Lake Kariba, Zambia,

Contaminant	EU EQS	Present study		
		Median wild fish, site S1 & S2 (long distance from the farms)	Wild fish, site 3 (near farms)	Farmed fish Farm F1 and F2
DDT		3.68	1.51	0.76
HCB	10	0.01	0.02	0.01
γ-HCH	-	0.009	0.022	0.016
Mirex	-	-	-	-
CHL	-	0.005	0.008	0.004
Heptachlor and heptachlor epoxide	0.0067	-	-	-
PCBs	0.6	0.04	0.07	-
DL-PCB: PCB-118*	6.5*	<0.001*	<0.001*	0.02
HBCDD	167	-	-	<0.001*
PBDEs	0.0085	0.16	0.09	F1:0.04; F2:0.11
PFOS	9.1	0.40	-	-

*: pg TEQ/g ww

5. Conclusion

The present study shows that in general, levels of OCPs, PCBs, BFRs and PFAS are lower in farmed fish compared to wild fish within the same lake. This indicates lower risk for adverse health effects in farmed fish than for wild fish in Lake Kariba. The study revealed a geographical trend with higher levels of DDTs, PCBs, PBDEs and PFASs from west to east of Lake Kariba. However, levels of HCB and HCHs were also higher in fish that had foraged near the farms. This needs further investigation to elucidate possible sources. The contribution of *p,p'*-DDD to ΣDDTs increased eastwards, possibly due to higher environmental impact of anaerobic processes and historic use. PCB levels were low, and PCB profiles were dominated by the most persistent PCB congeners PCB-118, -138, -153, and -180. This indicated exposure to historical used PCB, but to a different PCB mixture than in some other East African countries. The finding of the now banned PFOS and BDE-209 in the wild tilapia from the western part of the lake, warrant further research for determination of the possible sources.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. (Anuschka.polder@nmbu.no)

Declaration

Conflict of interest: We declare no conflict of interest.

Author Contribution Statement

509　Chalumba K Simukoko: Collected samples, did chemical analyses, treated data, wrote the text
510　and performed statistical analyses for original draft. Contribution to revised manuscript.
511　Eliezer B Mwakalapa: Collecting of samples, helping in analyzing data and writing the original
512　draft, design of map. Contribution to revised manuscript.
513　Kaampwe Muzandu: Supervision of whole study: Design, collection of samples, evaluation of
514　data, and manuscript writing. Contribution to revised manuscript.
515　Stephen Mutoloki: Study design, collection of samples, reviewing the writing.
516　Øystein Evensen: Study design, collection of samples, reviewing the writing.
517　Erik M Ræder: Analyses of PFAS and evaluation of PFAS results. Writing of PFAS chapter.
518　Mette B Müller: Evaluation of chemical data. Review and revision of the manuscript, revision
519　of statistical analyses, revision of tables and figures. Contributing with editing of revised
520　manuscript.
521　Anuschka Polder: Supervision and contribution with analyzing data and writing of original
522　draft. Editing of the revised manuscript. Corresponding author.
523　Jan L Lyche: Supervision of all stages of study steps: Design, collection of samples, evaluation
524　of data, and manuscript writing. Contribution to revised manuscript.
525

526　**References**
527　Allan, J. D., Abell, R., Hogan, Z., Revenga, C., Taylor, B. W., Welcomme, R. L.,
528　　　Winemiller, K. (2005). Overfishing of Inland Waters. *BioScience 55*(12):1041–1051.
529　　　https://doi.org/10.1641/0006-3568(2005)055[1041:OOIW]2.0.CO;2

530　Asante, K., A., Takahashi, S., Itai, T., Isobe, T., Devanathan, G., Muto, M., Agyakwah, S.,
531　　　K., Adu-Kumi, S., Subramanian, A., Tanabe, S. (2013). *Ecotoxicology and*
532　　　*Environmental Safety 94*:123-130. https://doi.org/10.1016/j.ecoenv.2013.05.008

533　Ashraf, M. A. (2017) Persistent organic pollutants (POPs): a global issue, a global challenge.
534　　　*Environ Sci Pollut Res 24*:4223–4227.

535　Ballester-Moltó, M. , Sanchez-Jerez, P., Aguado-Giménez, F. (2017). Consumption of
536　　　particulate wastes derived from cage fish farming by aggregated wild fish. An
537　　　experimental approach. *Marine Environmental Research 130*: 166-173.
538　　　doi:https://doi.org/10.1016/j.marenvres.2017.07.014

539　Banda, S. F. & Mundia, A. Y. (2009). An assessment of the demand for DDT (A case study
540　　　for Zambia). *Current World Environment 4*:293-298.

541 Barni, M. F. S., Ondarza, P. M., Gonzalez, M., Da Cuna, R., Meijide, F., Grosman, F.,
542   Sanzano, P., Lo Nostro, F. L., Miglioranza, K. S. B. (2016). Persistent organic
543   pollutants (POPs) in fish with different feeding habits inhabiting a shallow lake
544   ecosystem. *Sci Total Environ 550*:900-909.

545 Berg, H., Martina, K., Kautsky, N. (1992) DDT and Other Insecticides in the Lake Kariba
546   *Ecosystem, Zimbabwe. Ambio 21*:444-450.

547 Brevik, E. M. (1978). Gas chromatographic method for determination of organochlorine
548   pesticides in human milk. *Bull Environ Contam Toxicol 19***:281–286.

549 Brevik, E. M., Grande, M., Knutzen, J., Polder, A., Skaare, J.U. (1996). DDT contamination
550   of fish and sediments from Lake Ørsjøen, Southern Norway: Comparison of data from
551   1975 and 1994. *Chemosphere 33*(11):2189-2200.

552 Buah-Kwofie, A. & Humphries, M., S. (2017). The distribution of organochlorine pesticides
553   in sediments from iSimangaliso Wetland Park: Ecological risks and implications for
554   conservation in a biodiversity hotspot. *Environmental Pollution* 229:715-723.
555   https://doi.org/10.1016/j.envpol.2017.07.031

556 Burreau, S., Zebühr, Y., Broman, D., Ishaq, R. (2004). Biomagnification of polychlorinated
557   biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (Esox
558   lucius), perch (Perca fluviatilis) and roach (Rutilus rutilus) from the Baltic Sea.
559   *Chemosphere 55*:1043-1052.

560 Bustnes, J. O., Lie, E., Herzke, D., Dempster, T., Bjørn, P.A., Nygård, T., Uglem, I. (2010).
561   Salmon Farms as a Source of Organohalogenated Contaminants in Wild Fish. *Environ
562   Sci Technol 44*:8736-8743.

563 Chen, S. H., Xu, F. L., Dawson, R., Jiao, X. C., Tao, S. (2007). Adsorption and absorption of
564   dichlorodiphenyltrichloroethane (DDT) and metabolites (DDD and DDE) by rice roots..
565   *Environ Pollut 147*:256-261.

566 Covaci, A., Voorspoels, S., Roosens, L., Jacobs, W., Blust, R., Neels, H. (2008).
567   Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in
568   human liver and adipose tissue samples from Belgium. *Chemosphere 73*:170-175.

569 Deribe, E., Rosseland, B. O., Borgstrom, R., Salbu, B., Gebremariam, Z., Dadebo, E., Norli,
570   H. R., Eklo, O. M. (2011). Bioaccumulation of persistent organic pollutants (POPs) in
571   fish species from Lake Koka, Ethiopia: The influence of lipid content and trophic
572   position. *Sci Total Environ 410-411*:136-145.

573 EPA (2019). Environmental Protection Agency. PFOA, PFOS and Other PFASs. Basic
574   Information on PFAS. https://www.epa.gov/pfas/basic-information-pfas. Accessed on
575   05/01/2022.

576 European Commission (2013). Directive 2013/39/EU of the european parliament and of the
577   council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as
578   regards priority substances in the field of water policy. Official Journal of the European
579   Union.

580 FAO (2005). The State of Food and Agriculture. FAO Agriculture Series No. 36. ISSN 0081-
581   4539. Available at http://www.fao.org/catalog/inter-e.htm.

582 FAO (2018). The State of World Fisheries and Aquaculture - Meeting the Sustainable
583   Development Goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.. ISBN 978-92-5-
584   130562-1. Available at https://www.fao.org/3/I9540EN/i9540en.pdf.

585 FAO (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action.
586   Rome. https://doi.org/10.4060/ca9229en

587 Gbeddy, G., Glover, E., Doyi, I., Frimpong, S., Doamekpo, L. (2015). Assessment of
588   Organochlorine Pesticides in Water, Sediment, African Cat fish and Nile tilapia,
589   Consumer Exposure and Human Health Implications, Volta Lake, Ghana. *J Environ*
590   *Anal Toxicol, 5*: 1-8.

591 Genschick, S., Kaminski, A.M., Kefi, A.S., Cole, S.M. (2017). Aquaculture in Zambia- An
592   overview and evaluation of the sector's responsiveness to the needs of the poor. Penang,
593   Malaysia: *CGIAR Research Program on Fish Agri-Food Systems and Lusaka, Zambia:*
594   *Department of Fisheries. Working Paper: FISH-2017-08.*

595 Groffen, T., Wepener, V., Malherbe, W., Bervoets, L. (2018). Distribution of perfluorinated
596   compounds (PFASs) in the aquatic environment of the industrially polluted Vaal River,
597   South Africa. *Sci Tot Environ 627*:1334-1344.

598 Gronnestad, R., Villanger, G. D., Polder, A., Kovacs, K. M., Lydersen, C., Jenssen, B. M ,
599   Borga, K. (2017). Maternal transfer of perfluoroalkyl substances in hooded seals.
600   *Environ Toxicol Chem*, 36:763-770.

601 Hayward, D., Wong, J., Krynitsky, A.J. (2007). Polybrominated diphenyl ethers and
602   polychlorinated biphenyls in commercially wild caught and farm-raised fish fillets in
603   the United States. *Environ Res 103*:46-54.

604 Henry, L. & Kishimba, M.A. (2006). Pesticide residues in Nile tilapia (Oreochromis
605   niloticus) and Nile perch (Lates niloticus) from Southern Lake Victoria, Tanzania.
606   *Environ Pollut 140*:348-354.

607 Jürgens, M. D., Chaemfa, C., Hughes, D., Johnson, A. C., Jones, K. C. (2015). PCB and
608   organochlorine pesticide burden in eels in the lower Thames River (UK). *Chemosphere*
609   *118*:103-111.

610 Kidd, K. A., Stern, G., Lemoalle, J. (2004). Mercury and Other Contaminants in Fish from
611   Lake Chad, Africa. *Bull Environ Contam Toxicol 73*:249–256.

612 Kishimba, M. A., Henry, L., Mwevura, H., Mmochi, A. J., Mihale, M., Hellar, H. (2004). The
613   status of pesticide pollution in Tanzania. *Talanta 64*:48-53.

614 Lam, N-H., Cho, C-R., Lee, J-S., Soh, H-Y., Lee, B-C., Lee, J-A., Tatarozako, N., Sasaki, K.,
615   Saito, N., Iwabuchi, K., Kannan, K., Cho, H-S. (2014). Perfluorinated alkyl substances
616   in water, sediment, plankton and fish from Korean rivers and lakes: A nationwide
617   survey. *Sci Tot Environ 491-492*:154-162.

618 Letcher, R. J., Bustnes, J. O., Dietz, R., Jenssen, B.M., Jorgensen, E.H., Sonne, C., Verreault,
619   J., Vijayan, M. M., Gabrielsen, G. W. (2010). Exposure and effects assessment of
620   persistent organohalogen contaminants in arctic wildlife and fish. *Sci Total Environ*
621   *408*:2995-3043.

622 Li, X., Li, J., Wang, Y., Fu, L., Fu, Y., Li, B., Jiao, B. (2011). Aquaculture Industry in China:
623   Current State, Challenges, and Outlook. *Rev Fish Sci 19*:187-200.

624 Luo, Y-L., Luo, X-J., Ye, M-X., Lin, L.. Zeng, Y-H., Mai, B-X. (2019). Species-specific
625   debromination of polybromodiphenyl ethers determined by deiodinase activity in fish.
626   *Environ Pollut 246*:710-716.

627 Lycke, J. L., Berg, V., Kallenborn, R. (2018). |Prioriterte miljøgifter i norsk ferskvannsfisk
628   2013-2016 (EU Priority pollutants in Norwegian fresh water fish 2013-2016). *Report*
629   *Miljøovervåkning M-1075*.

630 Lyche, J. L., Rosseland, C., Berge, G., Polder, A. (2015). Human health risk associated with
631   brominated flame-retardants (BFRs). *Environ Int 74*:170-180.

632 Mdegela, R. H., Braathen, M., Pereka, A.E., Mosha, R. D., Sandvik, M., Skaare, J.U. (2009).
633   Heavy Metals and Organochlorine Residues in Water, Sediments, and Fish in Aquatic
634   Ecosystems in Urban and Peri-Urban Areas in Tanzania. *Water Air Soil Pollut*
635   *203*:369–379

636 Mhlanga, L., Phiri, C., Chifamba, P. (2014). Challenges surrounding water management
637   within Lake Kariba environs. In: Mhlanga L KN, Haller T (eds.) *Fragmentation of*
638   *Natural Resources Management: Experiences from Lake Kariba Lit-Verlag*
639   *Switzerland: PLAAS*.

640 Mudumbi, J. B. N., Ntwampe, S., Muganza, F. M., Okonkwo, O. (2014). Perfluorooctanoate
641   and perfluorooctane sulfonate in South African river water. *Water Sci Technol 69*:185-
642   94.

643 Mwakalapa, E. B., Mmochi, A.J., Müller, M. H. B., Mdegela, R.H., Lyche, J. L., Polder, A.
644   (2018). Occurrence and levels of persistent organic pollutants (POPs) in farmed and
645   wild marine fish from Tanzania. A pilot study. *Chemosphere 191*:438-449.

646 Nieuwoudt, C., Quinn, L. P., Pieters, R., Jordaan, I., Visser, M., Kylin, H., Borgen, A.R.,
647   Giesy, J. P., Bouwman, H. (2009). Dioxin-like chemicals in soil and sediment from
648   residential and industrial areas in central South Africa. *Chemosphere 76*:774-783

649 Nonga, H. E., Mdegela, R. H., Lie, E., Sandvik M., Skaare, J. U. (2011). Assessment of
650   farming practices and uses of agrochemicals in Lake Manyara basin, Tanzania. *Afr J
651   Agric Res 6*:2216-2230.

652 Pius, C., Sichilongo, K., Mswela, P. K., Dikinya, O. (2019). Monitoring polychlorinated
653   dibenzo-p-dioxins/dibenzofurans and dioxin-like polychlorinated biphenyls in Africa
654   since the implementation of the Stockholm Convention—an overview. *Environ Sci
655   Pollut Res 26*:101–113.

656 Polder, A., Müller, M. B., Lyche, J. L., Mdegela, R. H., Nonga, H. E., Mabiki, F. P., Mbise,
657   T. J., Skaare, J. U., Sandvik, M., Skjerve, E., Lie, E. (2014). Levels and patterns of
658   persistent organic pollutants (POPs) in tilapia (Oreochromis sp.) from four different
659   lakes in Tanzania: geographical differences and implications for human health. *Sci
660   Total Environ 488-489*:252-260.

661 fdecRodriguez-Hernandez, A., Camacho, M., Henriquez-Hernandez, L. A., Boada, L. D.,
662   Valeron P. F., Zaccaroni, A., Zumbado, M., Almeida-Gonzalez, M., Rial-Berriel, C.,
663   Luzardo, O. P. (2017). Comparative study of the intake of toxic persistent and semi
664   persistent pollutants through the consumption of fish and seafood from two modes of
665   production (wild-caught and farmed). *Sci Total Environ 575*:919-931.

666 Saavedra, M., Pereira, T. G., Carvalho, L. M., Pousão-Ferreira, P., Grade, A., Teixeira, B.,
667   Quental-Ferreira, H., Mendes, R., Bandarra, N., Gonçalves, A. (2017). Wild and farmed
668   meagre, Argyrosomus regius: A nutritional, sensory and histological assessment of
669   quality differences. *J Food Compos Anal 63*:8-14.

670 Santonen, T., Nielsen, G., Papameletiou, D., Klein, C. (2017). SCOEL/REC/188
671   Hexachlorobenzene - Recommendation from the Scientific Committee on Occupational
672   Exposure Limits. February 2017. Publications Office of the European UnionISBN:
673   978-92-79-64140-4. DOI:10.2767/35665

674 Sharma, C. M., Rosseland, B. O., Almvik, M., Eklo, O. M. (2009). Bioaccumulation of
675   organochlorine pollutants in the fish community in Lake Årungen, Norway.
676   *EnvironPollut 157*:2452-2458.
677 Simukoko, C. K., Mwakalapa, E. B., Bwalya, P., Muzandu, K., Berg, V., Mutoloki, S.,
678   Polder, A., Lyche, J. L. (2021). Assessment of heavy metals in wild and farmed tilapia
679   (*Oreochromis niloticus*) on Lake Kariba, Zambia: implications for human and fish
680   health. *Food Additives & Contaminants: Part A, 39*:1, 74-91.
681 Squadrone, S., Favaro, L., Prearo, M., Vivaldi, B., Brizio, P., Abete, M. C. (2013). NDL-
682   PCBs in muscle of the European catfish (Silurus glanis): An alert from Italian rivers.
683   *Chemosphere 93*:521–525.
684 Ssebugere, P., Kiremire, B. T., Kishimba, M., Wandiga, S. O., Nyanzi, S. A., Wasswa, J.
685   (2009). DDT and metabolites in fish from Lake Edward, Uganda. *Chemosphere,*
686   *76*:212-5.
687 Ssebugere, P., Sillanpää, M., Wang, P., Li, Y., Kiremire, B. T., Kasozi, G. N., Zhu, C., Ren,
688   D., Shang, H., Zhang, Q., Jiang, G. (2014). Polychlorinated dibenzo-p-dioxins,
689   polychlorinated dibenzofurans and polybrominated diphenyl ethers in sediments and
690   fish species from the Murchison Bay of Lake Victoria, Uganda. *Sci Tot Environ 500-*
691   *501*:1-10.
692 Stapleton, H. M., Letcher, R. J., Baker, J. E. (2004). Debromination of Polybrominated
693   Diphenyl Ether Congeners BDE 99 and BDE 183 in the Intestinal Tract of the Common
694   Carp (Cyprinus carpio). *Environ Sci Technol 38*:1054-1061.
695 Subasinghe, R. P. (2005). Epidemiological approach to aquatic animal health management:
696   opportunities and challenges for developing countries to increase aquatic production
697   through aquaculture. *Prev Vet Med 67*:117-124.
698 Stockholm Convention (2021). Stockholm Convention on Persistent Organic Pollutants.
699   Available at http:// chm. pops. int, Accessed 5 Januari 2022.
700 Varol, M. (2019). Impacts of cage fish farms in a large reservoir on water and sediment
701   chemistry. *Environ Pollut 252*:1448-1454.
702 Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M.,
703   Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D.,
704   Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.
705   (2006). The 2005 World Health Organization Reevaluation of Human and Mammalian
706   Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. *Toxicol Sci*
707   *93(2)*:223–241. doi: 10.1093/toxsci/kfl055.

708 Verhaert, V., Covaci, A., Bouillon, S., Abrantes, K., Musibono, D., Bervoets, L., Verheyen,
709   E., Blust, R. (2013). Baseline levels and trophic transfer of persistent organic pollutants
710   in sediments and biota from the Congo River Basin (DR Congo). *Environ Int 59*:290-
711   302.

712 Vijgen, J., de Borst, B., Weber, R., Stobiecki, T., Forter, M. (2019). HCH and lindane
713   contaminated sites: European and global need for a permanent solution for a long-time
714   neglected issue. *Environ Pollut 248*:696-705.

715 VKM (2014): Benefit-risk assessment of fish and fish products in the Norwegian diet – an
716   update. Scientific Opinion of the Scientific Steering Committee. *VKM Report 15 [293*
717   *pp] , ISBN: 978-82-8259-159-1*. Oslo, Norway.

718 Wania, F. & Mackay, D. (1993). Global Fractionation and Cold Condensation of Low
719   Volatility Organochlorine Compounds in Polar Regions. *Ambio, 22*.

720 WHO (2011). DDT in indoor residual spraying: human health aspects.
721   https://apps.who.int/iris/handle/10665/44560. *Environmental Health Criteria 241*.
722   ISBN 9789241572415 ; ISSN 0250-863X.

723 WorldFish (2022). https://www.worldfishcenter.org/where-we-work/africa/zambia. Accessed
724   5 January 2022.

725

Paper II

Assessment of heavy metals in wild and farmed tilapia (*Oreochromis niloticus*) on Lake Kariba, Zambia: implications for human and fish health

Chalumba Kachusi Simukoko, Eliezer Brown Mwakalapa, Patricia Bwalya, Kaampwe Muzandu, Vidar Berg, Stephen Mutoloki, Anuschka Polder & Jan Ludvig Lyche

Published online: 26 Oct 2021.

Submit your article to this journal ☑

View related articles ☑

View Crossmark data ☑

Taylor & Francis
Taylor & Francis Group

Assessment of heavy metals in wild and farmed tilapia (*Oreochromis niloticus*) on Lake Kariba, Zambia: implications for human and fish health

Chalumba Kachusi Simukoko[a,b], Eliezer Brown Mwakalapa[c], Patricia Bwalya[d], Kaampwe Muzandu[b], Vidar Berg[a], Stephen Mutoloki[d], Anuschka Polder[a], and Jan Ludvig Lyche[a]

[a]Department of Paraclinical Sciences, Norwegian University of Life Sciences, Ås, Norway; [b]Department, Biomedical Sciences, University, University of Zambia, Lusaka, Zambia; [c]Department of Natural Sciences, Mbeya University of Science and Technology, Mbeya, Tanzania; [d]Department of Basic Sciences and Aquatic Medicine, Section of Aquatic Medicine and Nutrition, Norwegian University of Life Sciences, Oslo, Norway

ABSTRACT

The aim of this study was to assess the levels of heavy metals in both wild and farmed tilapia on Lake Kariba in Zambia and to evaluate the impact of intensive fish farming on wild tilapia. Three sites for wild fish (2 distant and 1 proximal to fish farms) and two fish farms were selected. One hundred fish (52 from distant sites; 20 near fish farms; 28 farmed fish) were sampled and muscle tissues excised for analysis of heavy metals (Mg, Fe, Zn, Al, Cu, Se, Co, Mo, As, Cr, V, Ni, Hg, Pb, Li, Cd, and Ag) by acid (HNO$_3$) digestion and ICP-MS. All metals were found to be below the maximum limits (MLs) set by WHO/EU. Essential metals were higher in farmed tilapia, whereas non-essential metals were higher in wild tilapia. Significantly higher levels of essential metals were found in wild fish near the fish farms than those distant from the farms. Estimated weekly intake (EWI) for all metals were less than the provisional tolerable weekly intakes (PTWI). Target hazard quotients (THQ) and Hazard Indices (HI) were <1, indicating no health risks from a lifetime of fish consumption. Selenium Health Benefit Value (HBV$_{Se}$) was positive for all locations, indicating protective effects of selenium against mercury in fish. Total cancer risk (CR) due to As, Cr, Cd, Ni and Pb was less than 1×10^{-4}, indicating less than 1 in 10,000 carcinogenic risk from a lifetime consumption of tilapia from Lake Kariba. Hg levels (0.021 mg/kg) in wild tilapia at site 1 were higher than the Environmental quality standard (EQS = 0.020 mg/kg) set by EU, indicating possible risk of adverse effects to fish. Except for Hg, levels of metals in fish were safe for human consumption and had no adverse effects on fish.

Introduction

Aquaculture is a fast-growing sector that contributes significantly to food security, provides an important source of high-quality protein and omega 3 fatty acids and leads to economic development. In 2018, the FAO (2020) estimated the global human consumption of fish to be 156 million tonnes. By 2005, one-quarter of wild fish stocks were underexploited, half fully exploited and the rest had been overexploited or depleted (FAO 2007). The decline in wild fish stocks contributes to the need for a growing aquaculture sector for protection of food security. Since 1970, world aquaculture has grown by an average of 7.5% per year (FAO 2020). The fastest growth has been in Africa and Asia, which have recorded double-digit growth in the past 20 years (FAO 2018, 2020). In 2018, total fish production from both fisheries and

aquaculture reached 179 million tonnes (FAO 2020), and aquaculture contributed 46% (82 million tonnes) of the total production (FAO 2020). Apart from being a source of protein, essential fatty acids, minerals and vitamins (Béné et al. 2015; Chan et al. 2019; Vicente-Zurdo et al. 2019), fish also serve as a source of income for people in developing countries (Béné et al. 2015; FAO 2018). In 2018, the FAO (2020) estimated World fish trade at about USD 400 billion, with USD 250 billion coming from aquaculture production. Africa currently contributes about 7% to the total global fish production (FAO 2020), with Egypt being the largest producer of farmed fish on the African continent (Mohamed Shaalan et al. 2018). In 2014, Zambia was the sixth-largest producer of farmed fish on the continent and the biggest producer of tilapia in the Southern African

CONTACT Chalumba Kachusi Simukoko ✉ chalumba@yahoo.com; chalumba.kachusi.simukoko@nmbu.no 🏛 Department, Biomedical Sciences, University, University of Zambia, P. O. Box 32379, Lusaka, Zambia

Development Community (SADC) (Genschick et al. 2017). Zambian aquaculture produced 20,000 tonnes of fish in 2014, with commercial farms contributing 75% (FAO 2016; Genschick et al. 2017).

Despite the many positives recorded from the growth in aquaculture, it may also have negative impacts on the environment. Some of these are release of organic effluents or chemicals and antibiotics (from medical treatments) into waterbodies and being a source of diseases or genetic contamination of wild species (Berg et al. 1992; Kishimba et al. 2004; Subasinghe 2005; Jarić et al. 2011; Li et al. 2011; Nonga et al. 2011; Polder et al. 2014; Ssebugere et al. 2014; Mwakalapa. et al. 2018). The current aquaculture practices can lead to elevated levels of antibiotic residues, antibiotic-resistant bacteria, persistent organic pollutants, metals, parasites, and viruses in natural waters and fish (Sapkota et al. 2008; Basaran et al. 2010). Increased pollution with organic (persistent organic pollutants) and inorganic (heavy metals) contaminants can affect fish and human health (Vos et al. 2000; Watterson. et al. 2008).

Heavy metals are naturally occurring elements in the earth's crust. They have a relatively high density (at least 5 times) compared to water (Tchounwou et al. 2012). Heavy metal pollution occurs when the metals exceed the naturally occurring levels in the environment or are at levels that affect human and animal health (Nazir et al. 2015). Pollution can be due to natural processes such as erosion and volcanic eruption, or anthropogenic activities (Adriano 2001; Mansour and Sidky 2002; Nazir et al. 2015) such as mining, industry, improper waste management, etc. (Adriano 2001; Xiao et al. 2014; Maurya et al. 2019). Essential metals (Mg, Fe, Zn, Cu, Se, Co, Mo, Cr, and Ni) are required for normal biological functions in humans and animals but can be harmful when levels exceed threshold levels for toxicity (Waseem et al. 2014; Javed and Usmani 2017; Marengo et al. 2018). Non-essential metals (Al, As, V, Hg, Pb, Li, Cd, and Ag) have no known biological functions and can be toxic even in small amounts (Tchounwou et al. 2012; Waseem et al. 2014). They have the potential to disrupt normal biological functions such as endocrine signalling and enzyme activity, which may lead to adverse health effects in both humans and fish (Tchounwou et al. 2012; Javed and Usmani 2017; Arisekar et al. 2020). Freshwater pollution with

accumulation of environmental toxicants in aquatic organisms is a global issue (Amundsen et al. 1997; Copaja et al. 2017; Marengo et al. 2018; Mwakalapa et al. 2019; Nakayama et al. 2013; Zhang et al. 2016). Therefore, monitoring of potentially toxic metals in fish is important for both environmental and public health (Maurya et al. 2019; Mwakalapa et al. 2019). Fish are among the most frequently used bioindicators of pollution in aquatic ecosystems (Stankovic et al. 2014) and provide data on bioaccumulation of heavy metals in wild and farmed fish. These data are needed for knowing the contamination status of waterbodies and for assessment of the health risk to aquatic organisms and humans. Limited scientific data are available on bioaccumulation of metals in wild and farmed fish from Zambia as well as sub-Saharan Africa. Furthermore, risk assessment for heavy metals through consumption of farmed and wild freshwater fish from Zambia has not been reported.

In Zambia and the rest of Africa, aquaculture is practiced in fishponds, tanks and in cages on lakes. Lake Kariba on the Zambia–Zimbabwe border is one location where farming of *Oreochromis niloticus* (Nile tilapia) in cages is practiced on an intensive scale. The aims of this study were to (1) assess and compare the levels of heavy metals in both wild and farmed tilapia, (2) evaluate the impact of intensive fish farming on wild tilapia near the farms and (3) compare the results with existing maximum residue levels and threshold values set for humans and fish health.

Materials and methods

Description of sampling area and species

Lake Kariba is a man-made lake located on the southern border of Zambia with Zimbabwe (−17° S 28° E). It was built between 1958 and 1963 for the purpose of hydroelectric generation. The lake is 320 km long with an area of 5400 km², and an average depth of 29 m. The lake controls about 90% of the Zambezi river runoff, and water flows from west to east. The climate is sub-tropical with annual rainfall between 400 and 700 mm and temperature between 13°C and 40°C. Fish (*O. niloticus*) were collected from five locations (sites 1–3, and farms 1 and 2) along the lake (Figure 1). Sites 1 and
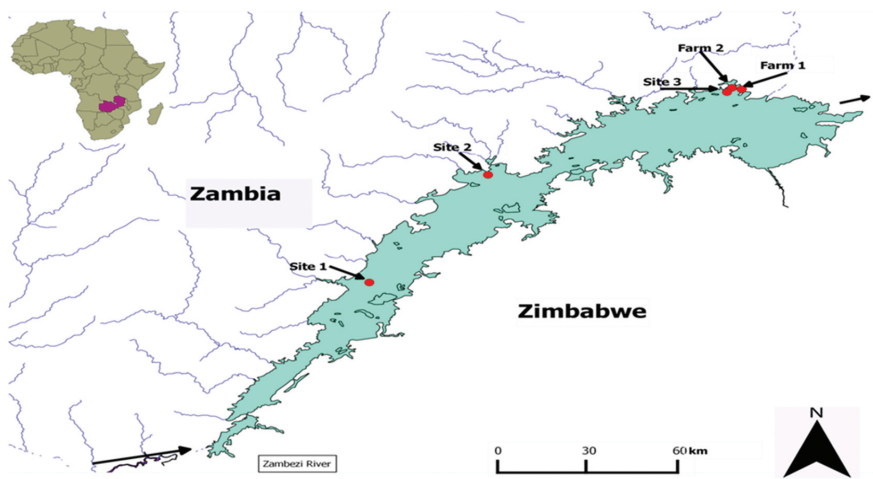
Figure 1. Map of Lake Kariba showing the 5 locations (sites 1–3 and farms 1 and 2) where tilapia was collected on the Zambian side. (Courtesy of Eliezer Brown Mwakalapa 2019).

2 are in Sinazongwe and Gweembe districts, respectively, over 100 km from site 3. The human populations in sites 1 and 2 are 98,246 and 50,136, respectively (Zambia Central Statistical Office 2012). Site 3 is in Siavonga district, on the eastern end of the lake and has a population of 58,864. Farms 1 and 2 are also located in Siavonga district and use cages for farming tilapia in the lake. *O. niloticus* is an omnivorous fish native to river Nile, with a worldwide distribution (FAO, 2005). It feeds on zooplankton and phytoplankton and higher plants (like algae). The fish thrives in tropical and subtropical climates with environmental temperatures of 9–42°C, living in shallow waters. It is found both as wild and farmed fish. Its fast growth and resistance to harsh conditions makes it favourable for aquaculture. Other activities on and around the lake are commercial fishing of kapenta (*Limnothrissa miodon*), farming, livestock, and wildlife managing. In addition, active coal mining is taking place at site 1. Site 3 also has a feed processing plant.

Ethical consideration and permission for the study

The study proposal was approved by the University of Zambia, School of Veterinary Medicine research committee. Wild tilapia was bought from fishermen. Permission from local district fisheries and veterinary officers was obtained before sampling in their areas. Managers at the fish farms gave their permission for samples to be collected from their farms.

Sample collection

A total of 172 wild and farmed tilapia were collected from June to July 2016. Live wild tilapia was bought from fishermen, placed in ice water and dissected back at the shore. Dip netting was used to catch farmed tilapia then dissected at the shore. Fish length and weight were recorded (Table 1). The scale used in the field could only weigh fish up to 1 kg, fish beyond this were assigned 1 kg. Therefore, only length was used for statistics. Stainless-steel forceps and scalpel blades were used to dissected muscle tissue of approximately 10 g. The tissue was placed in clean 15-ml Eppendorf tubes, then transported on ice in a cooler box to the University of Zambia, Veterinary Medicine School and stored at −20°C. Later, samples were transported on ice to the Laboratory of Environment Toxicology at the Norwegian University of Life Science (NMBU) in Oslo, Norway, and stored at −20°C until analysis. Permission to transport samples to Norway was obtained from the Ministry of Livestock and Fisheries, Zambia, and Norwegian Food Safety Authority.

Table 1. Location and fish characteristics: Sampling time, mean and range of individual length and number of pooled muscle samples from farmed (farms 1 and 2) and wild tilapia distant (sites 1 and 2) and near farms (site 3) from Lake Kariba. Zambia.

Location	Sampling time 2016	Mean of individual length (cm)	Range individual length (cm)	No. of individual samples	No. of fish per pool	No. of pooled/ individual samples
Site 1	June	35.57	28–45	29	2–3	10
Site 2	June	30.66	17–42	23	2–3	10
Site 3	June	27.6	24–33	20	2	10
Farm 1	June	23.6	13–29	16	1–3	10
Farm 2	June	27.13	22.5–36	12	1–2	10

Sample analysis

After selection of fish based on similar weight/length from each location, 100 muscle samples were pooled in equal amounts as shown in Table 1. Heavy metal analysis was done at the Laboratory for Soil and Water analysis, Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life Sciences (NMBU), Campus Ås, Norway.

Extraction and analysis of heavy metals

The following heavy metals were analysed: Mg, Fe, Zn, Al, Cu, Se, Co, Mo, As, Cr, V, Ni, Hg, Pb, Li, Cd, and Ag. Approximately 200 mg of muscle samples were weighed in ultrapure Teflon tubes (pre-rinsed in 7 M nitric acid (HNO_3) and in Milli-Q water®). Internal standard (^{74}Se, In) and 5 mL Ultrapure HNO_3 were added. The mixture was digested at 260°C in an UltraCLAVE (Milestone S.r.L, Sorisole (BG) – Italy). After digestion, 1 mL of UltraPure concentrated HCl was added (to prevent loss of Hg) and then diluted to 50 mL with distilled water. The samples were analysed using an Agilent 8800 ICP-MS.

Quality control and quality assurance

Method accuracy was verified by analysing certified reference materials in the same way, at the same time as the sample series. Fish Protein Certified Reference Material for Trace Metals (CRM Dorm-3, National Research Council Canada, Institute for National Measurement Standards, M-12, Ottawa, ON K1A 0R6, Canada) and fish muscle (ERM-BB422, European Commission – Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), 2440 Geel, Belgium) were used. The quantified values show good agreement with the certified values. LOD and LOQ were quantified from 3 to 10 times STDEV in the method blanks, $n = 6$. The mean of LOD and LOQ were calculated using average of the sample weights in a 50 ml dilution.

Human and fish health risk assessment

The mean metal concentrations in fish muscle (mg/kg ww) were compared to maximum limits (ML) set by WHO and EU (FAO/WHO 2002; EC EC 2006) for human consumption. Noncarcinogenic risks, estimated weekly intake (EWI), target hazard quotient (THQ) and hazard index (HI) were calculated as described by Mwakalapa et al. (2019), using formulas (1)–(3). EWIs were compared to provisional tolerable weekly intakes (PTWI) set by WHO. Target hazard quotient is the ratio between the estimated daily intake (EDI) and the oral reference dose (RfD, mg/kg bw/day) (Saha et al. 2016; USEPA 2018). RfD is an estimate of a daily oral exposure to a toxic substance that is likely to be without an appreciable risk of harmful effects during a lifetime (Varol et al. 2017; Mwakalapa et al. 2019). The HI also referred to total target hazard quotient (TTHQ), which is the sum of individual metal THQs. Values of THQ and HI >1 pose a risk of developing non-carcinogenic effects in one's lifetime (Saha et al. 2016; Copat et al. 2018). Selenium health benefit Values (HBV_{Se}) for all site were calculated as described by Ralston (Ralston et al. 2016), using formula (4). A positive HBV_{Se} indicates protective effects of selenium against mercury in the fish, while a negative value indicates that mercury poses a threat to human health (Ralston et al. 2019; Yabanli and Tay 2021). The daily average fish ingestion per person (fish ingestion rate) is 30.14 g/day (Kaminski et al. 2018; Tran et al. 2019), life expectancy (exposure duration) of 64 years,

exposure frequency to heavy metals of 365 days/year, and average body weight of an adult 70 kg were used in the calculations.

$$EWI = \frac{MC * IRD * 7}{BW} \quad (1)$$

where MC = mean metal concentration, IRD = daily average fish ingestion per person, and BW = average adult body weight.

$$THQ = \frac{E_F * E_D * F_{IR} * C}{RfD * W_{AB} * T_A} * 10^{-3} \quad (2)$$

where E_F = Exposure frequency, E_D = exposure duration, F_{IR} = fish ingestion rate, C = mean metal concentration, RfD = oral reference dose, W_{BA} = average body weight of an adult and T_A = average exposure time with noncarcinogenic effect (E_F*E_D).

$$HI = \sum_{i=1}^{n} THQi \quad (3)$$

$$HBV_{Se} = ([Se - Hg]/Se) * (Se + Hg) \quad (4)$$

The carcinogenic risk/cancer risks (CR) due to lifetime exposure to arsenic, chromium, cadmium, nickel and lead were calculated as described by (Saha et al. 2016), using formula (5). The total cancer risk (TCR) due to fish consumption from the lake, was calculated as the sum of the individual cancer risks (Bamuwamye et al. 2015), using formula (6). Carcinogenic risks (CR) are estimated as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to a potential carcinogen (Ahmed et al. 2016). According to USEPA (2018), acceptable lifetime cancer risk levels range from 10^{-4} (1 in 10,000) to 10^{-6} (1 in 1,000,000) chance of an individual developing cancer (Varol et al. 2017). Cancer risk values greater than 10^{-4} are unacceptable and those less than 10^{-6} indicate a negligible risk of cancer in an individual's lifetime. Cancer slope factors (CSF) $(mg/kg/day)^{-1}$ 1.5 (As), 6.3 (Cd), 0.5 (Cr), 1.7 (Ni) and 0.0085 (Pb) from the Integrated Risk Information System USEPA (2018) database were used in the calculation of CR (Raknuzzaman et al. 2016).

$$CR = CSF \times EDI \quad (5)$$

where CSF = Cancer slope factor, EDI = Estimated daily intake (EWI/7).

$$TCR = \sum_{i=1}^{n} CRi \quad (6)$$

Risk to fish health was assessed using Environmental Quality Standards (EQS) set by EU (Ec 2006). EQS are concentrations of a particular pollutant or group of pollutants in water, sediment or biota that should not be exceeded in order to protect humans, aquatic species (fish) and the environment (EC EC 2006; Lyche et al. 2019).

Statistical analysis

Data was organised in MS Excel 2016 spread sheets. JMP 14 statistical software was used for further analysis. Readings below the limit of detection (<LOD) were assigned a value of half the LOD. Since the data were not normally distributed, non-parametric tests Wilcoxon/Kruskal–Wallis test (rank sum) for differences among locations and Wilcoxon pair test for differences between locations were used. Spearman rank correlation was used to assess the correlation between variables; $p <.05$ values were considered significant.

Results

Concentration of heavy metals in muscle

Fish biometric data is presented in Table 1. Wild tilapia from sites 1 and 2 had significantly greater total length ($p < .01$) than those from site 3 and farmed tilapia. Descriptive statistics (mean, median, minimum and maximum) are shown in Table 2. Concentration of heavy metals in fish muscle in descending order was as follows: Mg >Fe >Zn >Al >Cu >Se >Co >Mo >As >Cr >V > Ni >Hg >Pb >Li >Cd >Ag. Except for Pb, all other metals were present in all samples.

Essential metals

Essential metals (Cu, Fe, Zn and Mo) were higher in farmed and the wild tilapia sampled near the fish farms (site 3) compared to wild fish sampled at locations away from the farms, sites 1 and 2 (Figure 2). The highest levels of Fe were in farmed fish farm 1

Table 2. Descriptive statistics of heavy metal concentrations in muscles of wild and farmed tilapia (mg/kg ww) and maximum limits (ML) set by WHO/FAO, EU, FDA.

Location		Essential metals									Non-essential metals							
		Mg	Cr	Fe	Co	Ni	Cu	Zn	Mo	Se	As	Al	Ag	Cd	Hg	Pb	Li	V
Site 1	Mean	249	0.095	5.23	0.033	0.018	0.25	4.76	0.003	0.15	0.022	4.81	0.0011	0.0007	0.021	0.009	0.006	0.015
	Median	250	0.015	5.15	0.034	0.012	0.25	4.45	0.003	0.16	0.022	4.4	0.0011	0.0006	0.006	0.009	0.006	0.016
	Min	210	0.009	3.1	0.018	0.009	0.2	3.6	0.002	0.13	0.018	2.4	0.0005	0.0005	0.004	0.008	0.005	0.013
	Max	270	0.8	9.4	0.041	0.068	0.32	7.1	0.004	0.17	0.028	8.5	0.0024	0.0018	0.16	0.011	0.008	0.017
Site 2	Mean	263	0.016	5.34	0.036	0.019	0.33	4.75	0.011	0.18	0.042	4.28	0.0016	0.0022	0.008	0.009	0.006	0.048
	Median	260	0.012	5.15	0.034	0.018	0.32	4.7	0.006	0.17	0.042	4.25	0.0012	0.0021	0.005	0.01	0.006	0.021
	Min	240	0.008	3.4	0.027	0.011	0.26	3.7	0.004	0.16	0.024	1.5	0.001	0.0009	0.004	0.0015	0.004	0.015
	Max	290	0.051	8.2	0.054	0.031	0.45	6.1	0.044	0.22	0.057	8.7	0.0034	0.0045	0.026	0.012	0.011	0.19
Site 3	Mean	231	0.021	8.16	0.044	0.009	2.14	8.49	0.054	0.14	0.023	0.78	0.0004	0.0005	0.009	0.004	0.009	0.005
	Median	225	0.02	8.2	0.042	0.009	2.15	8.1	0.045	0.14	0.023	0.91	0.0003	0.0005	0.01	0.002	0.008	0.005
	Min	180	0.007	4.3	0.027	0.006	1.2	6.6	0.03	0.11	0.012	0.3	0.0002	0.0003	0.006	0.002	0.006	0.002
	Max	290	0.039	14	0.061	0.011	3.2	11	0.086	0.16	0.04	1.4	0.0007	0.0006	0.012	0.009	0.013	0.011
Farm 1	Mean	244	0.02	9.38	0.066	0.01	2.57	6.99	0.058	0.18	0.043	1.3	0.0011	0.0008	0.007	0.008	0.006	0.016
	Median	240	0.016	9.55	0.054	0.008	2.55	6.75	0.054	0.18	0.032	1.25	0.0007	0.0004	0.002	0.01	0.006	0.007
	Min	180	0.007	4.8	0.024	0.006	0.78	5.6	0.025	0.13	0.021	0.47	0.0003	0.0003	0.001	0.002	0.004	0.004
	Max	320	0.053	17	0.17	0.019	4.3	9.3	0.092	0.28	0.15	2.7	0.005	0.0022	0.041	0.011	0.006	0.052
Farm 2	Mean	260	0.038	5.78	0.043	0.011	3.5	7.06	0.05	0.15	0.028	1.64	0.0004	0.0004	0.002	0.007	0.007	0.005
	Median	255	0.015	5.5	0.043	0.01	2.65	7.1	0.047	0.16	0.031	0.91	0.0004	0.0003	0.002	0.009	0.008	0.004
	Min	240	0.008	2.4	0.011	0.006	0.5	4.3	0.021	0.12	0.005	0.38	0.0001	0.0003	0.001	0.002	0.008	0.002
	Max	300	0.24	8.5	0.068	0.024	9	10	0.086	0.2	0.05	8.7	0.0011	0.0005	0.003	0.011	0.008	0.012
WHO and FAO	ML			45			30	30						0.1	0.5	0.3		
EU	ML													0.05	0.5	0.3		
FDA	ML															1		

(9.38 mg/kg) and wild fish sampled at site 3 (8.16 mg/kg). Fe levels in fish from these locations were significantly higher ($p < .05$) than in fish sampled from sites 1 and 2. Likewise, Zn levels in farmed fish and wild fish from site 3 were significantly higher ($p < .01$) than in wild fish from sites 1 and 2. The highest Zn level was at site 3 (8.49 mg/kg) and lowest at site 2

(4.75 mg/kg). Cu levels in farmed and wild fish from site 3 were also significantly higher ($p < .01$) than wild fish from sites 1 and 2. The highest Cu level was at farm 2 (3.5 mg/kg) and the lowest at site 1 (0.25 mg/kg). Se in wild fish from site 3 (0.14 mg/kg) had significantly lower ($p < .05$) levels than those from sites 1, 2 and farm 1. The highest Se level 0.178 mg/kg
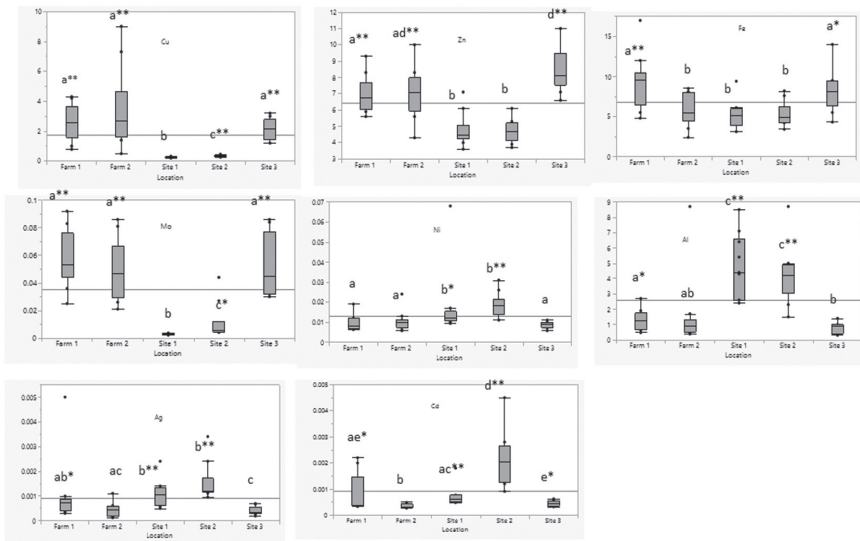

Figure 2. Concentration of heavy metals (Cu, Fe, Zn, Ni, Mo, Al and Cd) in muscle tissue of farmed and wild tilapia from Lake Karbia. Site with the same letter (a,b,c,d, or e) showed no significant difference in metal concentration at ** $p < .01$ and * $p < .05$.

was in farm 1. The highest Co level was at farm 1 (0.07 mg/kg) and lowest at site 1 (0.03 mg/kg). The Co levels in fish from farm 1 were significantly higher ($p < .01$) than wild fish from sites 1 and 2. Mo levels in farmed and wild fish from site 3 were significantly higher ($p < .01$) than wild fish from sites 1 and 2. Highest Mo level was at farm 1 (0.06 mg/kg) and lowest at site 1 (<0.01 mg/kg). Cr and Mg showed no significant difference among all locations.

Non-essential metals

Non-essential metals (Al, Ag, Ni and Cd) were higher in wild tilapia at sites 1 and 2 (Figure 2). Al was significantly higher in wild fish from sites 1 and 2 than farmed and wild fish from site 3. The highest level of Al was at site 1 (4.81 mg/kg) and lowest at farm 1 (0.78 mg/kg). As levels in wild fish from site 2 and farm 1 (site 3) were significantly higher than wild fish from sites 1 and 3. The highest As level was at farm 1 (0.04 mg/kg). V levels at sites 1, 2 and farm 1 (site 3) were significantly higher ($p < .01$) than other locations, with the highest level at site 2 (0.0476 mg/kg). Ni level at sites 1 and 2 was significantly higher than farmed and wild fish from site 3. The highest level was at site 2 (0.0186 mg/kg). Hg levels in wild fish (all sites) were significantly higher ($p < .05$) than farmed fish (site 3). The highest Hg level was at site 1 (0.021 mg/kg). The level of Pb in wild fish from site 3 was significantly lower ($p < .01$) than sites 1,2 and farm 1, with the highest level at site 2 (0.00925 mg/kg). Site 3 had significantly higher levels ($p < .01$) of Li than sites 1, 2 and farm 1, with the highest level of 0.00933 mg/kg. Cd levels at farm 2 were significantly lower ($p < .05$) than the other locations. Ag levels at sites 1 and 2 were higher ($p < .01$) than fish from sites 3 and farm 2. The highest level was at site 2 (0.00155 mg/kg).

Correlation among metals and fish length

Fish length had a significant positive correlation (Spearman's correlation) with Ni and Al, but significant negative correlation with Cu, Fe, Zn, Co and Mo. Significant positive correlation was also found for Fe, Cu, Zn, Co and Mo. Al had significant positive correlation with Cd, Ag, Hg and Ni. Al, Cd and Ni had significant negative correlation with Zn, Cu and Mo.

Risk assessment

Human health

Compared to maximum limits set by WHO/FA0, EU and FDA, all the measured metals in fish muscle were below these limits (Table 2). EWIs of all analysed muscles were also below the PTWI (Table 3). The calculated THQ and HI for all metals were less than the threshold of 1 (Table 4) posing no risk to human health. HBV_{Se} was positive for all locations, indicating protective effect of Se against Hg. Cancer risk due to consumption of As, Cr, Cd, Ni and Pb in fish were less than 1×10^{-4} (Table 5) showing no risk of cancer.

Fish health

The European Union has set an Environmental Quality Standard (EQS) of 0.02 mg/kg of mercury in fish in the environment for assessment of fish health (EC EC 2006). The mean Hg level in wild fish from site 1 (0.021 mg/kg) was higher than the EQS set by the EU (Table 2). This may cause adverse effects in the fish.

Discussion

Metal concentration in muscle of farmed and wild tilapia from Lake Kariba

The study found significant difference in metal concentrations, with essential metals higher in farmed tilapia and non-essential metals higher in wild tilapia. Essential metals (Cu, Zn, Fe, Co and Mo) were higher in farmed and wild fish (site 3) near the farms, than in wild fish (sites 1 and 2) far from the farms. Essential metals are necessary for various biological functions and are therefore added as trace elements to fish feed (Yildiz 2008; Fallah et al. 2011), which may explain the higher levels in farmed fish (Sapkota et al. 2008; Burridge et al. 2010). Feed spillage from the fish farms is accessed by the wild tilapia nearby, thereby exposing them to higher levels of essential metals than the wild fish far from the fish farms (Basaran et al. 2010; Ballester-Molto et al. 2017). Non-essential

Table 3. Estimated weekly intake (EWI) of heavy metals and Selenium health benefit values (HBV$_{Se}$) from consumption of muscle tissue from wild and farmed tilapia from Lake Kariba, Zambia.

	Site 1	Site 2	Site 3	Farm 1	Farm 2	PTWI
Li	1.91×10^{-5}	1.87×10^{-5}	2.81×10^{-5}	1.76×10^{-5}	2.18×10^{-5}	
Mg	7.5×10^{-1}	7.93×10^{-1}	6.96×10^{-1}	7.35×10^{-1}	7.84×10^{-1}	
Al	1.45×10^{-2}	1.29×10^{-2}	2.35×10^{-3}	3.92×10^{-3}	4.95×10^{-3}	1 (EFSA 2008)
V	4.64×10^{-5}	1.43×10^{-4}	1.52×10^{-5}	4.77×10^{-5}	1.45×10^{-5}	
Cr	2.86×10^{-4}	4.83×10^{-5}	6.43×10^{-5}	5.87×10^{-5}	1.16×10^{-4}	0.023 (Lin et al. 2004)
Fe	1.58×10^{-2}	1.61×10^{-2}	2.46×10^{-2}	2.83×10^{-2}	1.74×10^{-2}	5.6 (JECFA 2017)
Co	9.92×10^{-5}	1.09×10^{-4}	1.32×10^{-4}	2×10^{-4}	1.28×10^{-4}	0.21 (Finley et al. 2012)
Ni	5.43×10^{-5}	5.61×10^{-5}	2.6×10^{-5}	2.94×10^{-5}	3.19×10^{-5}	0.035 (JECFA 2017)
Cu	7.66×10^{-4}	1×10^{-3}	6.45×10^{-3}	7.74×10^{-3}	1.05×10^{-2}	3.5 (JECFA 2017)
Zn	1.43×10^{-2}	1.43×10^{-2}	2.56×10^{-2}	2.1×10^{-2}	2.13×10^{-2}	7 (JECFA 2017)
As	6.57×10^{-5}	1.26×10^{-4}	6.78×10^{-5}	1.3×10^{-4}	8.32×10^{-5}	0.01498 (JECFA 1989)
Se	4.58×10^{-4}	5.30×10^{-4}	4.07×10^{-4}	5.36×10^{-4}	4.58×10^{-4}	
Mo	8.95×10^{-6}	3.43×10^{-5}	1.62×10^{-4}	1.75×10^{-4}	1.5×10^{-4}	
Ag	3.34×10^{-6}	4.66×10^{-6}	1.2×10^{-6}	3.2×10^{-6}	1.33×10^{-6}	
Cd	2.19×10^{-6}	6.57×10^{-6}	1.4×10^{-6}	2.48×10^{-6}	1.1×10^{-6}	0.0056 (JECFA 2011)
Hg	6.33×10^{-5}	2.36×10^{-5}	2.66×10^{-5}	2.05×10^{-5}	6.69×10^{-6}	0.004 (EFSA 2012; JECFA, 2017)
Pb	2.75×10^{-5}	2.79×10^{-5}	1.1×10^{-5}	2.41×10^{-5}	2.01938E-05	0.0105 (EFSA 2010)
HBV$_{Se}$	1.92×10^{-3}	2.23×10^{-3}	1.71×10^{-3}	2.25×10^{-3}	1.92×10^{-3}	

Table 4. Total hazard quotient (THQ) and hazard index (HI) for analysed heavy metals from consumption of wild and farmed tilapia.

	Site 1	Site 2	Site 3	Farm 1	Farm 2	RfD (USEPA 2018)
Li	1.36×10^{-6}	1.33×10^{-6}	2.01×10^{-6}	1.26×10^{-6}	1.56×10^{-6}	0.002
Mg	2.19×10^{-6}	2.31×10-6	2.03×10^{-6}	2.14×10^{-6}	2.28×10^{-6}	
Al	2.07×10^{-6}	1.84×10^{-6}	3.36×10^{-7}	5.6×10^{-7}	7.07×10^{-7}	1
V	1.33×10^{-6}	4.1×10^{-6}	4.35×10^{-7}	1.36×10^{-6}	4.13×10^{-7}	0.005
Cr	1.36×10^{-5}	2.3×10^{-6}	3.06×10^{-6}	2.8×10^{-6}	5.51×10^{-6}	0.003
Fe	3.22×10^{-6}	3.28×10^{-6}	5.02×10^{-6}	5.77×10^{-6}	3.56×10^{-6}	0.7
Co	4.72×10^{-5}	5.2×10^{-5}	6.27×10^{-5}	9.52×10^{-5}	6.11×10^{-5}	0.0003
Ni	3.88×10^{-7}	4.0×10^{-7}	1.86×10^{-7}	2.1×10^{-7}	2.28×10^{-7}	0.02
Cu	2.73×10^{-6}	3.57×10^{-6}	2.30×10^{-5}	2.76×10^{-5}	3.77×10^{-5}	0.04
Zn	6.83×10^{-6}	6.82×10^{-6}	1.22×10^{-5}	1.0×10^{-5}	1.0×10^{-5}	0.3
As	3.13×10^{-5}	6.0×10^{-5}	3.23×10^{-5}	6.2×10^{-5}	3.96×10^{-5}	0.0003
Se	1.31×10^{-5}	1.52×10^{-5}	1.16×10^{-5}	1.53×10^{-5}	1.31×10^{-5}	0.005
Mo	2.56×10^{-7}	9.81×10^{-7}	4.62×10^{-6}	5.0×10^{-6}	4.29×10^{-6}	0.005
Ag	9.55×10^{-8}	1.33×10^{-7}	3.43×10^{-8}	9.15×10^{-8}	3.79×10^{-8}	0.005
Cd	3.13×10^{-7}	9.39×10^{-7}	2.01×10^{-7}	3.54×10^{-7}	1.52×10^{-7}	0.001
Hg	9.04×10^{-5}	3.37×10^{-5}	3.8×10^{-5}	2.92×10^{-5}	9.56×10^{-6}	0.0001
Pb	1.12×10^{-6}	1.14×10^{-6}	4.49×10^{-7}	9.84×10^{-7}	8.24×10^{-7}	
Hazard index	2.18×10^{-4}	1.9×10^{-4}	1.98×10^{-4}	2.6×10^{-4}	1.91×10^{-4}	

RfD: Reference dose set by USEPA (2018).

metals (Al, Ni, Cd, Hg) were higher in wild fish sampled far from the fish farms. The higher Hg level at site 1 (0.021 mg/kg) (Table 2) could possibly be due to coal mining in the area as coal mining has been reported to release Hg to the environment (Liu et al. 2014; Plessl et al. 2019). This calls for further studies. Furthermore, wild tilapia can live up to 9 years compared to farmed tilapia, which is harvested within 6 months of cage-rearing on the lake. The wild tilapia can therefore accumulate contaminants with long biological half-lives such as Hg and Cd over a longer life-span compared to farmed tilapia. Positive associations between Hg and Cd concentrations in tilapia and age as well as size are documented in other studies (Hamada et al. 2018). However, in the present study, we did not

find any association between fish size (length) and Hg, whereas Cd and length showed significant association.

Comparison of metal concentration in muscle of tilapia with other regions

Sub-Saharan Africa

Levels of Cu, Fe, Zn and Mo in wild tilapia were similar to results reported in an earlier study on the same lake (Nakayama et al. 2010) but 10 (Cu) and two (Zn) times lower than findings by Berg et al. (1995) (Table 6). The 21 years lapse between the study by Berg et al. (1995) and our study may account for changes in metal content in the lake. This downward temporal trend could be due to

changes in mining techniques along the drainage area of Lake Kariba, which are more environmentally friendly today. Berg et al. (1995) also sampled in an area where cage and pond aquaculture were being practiced, which could have influenced levels of metals in the wild tilapia. Wild tilapia from Tanzania (Mapenzi et al. 2020) and Kenya (Nyingi et al. 2016) had three to 20 times higher levels of Fe, Cu and Zn than the present study, which may be explained by their location in areas with more industrial mining, agriculture and human activities. Cr level was 10 times lower than an earlier study in Kariba (Nakayama et al. 2010). The level of Cd was similar to those detected 6 years earlier (Nakayama et al. 2010), but more than 20 times lower than 21 years earlier in the lake, suggesting a reduction of Cd levels the last 20 years (Berg et al. 1995). Hg has not previously been analysed in tilapia from Zambia, but the levels were about 20 times lower than in wild tilapia from Tanzania (Mshana Grayson 2015) and two times lower than Ethiopia (Dsikowitzky et al. 2012). Pb levels were similar to findings 6 years earlier in Lake Kariba (Nakayama et al. 2010), but over 100 times lower than 21 years earlier in the same lake (Berg et al. 1995). Pb was also more than seven times lower than in Tanzania (Mapenzi et al. 2020), Kenya (Nyingi et al. 2016) and Ethiopia (Dsikowitzky et al. 2012). Ni in this study was ten times lower than earlier findings in Lake Kariba (Berg et al. 1995; Nakayama et al. 2010). The decrease of Pb, Cr and Ni levels was contrary to expectations, since the number of kapenta fishing boats rose from 423 in 2009 to 962 in 2013; these are thought to be the sources of Pb, Cr and Ni (Chali et al. 2014; Paulet 2014). The levels of metals in wild tilapia from Lake Kariba were thus lower than the levels in tilapia from other countries in the region. We also report lower levels

of Cd (20x) and Pb (100x) compared with a previous study from the same lake. The Hg concentration was not measured in the previous study, but the levels found in the present study were lower than tilapia from Tanzania and Ethiopia.

As stated above, the farmed tilapia had significant higher levels of essential metals and significant lower levels of non-essential metals compared to the wild tilapia sampled far from the fish farms.

Farmed tilapia in this study had similar levels of the essential metals, Cu and Zn to those found earlier in fish from Lake Kariba (Berg et al. 1995), but five to 12 times higher than in farmed tilapia from Uganda (Birungi et al. 2007) (Table 7). This could be due to differences in composition of trace elements in the fish feeds. For Pb, Cd and Ni, levels were more than 200 times less than those found earlier in Lake Kariba (Berg et al. 1995). This is similar to observations above in wild tilapia where levels were also higher in the earlier study by Berg et al. (1995), which indicates a substantial decline in the contamination of non-essential metals in Lake Kariba.

North Africa

Levels of Cu, Fe, V, Al and Co in wild tilapia were similar to findings in Egypt (Ibrahim et al. 2020), whereas Se, Cr, As, Cd, Hg, Pb and Ni were two times lower than in Egypt (Hamada et al. 2018; Ibrahim et al. 2020) (Table 6). Tributaries that drain into river Nile carry effluents from industries and agriculture, as well as sewage. This may account for higher levels of Cd, Hg and Pb in Egypt.

The levels of Pb, Hg and Cd in farmed tilapia from Egypt (Authman et al. 2012; Hamada et al. 2018) were over 100 times higher than in the current study probably due to industrial and agricultural effluents as well as sewage, which have been reported

Table 5. Estimation of cancer risk (CR) posed by a lifetime of consumption of wild and farmed tilapia from Kariba due to As, Pb, Cd, Cr and Ni.

	Site 1	Site 2	Site 3	Farm 1	Farm 2	CSF (USEPA 2018)	Acceptable risk range (USEPA 1995)
As	1.41×10^{-5}	2.71×10^{-5}	1.45×10^{-5}	2.79×10^{-5}	1.78×10^{-5}	1.5	1×10^{-4} to 1×10^{-6}
Pb	3.34×10^{-8}	3.39×10^{-8}	1.34×10^{-8}	2.93×10^{-8}	2.45×10^{-8}	0.0085	
Cd	1.97×10^{-6}	5.92×10^{-6}	1.26×10^{-6}	2.23×10^{-6}	9.6×10^{-7}	6.3	
Cr	2.04×10^{-5}	3.45×10^{-6}	4.59×10^{-6}	4.19×10^{-6}	8.27×10^{-6}	0.5	
Ni	1.32×10^{-5}	1.36×10^{-5}	6.31×10^{-6}	7.15×10^{-6}	7.74×10^{-6}	1.7	
Total CR	4.97×10^{-5}	5.01×10^{-5}	2.67×10^{-5}	4.15×10^{-5}	3.48×10^{-5}		

CSF, cancer slope factor.

Table 6. Comparison of mean concentration of heavy metals in muscle (mg/kg ww) of wild tilapia from other studies.

Country	Location	Li	Mg	Al	V	Cr	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Ag	Cd	Hg	Pb	Reference
Zambia[1]	Lake	0.0063	249	4.81	0.0154	0.095	5.23	0.033	0.018	0.25	4.76	0.022	0.152	0.003	0.00111	0.001	0.021	0.009	This study
Zambia[2]	Lake	0.0062	263	4.28	0.0476	0.016	5.34	0.036	0.019	0.33	4.75	0.042	0.176	0.011	0.00155	0.002	0.008	0.009	This study
Zambia[3]	Lake	0.0093	231	0.78	0.0050	0.021	8.16	0.044	0.009	2.14	8.49	0.023	0.135	0.054	0.0004	0.001	0.009	0.004	This study
Zambia[a]	Lake					1.05		nd	0.174	0.42	4.45					0.0004		0.008	(Nakayama et al. 2010)
Zimbabwe[a]	Lake								2.18	1.45	10.29					0.671		1.51	(Berg et al. 1995)
Tanzania[a]	Lake									0.32	28.3		1.01					0.32	(Mapenzi et al. 2020)
Kenya	Lake						63.9			5.8	17.1					1.90		6.11	(Nyingi et al. 2016)
Egypt[a]	River			2.59	0.0064	0.326	2.03	0.004	0.481	0.11	0.85	0.367	0.159	0.034		<0.012		0.008	(Ibrahim et al. 2020)
Egypt	River															0.15	1.18	0.54	(Hamada et al. 2018)
Ethiopia[a]	Lake					0.07						0.055	0.046			0.044	0.051	0.063	(Dsikowitzky et al. 2012)
China	Lake					0.51			3.5	1.38	29.5	0.03				0.03		8.62	(Leung et al. 2014)
Malaysia[a]	Lake					1.208			0.636	0.5	9.54					0.006		0.03	(Taweel and Shuhaimi-Othman 2011)
Saudi Arabia[a]	River									0.37	4.72					0.083		0.35	(Mohammed 2009)
Saudi Arabia[a]	River					0.048				0.23						0.002	0.001	0.008	(Abdel-Baki et al. 2011)

Sites 1–3 from this study.
[a]Converted to wet weight from dry weight using the estimated percentage 78.8% as moisture content (Miao et al. 2010)
Nd, not detected.

Table 7. Comparison of mean concentration of heavy metals in muscle (mg/kg ww) of farmed tilapia from other studies.

Country	Location	Li	Mg	Al	V	Cr	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Ag	Cd	Hg	Pb	Reference
Zambia[1]	Cages	0.0058	244	1.3	0.0158	0.019	9.38	0.066	0.0098	2.568	6.99	0.043	0.178	0.0581	0.00106	0.00082	0.0068	0.008	This study
Zambia[2]	Cages	0.0072	260	1.643	0.0048	0.038	5.78	0.043	0.0106	3.5	7.06	0.028	0.152	0.0498	0.00044	0.00035	0.0022	0.007	This study
Zimbabwe[a]	Pond								2.29	1.62	11.69		0.45			0.78		1.51	(Berg et al. 1995)
Zimbabwe[a]	Cage			4.98					2.52	1.79	12.53		1.06			0.73		1.51	(Berg et al. 1995)
Egypt	Pond								1.19							3.72	1.23	1.23	(Authman et al. 2012)
Egypt	Canal															0.08	0.94	0.29	(Hamada et al. 2018)
Uganda	Cage					0.56				0.57	0.58								(Birungi et al. 2007)
Malaysia[a]	Pond					1.316			0.593	0.562	6.57					0.0021		0.023	(Taweel and Shuhaimi-Othman 2011)
Taiwan	Pond					2.46		1.57	2.27	3.17	61.4	1.27	2.7			0.01		0.14	(Ling et al. 2013)

Farms 1 and 2 in this study.
[a]Converted to wet weight from dry weight using the estimated percentage 78.8% as moisture content (Miao et al. 2010).

to cause metal contamination in water in these areas (Authman et al. 2012; Hamada et al. 2018). Farmed Egyptian tilapia had also 5–100 times higher levels of Al and Ni than in the present study (Authman et al. 2012).

Asia and Middle East

Levels of Cu, Zn and As in wild tilapia were similar to those found in Saudi Arabia (Mohammed 2009; Abdel-Baki et al. 2011), China (Leung et al. 2014) and Malaysia (Taweel and Shuhaimi-Othman 2011) (Table 6). Cr was comparable to levels in Saudi Arabia (Abdel-Baki et al. 2011), but five to 16 times lower in China (Leung et al. 2014) and Malaysia (Abdulali Taweel and Shuhaimi-Othman 2011). Levels of Cd were similar to findings in Saudi Arabia (Abdel-Baki et al. 2011) but three to 40 times lower than in China (Leung et al. 2014), Malaysia (Abdulali Taweel and Shuhaimi-Othman 2011) and Saudi Arabia (Mohammed 2009). Levels of Hg in wild tilapia were 10 to 20 times higher than those found in Saudi Arabia (Abdel-Baki et al. 2011) but 4 to 8 times lower than the Hg levels detected in wild tilapia from Malaysia (Naji et al. 2014). Pb levels were similar to those in Saudi Arabia (Abdel-Baki et al. 2011), but substantially lower than in China (Leung et al. 2014), Malaysia (Abdulali Taweel and Shuhaimi-Othman 2011) and Saudi Arabia (Mohammed 2009). It was noted that levels of Pb in China were almost 1000 times higher (Leung et al. 2014). In the present study, Ni was more than 30 times lower than in China (Leung et al. 2014) and Malaysia (Abdulali Taweel and Shuhaimi-Othman 2011), while As was comparable to levels in China (Leung et al. 2014). The higher levels of Pb, Ni, Hg and Cd in wild tilapia from Asia are probably due to discharge of untreated industrial, agricultural and domestic effluents to aquatic environments.

The Cu levels in farmed fish from Lake Kariba were comparable to those in Taiwan (Ling et al. 2013), but five times higher than in Malaysia (Taweel et al. 2013) (Table 7). Zinc levels were similar to those in Malaysia (Taweel et al. 2013), but nine times lower than in Taiwan (Ling et al. 2013). Co and Se levels were also lower than those in Taiwan (Ling et al. 2013). The variation in the levels of essential metals in farmed fish is probably due to differences in the content of these chemicals in the local fish feed. Levels of non-essential metals, As, Cd, Pb, Cr and Ni were substantially lower than findings in China (Cheung et al. 2008; Kwok et al. 2014; Geng et al. 2015), Malaysia (Taweel and Shuhaimi-Othman 2011) and Taiwan (Ling et al. 2013). The levels of Hg detected in farmed tilapia from China are also substantially higher than the levels detected in the present study as well as other studies from sub-Saharan Africa. The higher levels of heavy metals in farmed fish from Asia may be due to the higher level of industrial and agricultural activities, which increase the risk of pollution of aquatic environments. Difference in feed composition may also affect levels in different areas.

Correlations

In the present study, essential metals (Fe, Cu, Zn, Co and Mo) showed positive correlation, which may be explained by a common source of trace elements from the fish feed (Yildiz 2008; Fallah et al. 2011). Non-essential metals (Al, Cd, Ag, Hg and Ni) also had positive correlation but showed negative correlation with essential metals (Zn, Cu and Mo). The wild fish had higher levels of non-essential metals probably due to a longer life span resulting in longer exposure, whereas they had lower essential metals probably because they were not exposed to commercial feed. This may explain the negative correlation between non-essential and essential metals. Since wild fish were larger than the farmed fish (Table 1) and had lower levels of essential metals (Zn, Cu and Mo), this accounted for the negative correlation observed between length and Zn, Cu and Mo.

Possible health risk assessment

Human health risk

The concentrations of metals in the muscle of wild and farmed tilapia from Lake Kariba (Table 2) were below the threshold values for adverse effects set by WHO/FAO (FAO/WHO 2002) and EU(EC EC 2006). We estimated the EWI (Table 3) and showed that the EWI was much lower than the PTWI. The THQs, which is the ratio of the exposure levels (EWI) of the individual potential harmful metal and the level at which no adverse effects are expected (PTWI), were below 1 for all metals.

Likewise, the HI (Table 4), which is the sum of THQs for all metals was also below 1, indicating that the fish can be consumed regularly without any significant health risk from both single metal exposure and cumulative effect of exposure to multiple metals. HBV_{Se} from all sites were positive, indicating protecting effects of selenium against mercury in the fish (Ralston et al. 2016; Yabanli and Tay 2021). Mercury has been shown to inhibit selenium-dependent enzymes (selenoenzymes) that protect against and reverse oxidative brain damage and perform other vital functions such as foetal brain development, growth, thyroid hormone metabolism and calcium regulation (Squadrone et al. 2015; Ralston et al. 2016, 2019). Selenium sequesters mercury, forming an insoluble selenium-mercury (HgSe) compound, that is excreted from the body (Ralston et al. 2016; Yabanli and Tay 2021). Thus, a Se:Hg molar >1, indicates excess Se and its protective effect against Hg (Squadrone et al. 2015; Yabanli and Tay 2021). The estimated TCR for As, Cd, Cr, Ni and Pb was less than $1 \times 10^x{-}4$ (Table 5), indicating that the risk of humans developing cancer during their lifetime, as a result of consuming wild and farmed tilapia from Lake Kariba was less than 1:10,000 (Varol et al. 2017).

Fish health risk

The mean concentration of Hg at site 1 (0.021 mg/kg) was slightly higher than the EQS_{Biota} (0.02 mg/kg) set by the EU (EC EC 2006). An EQS is the concentration of a particular pollutant or group of pollutants in water, sediment or biota that should not be exceeded in order to protect human health and the environment (Lyche et al. 2019). Environmental quality standards (EQS) are therefore guidelines set by the EU for monitoring pollution in water and biota (aquatic organisms). Chemical pollution of surface water poses a threat to the aquatic environment and may induce acute and chronic toxicity in aquatic organisms (Ec 2006). Relatively small concentrations of Hg (<160 mg/kg ww) can adversely affect the reproduction, development, growth, metabolism, neurobehavior, and immune responses of fish (Weis 2009; Rhea et al. 2013). Though the mean at site 1 was above the EQS, only one sample (0.16 mg/kg) was above the EQS. However, this finding is still

a cause of concern, since it is above the EQS and may induce adverse effects in fish and other sensitive aquatic organisms.

Conclusions

The study presents the first comparison of heavy metals in wild and farmed tilapia on Lake Kariba in Zambia. This will serve as a baseline for future studies and developing of guidelines for the rapidly developing aquaculture sector in Zambia as well as sub-Saharan Africa. Heavy metal levels in all fish were below the ML set by WHO/EU. Essential metals (Cu, Zn, Fe, Mo and Co) were higher in farmed fish, while non-essential metals (Cd, Pb, Al, Ni and Hg) were higher in wild fish. Wild tilapia near the fish farms also had higher levels of essential metals compared to those distant from the farms suggesting that spill-over of feed and waste from the farms has the potential to contaminate the wild fish in the vicinity. It is therefore vital that proper guidelines are put in place to limit the effects on wild fish. Levels of Cd, Hg and Pb in Lake Kariba were also lower than those reported in North Africa and Asia. EWI of all metals were far below the PTWI. The THQ and HI were all below 1, showing no risk of non-carcinogenic effects from consumption of individual or combination of metals in fish, during a lifetime. HBV_{Se} were all positive, indicating protective effects of Se against Hg in fish. Total cancer risks were below 1×10^{-4}, showing less than 1:10,000 risk of carcinogenic effects from fish consumption during a lifetime. Levels of Hg in some wild fish were higher than the EQS set by the EU. Long-term exposure to Hg may therefore cause adverse effects in wild fish and other aquatic organisms. The findings of this study show that tilapia from the lake are safe for human consumption and there are no adverse effects on fish health.

Acknowledgments

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

References

Abdel-Baki AS, Dkhil MA, Al-Quraishy S. 2011. Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. AFR J BIOTECHNOL. 10:2541–2547. doi:10.5897/AJB10.1772

Adriano DC. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. 2nd ed. New York: Springer Verlag; p. 91–131.

Ahmed K, Baki MA, Kundu GK, Saiful I, Monirul I, Muzammel H. 2016. Human health risks from heavy metals in fish of Buriganga river, Bangladesh. SpringerPlus. 5(1):1697. doi:10.1186/s40064-016-3357-0.

Amundsen P-A, Staldvik FJ, Lukin AA, Kashulin NA, Popova OA, Reshetnikov YS. 1997. Heavy metal contamination in freshwater fish from the border region between Norway and Russia. Sci Total Environ. 201(3):211–224. doi:10.1016/S0048-9697(97)84058-2.

Arisekar U, Shakila RJ, Shalini R, Jeyasekaran G. 2020. Human health risk assessment of heavy metals in aquatic sediments and freshwater fish caught from Thamirabarani River, the Western Ghats of South Tamil Nadu. Mar Pollut Bull. 159:111496. doi:10.1016/j.marpolbul.2020.111496

Authman MM, Abbas WT, Gaafar AY. 2012. Metals concentrations in Nile tilapia Oreochromis niloticus () from illegal fish farm in Al-Minufiya Province, Egypt, and their effects on some tissues structures. Ecotoxicol Environ Saf. 84:163–172. doi:10.1016/j.ecoenv.2012.07.005

Ballester-Molto M, Sanchez-Jerez P, Aguado-Gimenez F. 2017. Consumption of particulate wastes derived from cage fish farming by aggregated wild fish. An experimental approach. Mar Environ Res. 130:166–173. doi:10.1016/j.marenvres.2017.07.014

Bamuwamye M, Ogwok P, Tumuhairwe V. 2015. Cancer and non-cancer risks associated with heavy metal exposures from street foods: evaluation of roasted meats in an urban setting. J Environ Pollut Human Health. 3(2):24–30. doi:10.12691/jephh-3-2-1.

Basaran AK, Aksu M, Egemen O. 2010. Impacts of the fish farms on the water column nutrient concentrations and accumulation of heavy metals in the sediments in the eastern Aegean Sea (Turkey). Environ Monit Assess. 162(1):439–451. doi:10.1007/s10661-009-0808-x.

Béné C, Barange M, Subasinghe R, Pinstrup-Andersen P, Merino G, Hemre G-I, Williams M. 2015. Feeding 9 billion by 2050 – putting fish back on the menu. Food Security. 7(2):261–274. doi:10.1007/s12571-015-0427-z.

Berg H, Kiibus M, Kautsky N. 1995. Heavy metals in tropical Lake Kariba, Zimbabwe. Water Air Soil Pollut. 83(3):237–252. doi:10.1007/BF00477355.

Berg H, Martina K, Kautsky N. 1992. DDT and other insecticides in the Lake Kariba ecosystem, Zimbabwe. Ambio. 21(7):444–450.

Birungi Z, Masola B, Zaranyika MF, Naigaga I, Marshall B. 2007. Active biomonitoring of trace heavy metals using fish (Oreochromis niloticus) as bioindicator species. The case of Nakivubo wetland along Lake Victoria. Phys Chem Earth Parts A/B/C. 32(15):1350–1358. doi:10.1016/j.pce.2007.07.034.

Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K. 2010. Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. Aquaculture. 306(1):7–23. doi:10.1016/j.aquaculture.2010.05.020.

Chali M, Musuka G, Confred, Nyimbili B. 2014. The impact of fishing pressure on Kapenta (Limnothrissa miodon) production in Lake Kariba, Zambia! A case study of Siavonga District. Int J Agric Fish. 2(6):107–116.

Chan CY, Tran N, Pethiyagoda S, Crissman CC, Sulser TB, Phillips MJ. 2019. Prospects and challenges of fish for food security in Africa. Global Food Security. 20:17–25. doi:10.1016/j.gfs.2018.12.002

Cheung KC, Leung HM, Wong MH. 2008. Metal concentrations of common freshwater and marine fish from the Pearl River Delta, South China. Arch Environ Contam Toxicol. 54(4):705–715. doi:10.1007/s00244-007-9064-7.

Copaja SV, Perez CA, Vega-Retter C, Veliz D. 2017. Heavy metal content in Chilean fish related to habitat use, tissue type and river of origin. Bull Environ Contam Toxicol. 99(6):695–700. doi:10.1007/s00128-017-2200-9.

Copat C, Grasso A, Fiore M, Cristaldi A, Zuccarello P, Signorelli SS, ... Ferrante M. 2018. Trace elements in seafood from the Mediterranean sea: an exposure risk assessment. Food Chem Toxicol. 115:13–19. doi:10.1016/j.fct.2018.03.001

DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, (2013).

Dsikowitzky L, Tsegaye M, Dadebo E, Carvalho C, Sindern S. 2012. Assessment of heavy metals in water samples and tissues of edible fish species from Awassa and Koka Rift Valley Lakes, Ethiopia. Environ Monit Assessment, 185. doi:10.1007/s10661-012-2777-8

[EC] European Commission. 2006. COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. IOP Publishing PhysicsWep. https://www.fsai.ie/uploadedFiles/Consol_Reg1881_2006.pdf

[EFSA] European Food safety. 2010. EFSA Panel on Contaminants in the Food Chain Scientific Opinion on Lead in Food. 8(4), 1570. doi: 10.2903/j.efsa.2010.1570.

[EFSA] European Food Safety Authority. 2008. Safety of aluminium from dietary intake1. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC). Adopted on 22 May 2008. The EFSA Journal.

[EFSA] European Food Safety Authority. 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. Panel on Contaminants in the Food Chain. 10(12), 2985. doi: 10.2903/j.efsa.2012.2985.

Fallah AA, Saei-Dehkordi SS, Nematollahi A, Jafari T. 2011. Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (Oncorhynchus mykiss) using ICP-OES technique. Microchem J. 98(2):275–279. doi:10.1016/j.microc.2011.02.007.

Finley, B. L, Monnot, A. D, Paustenbach, D. J, & Gaffney, S. H. (2012). Derivation of a chronic oral reference dose for cobalt. Regulatory Toxicology and Pharmacology, 64(3), 491–503. doi:10.1016/j.yrtph.2012.08.022

[FAO] Food and Agriculture Organisation. 2005-2019. Cultured aquatic species information programme. Oreochromis niloticus. Cultured aquatic species information programme. Text by Rakocy, J. E. In: FAO Fisheries and Aquaculture Department [online]. Rome. [Updated Oct 18 Feb 2005; cited 23 Oct 2019]. accessed 2019 Oct 23 www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en.

[FAO] Food and Agriculture Organisation. 2016. The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. Rome: 200.

[FAQ] Food and Agriculture Organisation. 2018. THE STATE OF WORLD FISHERIES AND AQUACULTURE - MEETING THE SUSTAINABLE DEVELOPMENT GOALS. Rome.

[FAO] Food and Agriculture Organisation. 2020. The state of world fisheries and aquaculture 2020. Sustainability in action. Rome. doi:10.4060/ca9229en

[FAO/WHO] Food and Agriculture Organisation/World Health Organisation. 2002. Codex Alimentarius, Schedule 1 of the proposed draft Codex general standards for contaminants and toxins in food. Joint FAO/WHO Food Standards Programme, Codex Committee, Rotterdam. PhysicsWep: http://www.fao.org/input/download/report/28/Al03_12e.pdf.

Geng -J-J, Li H, Liu J-P, Yang Y, Jin Z-L, Zhang Y-N, . . . Du Z-Y. 2015. Nutrients and contaminants in tissues of five fish species obtained from Shanghai markets: risk–benefit evaluation from human health perspectives. Sci Total Environ. 536:933–945. doi:10.1016/j.scitotenv.2015.06.057

Genschick S, Kaminski AM, Kefi AS, Cole SM. 2017. Aquaculture in Zambia- An overview and evaluation of the sector's responsiveness to the needs of the poor. Penang (Malaysia): CGIAR Research Program on Fish Agri-Food Systems and Lusaka, Zambia: Department of Fisheries. Working Paper: FISH-2017-08.

Hamada M, Elbayoumi Z, Khader R, Elbagory A. 2018. Assessment of heavy metal concentration in fish meat of wild and farmed nile tilapia (Oreochromis Niloticus), Egypt. Alexandria J Vet Sci. 57:30. doi:10.5455/ajvs.295019

Ibrahim M, Sayed E-S, Shaban A, Abdel-Ghafour S, Abouzied S, Abdel-Rahman R, Hassan M, El-Lahamy A, Adel, Abo–Zeid S, et al. 2020. Effect of location and grilling process on heavy metals concentration in muscles of different fish species, Egypt %J. Egypt J Aquat Biol Fish. 24 (6):15–24. doi:10.21608/ejabf.2020.109083.

Jarić I, Višnjić-Jeftić Ž, Cvijanović G, Gačić Z, Jovanović L, Skorić S, Lenhardt M. 2011. Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (Acipenser ruthenus) from the Danube River in Serbia by ICP-OES. Microchem J. 98 (1):77–81. doi:10.1016/j.microc.2010.11.008.

Javed M, Usmani N. 2017. An overview of the adverse effects of heavy metal contamination on fish health. Proc Nat Acad Sci In Sec B. 89(2):389–403. doi:10.1007/s40011-017-0875-7.

[JECFA] Joint FAO/WHO Expert Committee on Food Additives. 2011. Evaluation of Certain Food Additives and Contaminants Seventy-Third Report of the Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives and Contaminants.

[JECFA] Joint FAO/WHO Expert Committee on Food Additives. 2017. Food Contaminants. http://apps.who.int/foodadditives-contaminants-jecfa-database/search.aspx

Food and Agriculture Organisation (FAO). 2007. THE STATE OF WORLD FISHERIES AND AQUACULTURE 2006. Rome: FAO (Fisheries and Aquaculture Department; p. 1–180. Rome.

[JECFA] Joint FAO/WHO Expert Committee on Food Additives, Food Agriculture Organization of the United, Nations World Health, Organization. 1989. Evaluation of certain food additives and contaminants: thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives [meeting held in Geneva from 21 to 30 March 1988]. Geneva: World Health Organization.

Kaminski AM, Genschick S, Kefi AS, Kruijssen F. 2018. Commercialization and upgrading in the aquaculture value chain in Zambia. Aquaculture. 493:355–364. doi:10.1016/j.aquaculture.2017.12.010

Kishimba MA, Henry L, Mwevura H, Mmochi AJ, Mihale M, Hellar H. 2004. The status of pesticide pollution in Tanzania. Talanta. 64(1):48–53. doi:10.1016/j.talanta.2003.11.047.

Kwok CK, Liang Y, Wang H, Dong YH, Leung SY, Wong MH. 2014. Bioaccumulation of heavy metals in fish and Ardeid at Pearl River Estuary, China. Ecotoxicol Environ Saf. 106:62–67. doi:10.1016/j.ecoenv.2014.04.016

Leung HM, Leung AOW, Wang HS, Ma KK, Liang Y, Ho KC, Yung KKL, Tohidi F, Yung KKL. 2014. Assessment of heavy metals/metalloid (As, Pb, Cd, Ni, Zn, Cr, Cu, Mn) concentrations in edible fish species tissue in the Pearl River Delta (PRD), China. Mar Pollut Bull. 78(1):235–245. doi:10.1016/j.marpolbul.2013.10.028.

Li X, Li J, Wang Y, Fu L, Fu Y, Li B, Jiao B. 2011. Aquaculture industry in China: current state, challenges, and outlook. Rev Fish Sci. 19(3):187–200. doi:10.1080/10641262.2011.573597.

LIN, H. T., WONG, S. S. & LI, G.-C. 2004. Heavy metal content of rice and Shellfish in Taiwan. Journal of Food and Drug Analysis, 12, 167–174.

Ling M-P, Wu -C-C, Yang K-R, Hsu H-T. 2013. Differential accumulation of trace elements in ventral and dorsal muscle tissues in tilapia and milkfish with different feeding habits from the same cultured fishery pond. Ecotoxicol Environ Saf. 89:222–230. doi:10.1016/j.ecoenv.2012.12.002

Liu J, Xu X, Yu S, Cheng H, Hong Y, Feng X. 2014. Mercury pollution in fish from South China Sea: levels, species-specific accumulation, and possible sources. Environ Res. 131:160–164. doi:10.1016/j.envres.2014.03.004

Lyche J, Ludvig., Nøstbakken O, Jakob., Berg V. 2019. EU water framework-directive priority contaminants in Norwegian freshwater fish. Norway.

Mansour SA, Sidky MM. 2002. Ecotoxicological Studies. 3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. Food Chem. 78(1):15–22. doi:10.1016/S0308-8146(01)00197-2.

Mapenzi LL, Shimba MJ, Moto EA, Maghembe RS, Mmochi AJ. 2020. Heavy metals bio-accumulation in tilapia and catfish species in Lake Rukwa ecosystem Tanzania. J Geochem Explor. 208:106413. doi:10.1016/j.gexplo.2019.106413

Marengo M, Durieux EDH, Ternengo S, Lejeune P, Degrange E, Pasqualini V, Gobert S. 2018. Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health. Ecotoxicol Environ Saf. 158:204–212. doi:10.1016/j.ecoenv.2018.04.034

Maurya PK, Malik DS, Yadav KK, Kumar A, Kumar S, Kamyab H. 2019. Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: possible human health risks evaluation. Toxicol Rep. 6:472–481. doi:10.1016/j.toxrep.2019.05.012

Miao, L, Liu, B, He, J, Xie, J, Ge, X, Xu, P, . . . Liang, Z. (2010). Evaluation of nutritive quality and nutritional components in the muscle of GIFT strain of Nile tilapia (Oreochromis niloticus). Journal of Shanghai Ocean University, 19(5), 635–641

Mohammed AA-K. 2009. Accumulation of heavy metals in Tilapia Fish (Oreochromis niloticus) from Al-Khadoud Spring, Al-Hassa, Saudi Arabia. Am J Appl Sci. 6(12). doi:10.3844/ajassp.2009.2024.2029.

Mshana Grayson J. 2015. Mercury and lead contamination in three fish species and sediments from lake rukwa and catchment areas in Tanzania. J Health Pollut. 5(8):7–18. doi:10.5696/i2156-9614-5-8.7.

Mwakalapa. EB, Mmochi. AJ, Müller. MHB, Mdegela. RH, Lyche. JL, Polder. A. 2018. Occurrence and levels of persistent organic pollutants (POPs) in farmed and wild marine fish from Tanzania. A pilot study. Chemosphere. 191(438–449):438–449. doi:10.1016/j.chemosphere.2017.09.121.

Mwakalapa EB, Simukoko CK, Mmochi AJ, Mdegela RH, Berg V, Bjorge Muller MH, Polder A, Polder A. 2019. Heavy metals in farmed and wild milkfish (Chanos chanos) and wild mullet (Mugil cephalus) along the coasts of Tanzania and associated health risk for humans and fish. Chemosphere. 224:176–186. doi:10.1016/j.chemosphere.2019.02.063

Naji A, Ismail A, Kamrani E, Sohrabi T. 2014. Correlation of MT levels in livers and gills with heavy metals in wild tilapia (Oreochromis mossambicus) from the Klang River, Malaysia. Bull Environ Contam Toxicol. 92(6):674–679. doi:10.1007/s00128-014-1243-4.

Nakayama SM, Ikenaka Y, Muzandu K, Choongo K, Yabe J, Muroya T, Ishizuka M, Minagawa M, Umemura T, Ishizuka M. 2013. Geographic information system-based source estimation of copper pollution in Lake Itezhi-tezhi and metal-accumulation profiles in Oreochromis spp. from both field and laboratory studies. Arch Environ Contam Toxicol. 64(1):119–129. doi:10.1007/s00244-012-9802-3.

Nakayama SMM, Ikenaka Y, Muzandu K, Choongo K, Oroszlany B, Teraoka H, Ishizuka M, Ishizuka M. 2010. Heavy metal accumulation in lake sediments, fish (Oreochromis niloticus and Serranochromis thumbergi), and Crayfish (Cherax quadricarinatus) in Lake Itezhi-tezhi and Lake Kariba, Zambia. Arch Environ Contam Toxicol. 59(2):291–300. doi:10.1007/s00244-010-9483-8.

Nazir R, Khan M, Masab M, Rehman HU, Rauf NU, Shahab S, . . . Shaheen Z. 2015. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. J Pharmaceut Sci Res. 7:89–97.

Nonga HE, Mdegela RH, Lie E, Sandvik M, Skaare JU. 2011. Assessment of farming practices and uses of agrochemicals in Lake Manyara basin, Tanzania. Af J Agric Res. 6:2216–2230. doi:10.5897/AJAR11.271

Nyingi B, Gitahi KJ, Kiptoo M, Jackson K. 2016. Heavy metal concentrations in water and selected fish species (tilapia, catfish and lungfish) from Lake Baringo, Kenya. Int J Sci Environ. 5:4288–4295.

Paulet G. 2014. KAPENTA RIG SURVEY OF THE ZAMBIAN WATERS OF LAKE KARIBA. Ebene (Mauritius).

Plessl C, Gilbert BM, Sigmund MF, Theiner S, Avenant-Oldewage A, Keppler BK, Jirsa F. 2019. Mercury, silver, selenium and other trace elements in three cyprinid fish species from the Vaal Dam, South Africa, including implications for fish consumers. Sci Total Environ. 659:1158–1167. doi:10.1016/j.scitotenv.2018.12.442

Polder A, Muller MB, Lyche JL, Mdegela RH, Nonga HE, Mabiki FP, Lie E, Skaare JU, Sandvik M, Skjerve E. 2014. Levels and patterns of persistent organic pollutants (POPs) in tilapia (Oreochromis sp.) from four different lakes in Tanzania: geographical differences and implications for human health. Sci Total Environ. 488-489:252–260. doi:10.1016/j.scitotenv.2014.04.085

Raknuzzaman M, Ahmed MK, Islam MS, Habibullah-Al-Mamun M, Tokumura M, Sekine M, Masunaga S. 2016. Trace metal contamination in commercial fish and crustaceans collected from coastal area of Bangladesh and health risk assessment. Environ Sci Pollut Res. 23 (17):17298–17310. doi:10.1007/s11356-016-6918-4.

Ralston NVC, Kaneko JJ, Raymond LJ. 2019. Selenium health benefit values provide a reliable index of seafood benefits vs. risks. J Trace Elements Medi Biol. 55:50–57. doi:10.1016/j.jtemb.2019.05.009

Ralston NVC, Ralston CR, Raymond LJ. 2016. Selenium health benefit values: updated criteria for mercury risk assessments. Biol Trace Elem Res. 171(2):262–269. doi:10.1007/s12011-015-0516-z.

Rhea DT, Farag AM, Harper DD, McConnell E, Brumbaugh WG. 2013. Mercury and selenium concentrations in biofilm, macroinvertebrates, and fish collected in the Yankee fork of the Salmon River, Idaho, USA, and their potential effects on fish health. Arch Environ Contam Toxicol. 64(1):130–139. doi:10.1007/s00244-012-9816-x.

Saha N, Mollah MZI, Alam MF, Safiur Rahman M. 2016. Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. Food Control. 70:110–118. doi:10.1016/j.foodcont.2016.05.040

Sapkota A, Sapkota AR, Kucharski M, Burke J, McKenzie S, Walker P, Lawrence R. 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ Int. 34(8):1215–1226. doi:10.1016/j.envint.2008.04.009.

Shaalan M, El-Mahdy M, Saleh M, El-Matbouli M. 2018. Aquaculture in Egypt: insights on the current trends and future perspectives for sustainable development. Rev Fish Sci Aquacult. 26(1):99–110. doi:10.1080/23308249.2017.1358696.

Squadrone S, Benedetto A, Brizio P, Prearo M, Abete MC. 2015. Mercury and selenium in European catfish (Silurus glanis) from Northern Italian Rivers: can molar ratio be a predictive factor for mercury toxicity in a top predator? Chemosphere. 119:24–30. doi:10.1016/j.chemosphere.2014.05.052

Ssebugere P, Sillanpää M, Wang P, Li Y, Kiremire BT, Kasozi GN, … Jiang G. 2014. Polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polybrominated diphenyl ethers in sediments and fish species from the Murchison Bay of Lake Victoria, Uganda. Sci Total Environ. 500-501(1–10). doi:10.1016/j.scitotenv.2014.08.076.

Stankovic S, Kalaba P, & Stankovic A. R. (2014). Biota as toxic metal indicators. Environmental Chemistry Letters, 12(1), 63–84. doi: 10.1007/s10311-013-0430-6.

Subasinghe RP. 2005. Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. Prev Vet Med. 67(2):117–124. doi:10.1016/j.prevetmed.2004.11.004.

Taweel A, Shuhaimi-Othman M. 2011. Heavy metals concentration in different organs of tilapia fish (Oreochromis niloticus) from selected areas of Bangi, Selangor, Malaysia. Afr J Biotechnol. 10(55).

Taweel A, Shuhaimi-Othman M, Ahmad AK. 2013. Assessment of heavy metals in tilapia fish (Oreochromis niloticus) from the Langat River and Engineering Lake in Bangi, Malaysia, and evaluation of the health risk from tilapia consumption. Ecotoxicol Environ Saf. 93:45–51. doi:10.1016/j.ecoenv.2013.03.031

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. 2012. Heavy metal toxicity and the environment. Exp Suppl. 101:133–164. doi:10.1007/978-3-7643-8340-4_6

Tran N, Chu L, Chan CY, Genschick S, Phillips MJ, Kefi AS. 2019. Fish supply and demand for food security in Sub-Saharan Africa: an analysis of the Zambian fish sector. Marine Pol. 99:343–350. doi:10.1016/j.marpol.2018.11.009

[USEPA] U.S. Environmental Protection Agency. 1995. Final water quality guidance for the Great Lakes system. Fed. Regist. 60:15365–15425.

[USEPA] United States Environmental Protection Agency. 2018. US EPA Regional Screening Levels (RSLs) (November 2018)-Dataset- California open data. [accessed 2021 Jul 28] https://data.ca.gov/dataset/us-epa-regional-screening-levels-rsls-november-2018/resource/b66c9172-a61a-4131-997f-01f45a473a08

Varol M, Kaya GK, Alp A. 2017. Heavy metal and arsenic concentrations in rainbow trout (Oncorhynchus mykiss) farmed in a dam reservoir on the Firat (Euphrates) River: risk-based consumption advisories. Sci Total Environ. 599-600:1288–1296. doi:10.1016/j.scitotenv.2017.05.052

Vicente-Zurdo D, Gómez-Gómez B, Pérez-Corona MT, Madrid Y. 2019. Impact of fish growing conditions and cooking methods on selenium species in swordfish and salmon fillets. J Food Compos Anal. 83:103275. doi:10.1016/j.jfca.2019.103275

Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, … Vethaak AD. 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicol. 30(1):71–133. doi:10.1080/10408440091159176.

Waseem A, Arshad J, Iqbal F, Sajjad A, Mehmood Z, Murtaza G. 2014. Pollution status of Pakistan: a retrospective review on heavy metal contamination of water, soil, and vegetables. Biomed Res Int. (2014:813206. doi:10.1155/2014/813206

Watterson. A, Little. D, Young. JA, Boyd. K, Azim. E, Murray. F. 2008. Towards integration of environmental and health impact assessments for wild capture fishing and farmed fish with particular reference to public health and occupational health dimensions. Int J Environ Res Public Health. 5(258–227):258–277. doi:10.3390/ijerph5040258.

Weis JS. 2009. Reproductive, developmental, and neurobehavioral effects of methylmercury in fishes. J Environ Sci Health Part C. 27(4):212–225. doi:10.1080/10590500903310088.

Xiao H, Zang S, Guan Y, Liu S, Gao Y, Sun Q, Pei X, Li M, Wang J, Pei X. 2014. Assessment of potential risks associated with heavy metal contamination in sediment in Aobaopao Lake, China, determined from sediment cores. Ecotoxicology. 23(4):527–537. doi:10.1007/s10646-014-1220-z.

Yabanli M, Tay S. 2021. Selenium and mercury balance in sea bream obtained from different living environments in Turkey: a risk assessment for the consumer health. Environ Sci Pollut Res Int. 28(27):36069–36075. doi:10.1007/s11356-021-13366-y.

Yildiz M. 2008. Mineral composition in fillets of sea bass (Dicentrarchus labrax) and sea bream (Sparus aurata): a comparison of cultured and wild fish. J Appl Ichthyol. 24(5):589–594. doi:10.1111/j.1439-0426.2008.01097.x.

Zambia Central Statistical Office. (2012). Zambia 2010 Census of Population and Housing. Lusaka, Zambia.

Zhang W, Jin X, Di Z, Zhu X, Shan B. 2016. Heavy metals in surface sediments of the shallow lakes in eastern China: their relations with environmental factors and anthropogenic activities. Environ Sci Pollut Res. 23(24):25364–25373. doi:10.1007/s11356-016-7643-8.

Paper III

Heavy metals in farmed and wild milkfish (*Chanos chanos*) and wild mullet (*Mugil cephalus*) along the coasts of Tanzania and associated health risk for humans and fish

Eliezer Brown Mwakalapa [a, b, e], Chalumba Kachusi Simukoko [d], Aviti John Mmochi [b], Robinson Hammerthon Mdegela [c], Vidar Berg [a], Mette Helen Bjorge Müller [a], Jan Ludvig Lyche [a], Anuschka Polder [a, *]

[a] Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, P. O. Box 8146 Dep, N-0033, Oslo, Norway
[b] Institute of Marine Sciences, University of Dar Es Salaam, P. O. Box 668, Mizingani Road, Zanzibar, Tanzania
[c] Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P. O. Box, 3021, Morogoro, Tanzania
[d] Department, Biomedical Sciences, University, University of Zambia, P. O. Box 32379 Lusaka, Zambia
[e] Department of Natural Sciences, Mbeya University of Science and Technology, P. O. Box 131, Mbeya, Tanzania

HIGHLIGHTS

- Pb and Ni were detected in 100% in the muscles and livers of all the analysed fish.
- Pb pollution is more prevalent in wild fish than in farmed fish.
- Pb concentrations was above the ML set by FAO/WHO for human consumption.
- EDI, THQ and HI values indicated no risk to human health.
- Pb and Hg levels questions the food safety for human consumption and health of fish.

GRAPHICAL ABSTRACT
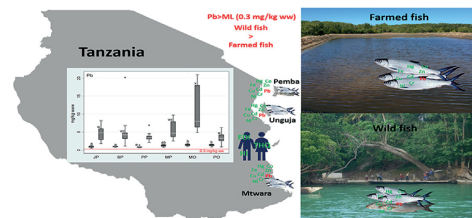
ARTICLE INFO

ABSTRACT

In 2016, farmed milkfish (*Chanos chanos*) from Tanzania mainland (Mtwara), and Zanzibar islands (Pemba and Unguja) and wild milkfish and mullet (*Mugil cephalus*) from the Indian Ocean were collected for analyses of heavy metals (Pb, Cd, Hg, As, Al, Fe, Zn, Cu, Ni, Co and Cr) in muscles and livers. High concentrations of Pb were detected in muscles and livers from wild and farmed milkfish and wild mullet from all sites. The highest concentration of Pb was detected in wild milkfish liver from Mtwara (47.4 mg/kg ww). The Pb concentrations in fish muscle exceeded maximum levels (ML) set by FAO/WHO (0.3 mg/kg ww) in 100% of the analysed fish. Concentrations of Pb were higher in wild fish than in farmed fish. Cd concentrations were generally low. The comparison of the Hg concentration with EQS_{Biota} indicated that Hg might pose potential health risk to 22% of the analysed fish. Median concentrations of Fe in livers from farmed milkfish from Jozani and Shakani, Zanzibar, were 40–80 times higher than the other sites. Assessment of human health risk and exposure to heavy metals indicated no potential risk from consuming the fish from the present study locations. However, the Pb concentrations exceeding ML in the fish suggests that Pb may affect the health of fish. Future investigations should include regular

---

* Corresponding author.
E-mail address: anuschka.polder@nmbu.no (A. Polder).

monitoring of heavy metals in farmed and wild fish in Tanzania for further development of sustainable aquaculture and the welfare of the wild fish stock in the coastal waters.

1. Introduction

Heavy metals are ubiquitous and persistent in nature, and may be toxic to biota at low concentrations (Jaishankar et al., 2014). They are released to the environment from various sources, including industrial, mining and agricultural activities, improper waste disposal (Biney et al., 1994; Henry et al., 2006; Su et al., 2014; Zhang et al., 2010) and volcanic eruption (Vigneri et al., 2017). Heavy metals may be distributed by air (AMAP, 2005; EMEP, 2015) and water through run-off, river flow and oceanic currents (Echegoyen et al., 2014; Khan et al., 2017). Atmospheric transported heavy metals are deposited on terrestrial organisms (Steinnes et al., 1994) or in aquatic ecosystems (Baki et al., 2018; Burger et al., 2007; Echegoyen et al., 2014).

Because of potential harmful effects in humans and biota, international bodies aim to reduce emission (Convention of the United Nations Economic Commission for Europe (UNECE) and Long-range Transboundary Air Pollution (CLRTAP)) of heavy metals. The Convention focuses on mercury (Hg), lead (Pb) and cadmium (Cd). Due to actions taken the Hg emissions decreased in Europe, Caucasus and Central Asia (EECCA) and in North America during the period 1990—2010, but increased significantly in Sub Saharan Africa and Southeast Asia during the same period due to release from artisanal and small-scale gold mining (EMEP, 2015). The rapidly decline in Pb emission in US and Europe has been related to phasing out of leaded gasoline since 2006 (EMEP, 2015). However, in Africa it is more challenging to find efficient actions for reducing lead because communities often are exposed to multiple sources of Pb including usage of lead-based paint (WHO, 2015).

Fish is an important source of protein, vitamins, essential minerals and unsaturated fatty acids for humans worldwide and important for income generation (FAO, 2014). Due to the fast growing human population, especially in African countries, there is increased need for food security (FAO, 2015). The wild fish stocks are declining globally, and the proportion of the global fish consumption constitute more and more farmed fish. In 2016, the aquaculture production in Africa increased by 18% (FAO, 2018). However, there is a concern on pollution of aquatic and oceanic environments by chemical contaminants, such as heavy metals because of potential leakage of contaminants to water bodies. Fish farms placed along the coast lines as in this study, gradually replace pond water with fresh sea water that may be contaminated with heavy metals originating from urban waste disposal and from natural sources (Baki et al., 2018; Biney et al., 1994; Jaishankar et al., 2014). Heavy metals can bioaccumulate in fish and biomagnify in the food chain, and result in negative health effects in humans, such as neurological, cardiovascular and renal system disorders (Ahmed et al., 2016; Fang et al., 2014; Goldhaber, 2003; WHO, 1995). Because of the nutritional importance of fish and public health concerns, there is a need to assess the health impact of heavy metals in fish for human consumption (Bosch et al., 2016; Fakhri et al., 2018; Hylland et al., 2006; Saria, 2016; Yi et al., 2011).

In Tanzania, studies from coastal regions revealed heavy metal contamination in crustaceans (Rumisha et al., 2017, 2012), and wild fish (Mziray and Kimirei, 2016; Saria, 2016; Shilla, 2016). However, no study has measured and compared the levels of heavy metals in farmed and wild marine fish. Therefore, the aims of the present study were to document the levels of heavy metals in farmed and wild fish along the coast of Tanzania, to compare these with the Environmental Quality Standards for biota EQS$_{Biota}$ and to assess possible health risks for humans. EQS$_{Biota}$ are scientifically defined values intended to protect fish from adverse health effects of contaminants. In this study, we measured the levels of heavy metals in wild and farmed fish and assessed possible health risks for fish and humans.

2. Materials and method

2.1. Study area and sampling

Details of fish species description, sampling sites, sample collection and processing were previously described in a study of persistent organic pollutants (POPs) in fish from Tanzania (Mwakalapa et al., 2018). Briefly, a total of 121 farmed and wild milkfish and mullets were collected from the Zanzibar islands Unguja and Pemba and Ndumbwe village in Mtwara (mainland) in Tanzania between January and April 2016 (Fig. 1). Collection of biometric information including weight and length, dissection and extraction of muscle and liver tissue were conducted onsite. When dissection was not possible in the field, live fish were collected and transferred in water containers to the laboratory for extraction of muscle and liver. In the field, muscle and liver samples were stored in a cool box and thereafter transferred to the Institute of Marine Science and preserved at $-20\,°C$ until transportation to Zambia and Norway for analysis. The samples were kept frozen at $-20\,°C$ until analysis.

2.2. Ethical consideration

Fish farmers and local authorities were informed about the aim of this study, and sampling of fish samples from their farmers was done upon their consent. The permission to conduct this research was granted by the management of the Institute of Marine Sciences, University of Dar es Salaam and the permit to transport samples from Tanzania to Zambia was given by the Ministry of Livestock and Fisheries Development in Tanzania. The permission to transport samples from Tanzania to Norway was granted by The Ministry of Agriculture, Livestock and Fisheries and The Norwegian Food Safety Authority.

2.3. Sample analysis

At each location the fish was selected on weight and length and grouped (Table 1). The intention was to get a sufficient number of fish with the same weight per site and per species, however, at some sites the available fish were smaller, as in Mtwara (Mwakalapa et al., 2018). Out of 121 fish, 48 fish were selected based of their weight for analysis of the following heavy metals in muscle and liver, the toxic, non-essential metals: mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As), aluminium (Al), and the essential metals iron (Fe), zinc (Zn), copper (Cu) nickel (Ni), cobalt (Co) and chromium (Cr). Hg was only analysed in 18 fish muscle samples. The analysis of Pb, Cd, As, Al, Fe, Zn, Cu, Ni, Co and Cr was performed at the laboratory of the Central Veterinary Research
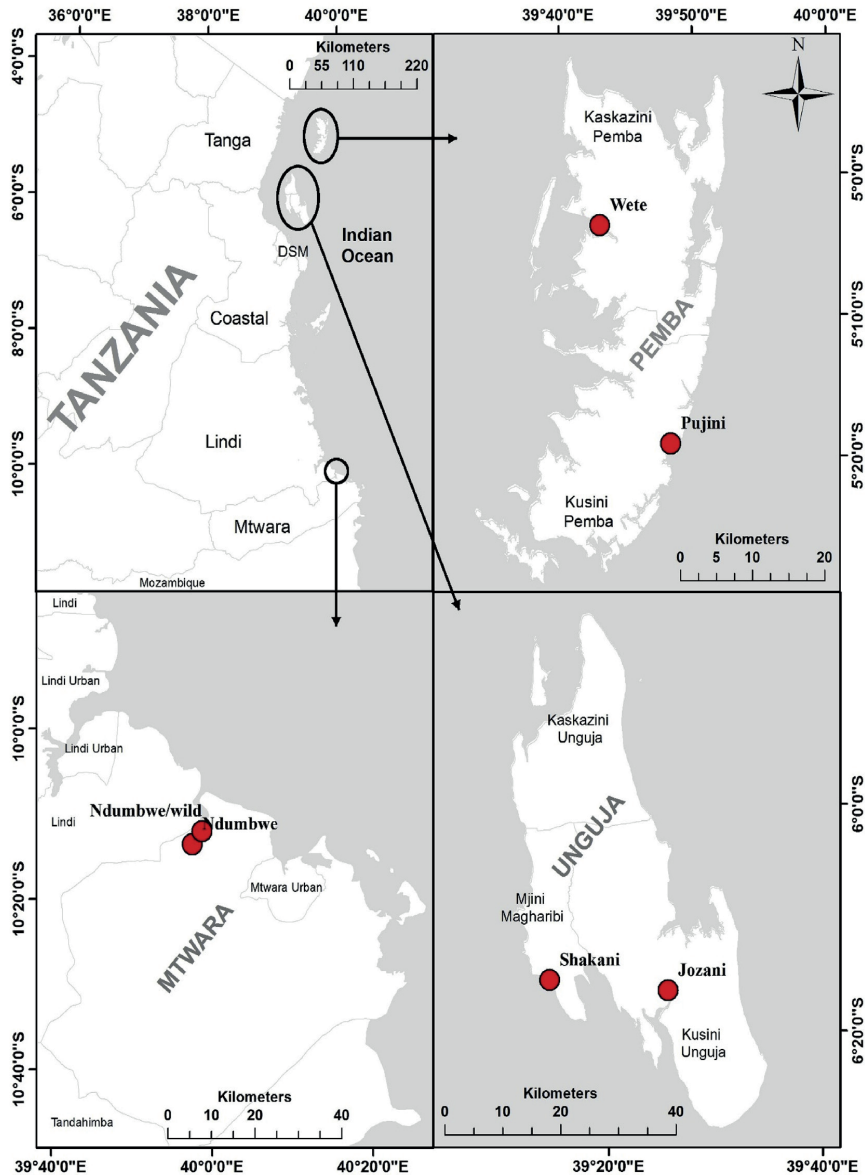
Fig. 1. Map of Tanzanian coasts showing location of sampling sites.

Institute in Zambia (CVRI). The laboratory was accredited by the Southern African Development Community Accreditation Services (SADCAS) on 16th November 2017. The analysis of Hg was done at the Laboratory for Soil and Water analysis, Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life Sciences (NMBU), Campus Ås, Norway.

2.4. Extraction and analysis of heavy metal

The standard operating procedure for heavy metals at CVRI, Zambia, was performed using The Analyst 400 series of Perkin Elmer Atomic Absorption Spectrophotometer (Nchima, 2014). After

thawing at room temperature, the samples of livers and muscles were macerated into small pieces and mixed in a blender to homogeneous mixture. After mixing, approximately 1 g of liver and 5 g of muscles from each sample were transferred quantitatively into a Kjeldahl flask. Digestion of the sample was followed by addition of 20 ml of concentrated nitric acid (HNO_3, analytical grade) followed by heating at the temperature between 210 and 350 °C for about 10–20 min. Thereafter, 10 ml of concentrated sulphuric acid (H_2SO_4, analytical grade) was added into the sample. The boiling continued until the samples were digested. For separation, 5 ml portions of concentrated nitric acid were added gradually. The clear sample was left to cool down to room temperature

Table 1
Characteristics of muscle and liver samples of milkfish from Jozani and Shakani (Unguja), Mtwara and Pemba and wild milkfish from Mtwara and wild mullets from Pemba, Tanzania, for the analysis of Pb, Cd, Fe, Zn, Cu, Ni, Co and Cr (A), and muscles from farmed milkfish from Jozani and Mtwara and wild mullets from Pemba for Hg analysis (B).

Site	Sampling month/year	Salinity (ppt)	Mean weight (g)	Weight range (g)	Mean length (cm)	No of samples (Muscle)	No of samples (Liver)
(A)							
Jozani pond	Jan-16	36	662	413–826	44.1	8	7
Shakani pond	Jan-16	40	683	533–936	43.5	8	9
Pemba pond	Mar-16	25	212	196–226	29.5	8	8
Mtwara pond	Apr-16	22	189	84–309	25.9	8	8
Mtwara wild	Apr-16	29	108	59–185	22	8	8
Pemba wild	Mar-16	30	612	542–711	39.6	8	8

Site	Sampling month/year	Salinity (ppt)	Mean weight (g)	Weight range (g)	Mean length (cm)	No of samples (Muscle)
(B)						
Jozani pond	16-Jan	36	572	232–814	42	6
Mtwara pond	16-Apr	22	188	84–338	26	6
Pemba wild	16-Mar	30	588	542–671	39	6

and washed using distilled water. The samples were filtered using 15 mm Whatman® filter into 25 or 50 ml volumetric flask and distilled water was used to adjust the volume. The concentration of heavy metals was measured by using the Atomic Absorption Spectrophotometer (Analyst 400 AAS, Perkin Elmer, USA) after preparation of the calibration standard. This method did not include measurement of dry weight. Therefore, results are expressed as mg/kg wet weight (ww).

The Hg analysis, performed at MINA, NMBU, Norway, was as following: Approximately 200 mg of muscle samples were weighed in ultrapure teflon tubes that were pre-rinsed in 7 M nitric acid (HNO_3) and in Milli-Q water® digested in 5 mL of Ultrapure HNO_3 at 260 °C in an UltraCLAVE (Milestone S.r.L, Sorisole (BG) — Italy). The samples were added 1 mL of UltraPure concentrated HCl after digestion to prevent loss of Hg and diluted to 50.0 mL with distilled water. Gold (Au) was used as internal standard. Hg was quantified at mass 200, 201 and 202 with an Agilent 8900.

2.5. Quality control and quality assurance

CVRI, Zambia: Contamination was avoided by cleaning the instrument prior to use. Reagent blanks were used to check contamination. Accuracy and precision of the analytical method and digestion was verified by performing in-house spike-recovery tests on random fish samples for the analytes included in the study. The laboratory performs Intra-Analyst comparisons (IAC) at least twice every year and participated in Proficiency Testing and Inter-Laboratory Comparisons at least once per year. The overall recoveries for Pb, Cd, As, Cr, Co, Ni, Fe, Cu, Zn and Al were above 80% and the limit of detection was 0.006 mg/kg ww for all the analysed metals.

MINA, NMBU, Norway: The LOD and LOQ for Hg were calculated from 3 times and 10 times standard deviation (SD) of blank samples (n = 5) and were 0.001 and 0.004 mg/kg dry weight (dw), respectively, based on mean weight of 0.185 g. For this study series, DORM-3 (Fish Protein Certified Reference Material for Trace Metals) from National Research Council Canada was digested and analysed at the same time. The quantified value of Hg showed good agreement with the certified value.

2.6. Estimation of potential health risks for human and fish

Potential human health risks related to heavy metal exposure from fish consumption were assessed by comparing the concentrations of heavy metals in fish muscles in mg/kg ww to maximum limit (ML) and calculating the Estimated Weekly Intake (EWI), Target Hazard Quotient (THQ) and Hazard Index (HI) (USEPA, 1989).

For Cd, the Estimated Monthly Intake (EMI) was calculated as recommended (EFSA, 2009). A THQ is the ratio of the potential exposure to a substance, i.e. estimated daily intake (EDI) of a heavy metal, and the level at which no adverse effects are expected, i.e. Reference doses (RfD) set by United States Environmental Protection Agency (USEPA) (USEPA, 2018). RfD is an estimate of a daily oral exposure to a toxic substance that is likely to be without an appreciable risk of harmful effects during a lifetime (Varol et al., 2017). The RfD values are given in Table 5. If the THQ is calculated to be less than 1, then no adverse health effects are expected as a result of exposure. If the THQ is greater than 1, then adverse health effects are possible.

The HI is the sum of all the THQ from individual metal. EWI, THQ and HI were calculated using equations below:

$$EWI = \frac{MC * IRd * 7}{BW} \tag{1}$$

where, MC is the median metal concentration in fish muscles (mg/kg ww); IRd is the daily average and maximum fish ingestion by an adult person (24.66 g/day (MALF, 2016) and 38.35 g/day (FAO, 2018)) and BW is an average body weight for an adult individual (70 kg)

$$THQ = \frac{E_F * E_D * F_{IR} * C}{RfD * W_{AB} * T_A} * 10^{-3} \tag{2}$$

E_F is the exposure frequency to heavy metals (365 days/yr.); E_D is the exposure duration (61.8 years) equivalent to life expectancy; F_{IR} is the fish ingestion rate (24.66 g/day); C is the median metal concentration in fish muscle (mg/kg ww); RfD is the oral reference dose (mg/kg/day); W_{AB} is the average body weight of an adult person (70 kg) and T_A is the average exposure time with non-carcinogenic effect ($E_F * E_D$)

$$HI = \sum_{i=1}^{n} THQ_i \tag{3}$$

THQ_i is the THQ of the individual metal and n is the number of heavy metals analysed included in the health risk assessment.

Potential health risk for fish was assessed by comparing the concentration of heavy metals in fish liver to EQS_{Biota}.

2.7. Statistical analysis

Collected data were organized using Microsoft Excel 2016. Statistical analyses were done using JMP Pro 13. Shapiro-Wilk Test W was used to test for the distribution of the data. Data were not

Table 2
Concentrations of Pb, Cd, Hg, Fe, Zn, Cu, Ni, Co, and Cr (mg/kg ww) in (1) muscles and (2) livers in farmed milkfish from Jozani, Shakani, Pemba and Mtwara, in wild milkfish from Mtwara and wild mullets from Pemba wild and Hg in muscles from the farmed milkfish from Jozani and Mtwara and wild mullets from Pemba, Tanzania.

Heavy Metals	Farmed Milkfish																Wild Milkfish				Wild Mullet			
	Milkfish Jozani Pond				Milkfish Shakani pond				Milkfish Pemba pond				Milkfish Mtwara pond				Milkfish Mtwara wild				Mullet Pemba wild			
	N	Mean	Median	Range (Min-Max)	N	Mean	Median	Range (Min-Max)	N	Mean	Median	Range (Min-Max)	N	Mean	Median	Range (Min-Max)	N	Mean	Median	Range (Min-Max)	N	Mean	Median	Range (Min-Max)
1.Muscle																								
Pb	8/8	0.97	1.02	0.54–1.35	8/8	0.94	0.91	0.73–1.11	8/8	0.91	0.89	0.65–1.42	8/8	1.20	1.18	0.76–1.57	8/8	1.44	1.40	1.05–1.96	8/8	1.39	1.43	0.89–1.74
Cd	7/8	0.01	0.01	<LOD–0.02	8/8	0.01	0.01	0.003–0.02	3/8	<LOD	<LOD	<LOD–0.005	7/8	0.01	0.01	0.004–0.02	5/8	0.01	0.01	<LOD–0.02	8/8	0.020	0.021	0.01–0.02
Hg	6/6	0.02	0.02	0.01–0.02	–	–	–	–	–	–	–	–	6/6	0.003	0.003	0.002–0.004	–	–	–	–	6/6	0.012	0.013	0.01–0.02
Fe	5/8	0.93	0.35	<LOD–2.80	7/8	3.34	1.98	<LOD–11.96	2/8	0.16	<LOD	<LOD–0.67	7/8	0.85	1.05	<LOD–1.49	7/8	0.83	0.68	<LOD–2.97	6/8	1.79	1.95	<LOD–3.56
Zn	8/8	0.93	0.82	0.09–1.78	7/8	1.06	1.01	<LOD–2.51	7/8	0.67	0.53	<LOD–2.45	2/8	0.18	<LOD	<LOD–1.24	5/8	0.36	0.24	<LOD–1.11	5/8	0.88	0.77	<LOD–2.81
Cu	1/8	0.29	<LOD	<LOD–2.31	1/8	0.22	<LOD	<LOD–1.79	0/8	<LOD	<LOD	<LOD	1/8	0.06	<LOD	<LOD–0.43	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD
Ni	8/8	0.04	0.05	0.02–0.06	8/8	0.07	0.07	0.04–0.09	8/8	0.07	0.07	0.04–0.09	8/8	0.06	0.06	0.04–0.1	8/8	0.07	0.07	0.05–0.09	8/8	0.05	0.05	0.04–0.07
Co	8/8	0.05	0.05	0.03–0.06	7/8	0.007	0.01	<LOD–0.03	8/8	0.051	0.05	0.02–0.09	8/8	0.04	0.04	0.03–0.05	8/8	0.02	0.02	0.01–0.03	8/8	0.03	0.03	0.01–0.05
Cr	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD–0.08	2/8	0.01	<LOD	<LOD–0.08
2.Liver																								
Pb	7/7	4.87	4.84	1.83–8.21	9/9	5.68	4.29	1.12–20.16	8/8	3.73	3.46	2.16–6.92	8/8	5.64	4.74	2.55–9.75	8/8	14.97	8.03	4.91–47.37	8/8	3.46	3.34	0.92–6.26
Cd	1/7	0.0004	<LOD	<LOD–0.003	5/9	0.004	0.002	<LOD–0.01	0/8	<LOD	<LOD	<LOD	3/8	0.001	<LOD	<LOD–0.004	3/8	0.003	<LOD	<LOD–0.01	7/8	0.01	0.01	<LOD–0.01
Hg*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fe	6/7	73.13	88.28	0–118.9	8/9	71.40	81.22	<LOD–147.9	5/8	11.46	4.74	<LOD–29.54	1/8	0.51	<LOD	<LOD–4.05	3/8	3.57	<LOD	<LOD–14.05	2/8	0.26	<LOD	<LOD–1.62
Zn	6/7	2.20	2.19	<LOD–5.35	8/9	2.01	1.95	<LOD–5.93	3/8	0.29	<LOD	<LOD–1.18	1/8	1.53	<LOD	<LOD–12.24	0/8	<LOD	<LOD	<LOD	6/8	1.42	0.81	<LOD–5.41
Cu	7/7	8.58	8.05	3.95–15.08	9/9	7.15	8.02	1.75–14.89	8/8	6.87	7.86	0.61–11.95	7/8	1.97	1.21	<LOD–5.48	7/8	4.65	4.62	<LOD–6.97	7/8	0.9	0.98	<LOD–1.81
Ni	7/7	0.06	0.05	0.03–0.08	9/9	0.07	0.07	0.06–0.08	8/8	0.07	0.07	0.06–0.08	8/8	0.06	0.06	0.05–0.09	8/8	0.06	0.05	0.04–0.09	8/8	0.06	0.06	0.03–0.09
Co	5/7	0.01	0.004	0–0.03	9/9	0.01	0.01	0.004–0.02	7/8	0.01	0.01	<LOD–0.022	8/8	0.02	0.02	0.01–0.03	8/8	0.01	0.01	0.002–0.02	8/8	0.01	0.01	0.004–0.02
Cr	0/7	<LOD	<LOD	<LOD	0/9	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD

Hg*: mercury was not analysed in the livers of the studied fish.
LOD for all metals was 0.006 mg/kg ww.

Table 3
Median concentrations (mg/kg ww) of heavy metals in the muscles of farmed and wild milkfish and wild mullets and maximum Limit (MLs) in mg/kg ww.

Metals	Farmed Milkfish	Wild Milkfish	Wild Mullet	WHO/FAO (ML)
Pb	0.963	1.387	1.426	0.3
Cd	0.007	0.01	0.021	0.1
Hg	0.008	–	0.013	0.5
Fe	0.687	0.625	1.953	43
Zn	0.46	0.181	0.772	30
Cu	<LOD	<LOD	<LOD	30
Ni	0.061	0.073	0.052	
Co	0.041	0.019	0.028	
Cr	<LOD	<LOD	<LOD	

-: Not analysed.

Table 5
Total Hazard Quotient (THQ) and Hazard Index (HI) for analysed heavy metals from consumption of farmed and wild milkfish and wild mullets.

	Farmed milkfish	Wild milkfish	Wild mullets	RfD
Pb	0.097	0.14	0.144	0.0035[a]
Hg	0.028	–	0.045	0.0001[a]
Cd	0.003	0.004	0.007	0.0001[a]
Fe	0.0004	0.0003	0.001	0.7[a]
Zn	0.0005	0.0002	0.0009	0.3[a]
Cu	<LOD	<LOD	<LOD	0.04[a]
Ni	0.0011	0.0013	0.0009	0.02[a]
Co	0.048	0.022	0.0323	0.0003[a]
Cr	<LOD	<LOD	<LOD	0.003[a]
Hazard Index	0.177	0.167	0.231	

-: Not analysed.
[a] RfD, Oral Reference Dose for different heavy metals in fish obtained from (USEPA, 2018).

normally distributed even after log transformation. Statistical differences between sites were assessed using non-parametric Kruskal Wallis Test and Post hoc analysis to determine differences between sites. Mann Whitney U Test was used to test the differences between muscles and livers. Pearson correlation coefficient was used to examine the relationship among heavy metals and between heavy metals and biometric parameters. The levels of significance were set to $p \leq 0.05$.

3. Results

3.1. Concentration of heavy metals

Characteristics of the fish and sampling sites are presented in Table 1A, B. The concentrations (mean, median and range) of non-essential metals (Pb, Cd, Hg) and essential metals (Fe, Zn, Cu, Ni, Co, Cr) in the muscles and livers of the studied fish are presented in Table 2. The concentrations of Pb, Cd and Hg in the muscles and livers were presented with percentiles in Table S1. Al and As were not detected above LOD in any of the analysed samples, and are not discussed hereafter.

3.1.1. Metal concentrations in muscles

Of the non-essential metals, Pb was detected in 100% of the muscle samples from farmed and wild milkfish and from wild mullets (Table 2). In general, the wild fish contained higher concentration of Pb in the muscles compared to farmed fish. The highest median concentrations of Pb were found in wild and farmed milkfish from Mtwara (1.44 and 1.18 mg/kg ww, respectively) and in wild mullet from Pemba (1.43 mg/kg ww). Hg was detected in 100% of the muscles from farmed milkfish from Jozani and Mtwara and in wild mullets (other sites not analysed), and the highest median concentration was found in muscle from farmed

milkfish from Jozani (0.02 mg/kg ww). Cd was detected in more than 70% in the muscles of farmed and wild milkfish and wild mullet. The highest median concentration of Cd was found in muscle of wild mullet from Pemba (0.02 mg/kg ww). Of the essential metals, Ni was detected in 100% and Fe, Zn and Co were detected in more than 70% in the muscles from farmed milkfish and wild milkfish and mullet. Concentrations of Ni in the muscles were in the same range in all species and sites (0.02–0.09 mg/kg ww). The highest median concentration of Fe was found in muscle of farmed milkfish from Shakani (3.34 mg/kg ww, range < LOD – 11.96 mg/kg ww). Cu and Cr were detected in less than 7% in the muscles of the analysed samples.

3.1.2. Metal concentrations in livers

Pb was detected in 100% of the liver samples from farmed and wild milkfish and from wild mullets (Table 2). The highest median concentration of Pb in liver was found in wild milkfish from Mtwara (8.03 mg/kg ww, range 4.91–47.37 mg/kg ww), followed by farmed milkfish from Shakani (5.68 mg/kg ww, range 1.12–20.16 mg/kg ww). The highest Pb concentration in Shakani was regarded as an outlier. When this outlier was removed from the dataset, the median concentration for Shakani was 3.77 mg/kg ww. The median concentration of Pb in the farmed milkfish from Mtwara was 4.74 mg/kg ww. Cd was detected in low levels in only 35% of the liver samples with highest frequency in farmed milkfish from Shakani and wild mullet from Pemba. Hg was not analysed in fish livers. Ni was detected in 100% in all fish livers from all sites. The concentrations of Ni were in the same range in all species and sites (0.03–0.09 mg/kg ww). Fe was detected in >85% of farmed milkfish from Jozani and Shakani, but in lower frequency at the other sites.

Table 4
Estimated Weekly Intake (EWI) for Hg, Fe, Zn, Cu, Ni, Co and Cr and Estimated Monthly Intake (EMI) for Cd in mg/kg bw/day from consumption of muscle from farmed and wild milkfish and wild mullets by an adult Tanzanian person, weighing 70 kg.

	Farmed milkfish	Wild milkfish	Wild mullet	PTWI/PTMI
Hg	1.96×10^{-5}	–	3.15×10^{-5}	0.004 (EFSA, 2012; JECFA, 2017)
Fe	1.69×10^{-3}	1.54×10^{-3}	4.82×10^{-3}	5.6 (JECFA, 2017)
Zn	1.13×10^{-3}	4.45×10^{-4}	1.90×10^{-3}	7 (JECFA, 2017)
Cu	<LOD	<LOD	<LOD	3.5 (JECFA, 2017)
Ni	1.5×10^{-4}	1.79×10^{-4}	1.28×10^{-4}	0.035 (JECFA, 2017)
Co	9.99×10^{-5}	4.56×10^{-5}	6.78×10^{-5}	0.21 (Finley et al., 2012)
Cr	<LOD	<LOD	<LOD	0.023 (Lin et al., 2004)
Cd[a]	7.4×10^{-5}	1.06×10^{-4}	2.17×10^{-4}	0.025 (JECFA, 2017)

Farmed milkfish: Jozani, Shakani, Pemba and Mtwara ponds.
Wild milkfish: Mtwara, Indian Ocean.
Wild Mullet: Pemba, Indian Ocean.
-: Not analysed.
[a] EMI and PTMI for Cd were calculated based on 30 days.

The highest median concentrations of Fe were found in farmed milkfish from Jozani and Shakani (88.28 and 81.22 mg/kg ww, respectively). Zn was detected in >85% of farmed milkfish from Jozani and Shakani and in 75% in mullet from Pemba, but in less than 15% in farmed and wild milkfish from Mtwara. The highest median concentration of Zn was found in farmed milkfish from Jozani (2.19 mg/kg ww). Cu and Co were detected in 70%–100% of the liver samples. The highest median concentrations of Cu were found in farmed milkfish from Jozani and Shakani (8.05 and 7.15 mg/kg ww, respectively). The concentrations of Co were in the same range in all species and sites (<LOD − 0.03 mg/kg ww). Cr was not detected in any of the liver samples.

3.1.3. Comparison of metal concentrations between muscle and liver within sites

Mann Whitney U test showed significantly higher concentrations of Pb and Cu ($p < 0.05$) in the liver than in the muscle for all species and sites (Fig. 2). The concentrations of Fe ($p < 0.05$) were significantly higher in the liver than in the muscle for farmed and wild milkfish in Jozani, Shakani and Pemba, whereas the Fe concentrations in the muscles of farmed milkfish from Mtwara and wild mullet from Pemba were higher than in the liver. The concentrations of Cd were significantly higher in muscles than in the livers for all fish and all sites (Fig. 2). The concentrations of Co were significantly higher in the muscles than in the liver in farmed milkfish from Jozani, Pemba and Mtwara and wild milkfish from Mtwara and wild mullet from Pemba. In contrast, the concentrations of Co were higher in the liver of farmed milkfish from Shakani (Fig. 2).

3.2. Correlations between heavy metals in muscles, fish weight and length

A significant positive correlation (using Pearson correlation test) was observed between fish weight and fish length for all fish (Table S2A, S2B and S2C). Correlation between the heavy metals and between heavy metals and fish weight and fish length is presented in tables S2A, S2B, S2C and S2D.appsec1

3.3. Human health risk assessment

3.3.1. Maximum limits (MLs)

The present study used MLs for heavy metals in fish defined by the WHO/FAO (Table 3). In the present study, the concentrations of Pb were above ML of 0.3 set by WHO/FAO (FAO/WHO, 1995), in 100% of the analysed fish. The concentrations of all other heavy metals were below the MLs.

3.3.2. Estimated weekly (EWI) and monthly intake (EMI)

The median concentrations in the muscles were used for estimating the EWIs of Hg, Fe, Zn, Co, Cr, and Ni from fish consumption in Tanzania (Table 3). For Cd the EMI was calculated according to Joint FAO/WHO (JECFA, 2011). None of the EWIs for Hg, Fe, Zn, Co, Cr, and Ni and EMI for Cd exceeded the (PTWI) and (PTMI) (Table 4). According to EFSA the PTWI for Pb were removed and assessment of risk of Pb is now expressed as (THQ) (JECFA, 2011).

3.3.3. Target Hazard Quotient (THQ) and Hazard Index (HI)

THQ values for the analysed heavy metals in muscles are presented in Table 5. All the THQs from individual heavy metals were less than 1. However, the highest THQ was observed for Pb in wild milkfish followed by wild mullets (Table 5). The Hazard Index (HI), which is the summation of the THQs of all the heavy metals, was 0.177, 0.167 and 0.231 (median levels), for farmed milkfish, wild milkfish and wild mullets, respectively. These HI values are below

the threshold value of 1, and thus reflect no health risk related to human consumption of the studied fish. The contributions of Pb, Hg and Co to the HI were Pb>54%, Hg>15%, Co>26%, in farmed milkfish and Pb>83%, Co>13% in wild milkfish and Pb>62%, Hg>19%, Co>13% in wild mullets, Hg was not measured in wild milkfish (Table 5). Using median Pb concentrations for average and high consumers show that the highest THQ was observed for wild mullet from Pemba (Table S5 A, B), contributing 14.4% and 22.3%, respectively, to the RfD. Using the 95th percentile of Pb concentrations for average and high consumers shows that the highest THQ was observed for wild milkfish from Mtwara, contributing 19.8% and 30.7%, respectively, to the RfD.

3.4. Fish health assessment

European Union has set Environmental Quality Standards (EQS) for various chemicals including POPs and mercury in fish muscle. The mean Hg concentrations in the muscles of the fish in the present study were below EQS (0.02 mg/kg ww). However, three individual farmed milkfish from Jozani exceeded the EQS for Hg in fish as set by EU. No EQS has been set for other heavy metals.

4. Discussion

4.1. Metal concentrations in muscle and liver, in different study sites and possible sources

The main findings of this study were the high concentrations of Pb in muscle and liver from wild and farmed milkfish and wild mullet from all sites (Fig. 2). This suggests wide distribution of Pb in the Tanzanian coastal environment. All the concentrations of Pb in fish muscle of milkfish and mullet exceeded the ML set by WHO/FAO for fish muscle (FAO/WHO, 1995) (Table 3). The significantly higher concentrations of Pb in wild milkfish muscle from Mtwara and wild mullet muscle from Pemba compared to the farmed fish suggest that fish from the Indian Ocean coastline is more exposed to Pb than farmed fish (Fig. 2). The study of Echegoyen et al. (2014) describes distribution of Pb in the Indian Ocean, which reflects regional emissions, deposition and its horizontal and vertical movement. Furthermore, this study shows that the surface waters of the Indian Ocean are contaminated by extremely high Pb concentrations resulting from emissions from recent rapid growing industrial activities in the region and a late phase-out of leaded gasoline (Echegoyen et al., 2014). Wild fish is part of the food chain in the coastal ocean. Therefore, the South Equatorial Current (east-west), combined with the East Africa Coastal Current (south-north) could be a possible additional exposure route for Pb in the coastal waters of Tanzania, and could be the cause of the high concentrations of Pb found in the wild fish in the present study. In addition, wild milkfish from Mtwara were collected in the brackish water from the estuaries of the Mambi River, within the mangroves, close to Ndumbwe village (Fig. 1). The main road from Southern to Northern Tanzania is situated close to the Mambi River and the Mtwara ponds. Leaded gasoline has been forbidden since 2006 in Tanzania, but earlier run-off from this road might have contributed to increased Pb concentrations in Mtwara estuaries and ponds. The wild mullets from Pemba were collected in the open ocean, and the bay nearby the town of Wete, which is one of the most populated areas of Pemba. Mullets migrate between the open ocean and brackish water in the mangroves and may therefore be contaminated due to anthropogenic activities, and the exposure from the surface water from the East Africa Coastal Current.

Although in lower concentrations, Cd was found at the highest levels in muscles of wild mullet from Pemba (Fig. 2). Use of mineral fertilizers in the intensive clove production at Pemba may be a
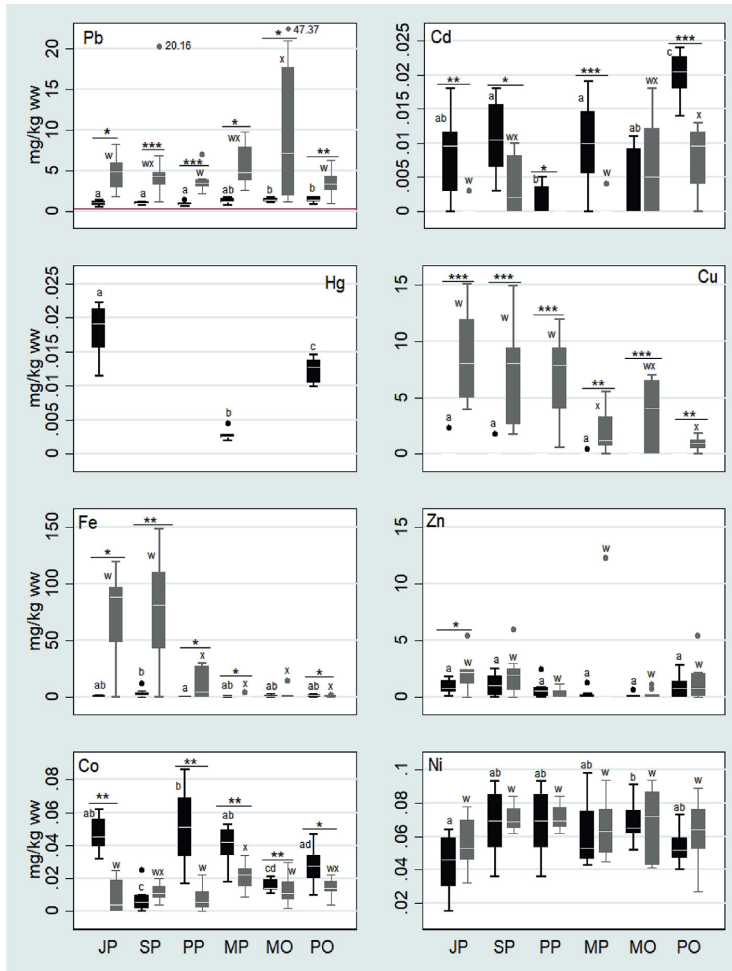
*p≤0.05, **p≤0.001, *** p≤0.0001.

Fig. 2. Heavy metal concentrations in the muscles (black boxes) and livers (grey boxes) of farmed milkfish from Jozani (JP), Shakani (SP), Pemba (PP) and Mtwara (MP) and wild milkfish from Mtwara (MO) and wild mullet from Pemba (PO). The red discontinued line represents the ML set for Pb in fish by WHO/FAO. The letters (a, b, c, d) and (w,x) groups site by variables for the muscles and livers respectively. Sites connected with the same letters indicate no significant differences in metal concentration. The stars indicate the differences between muscles and livers within site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

source for Cd in the area (OSPAR, 2017). Hg was detected in all the analysed fish muscles, indicating a wide distribution in the region. However, the concentration ranges within sites were quite low, which might indicate background levels. The Fe concentrations in liver showed significant geographic variations (Fig. 2), with highest concentrations in Jozani and Shakani at Unguja. This geographic difference may be caused by geogenic sources, especially for the Unguja region, but also because of different feeding regime. Although high quality fish feed is imported to Tanzania, the production of local quality controlled fish feed is not established yet (Rothuis et al., 2014). Some fish farmers use commercially produced fish feed while others use cattle manure to fertilise the ponds to obtain algae for fish feed. Others use left-over of food like corn and vegetables (pers. obs.). This practise gives fewer possibilities to trace the sources of contamination. The concentrations of Co in muscle of farmed milkfish from Jozani, Pemba and Mtwara were

significantly higher than in Shakani (Fig. 2). The ponds in Jozani, Pemba and Mtwara are manmade in mangroves and supplied with water from the ocean, whereas Shakani ponds are situated in a quarry area were seawater is supplied from the ocean by seepage. This might be the reason for the differences in Co between the sites.

4.2. Comparison of heavy metal concentrations in the studied fish muscle with other studies in Africa and elsewhere

Compared to other studies Pb concentrations in muscle of the farmed and wild fish in the present study were higher than those reported in the muscles of wild tilapia (Mdegela et al., 2009) and wild snapper (Saria, 2016) from Tanzania, and wild tilapia from Zambia (Nakayama et al., 2010), but lower than wild tilapia from Kenya (Nyingi et al., 2016) (Table 6). They were higher than wild and farmed carp from China (Qin et al., 2015; Wei et al., 2014), wild

mullet and farmed rainbow trout from Turkey (Tuzen, 2009; Varol et al., 2017), farmed carp from Pakistan (Chatta et al., 2016) and wild mullet from Palestine (Elnabris et al., 2013). They were much lower than in wild tilapia from China (Leung et al., 2014), and farmed milkfish from Taiwan (Chen et al., 2000). The Cd concentrations in the studied fish muscles were lower than the wild tilapia from Kenya (Nyingi et al., 2016), comparable to wild tilapia from Tanzania (Mdegela et al., 2009), but higher than tilapia from Zambia (Nakayama et al., 2010). They were also comparable to wild and farmed carp and wild tilapia from China (Leung et al., 2014; Qin et al., 2015; Wei et al., 2014) and farmed carp from Palestine (Chatta et al., 2016), higher than farmed trout from Turkey (Varol et al., 2017) but much lower than wild mullet from Turkey (Tuzen, 2009) and farmed milkfish from Taiwan (Chen et al., 2000). Hg concentrations in the muscle from wild and farmed fish in the present study were in the same range as in wild tilapia from Tanzania (Mdegela et al., 2009) and in wild and farmed carp in China (Qin et al., 2015; Wei et al., 2014). The concentrations of Fe, Zn, Cu, Co and Cr in the present study were lower than in the other studies. Ni concentrations in the studied fish were higher than in wild carp from China (Wei et al., 2014), but lower than in the other studies (Table 7).

4.3. Possible health risk implications for fish and humans with regard to heavy metals

4.3.1. Human health

In general, the present study showed that the concentrations of Pb in all the studied fish muscles exceeded the maximum levels (MLs) set by FAO/WHO, while other heavy metals were below the respective MLs (Table 3) (FAO/WHO, 1995).

The calculated EWIs for Hg, Fe, Zn, Cu, Ni, Co and Cr were far below the PTWIs set by various parties (Table 4). In the present study, the EMI for Cd was far below the PTMI (Table 4). The THQs and HIs were less than 1 for all fish suggesting that consumption of the fish will not pose any health risk in a lifetime for a healthy, adult person in Tanzania. (Table 5).

Pb is a very toxic heavy metal. Human exposure to Pb can result in neurological and renal problems, haematological effects, hypertension and cancer (Bosch et al., 2016). Pb is retained longer in the bodies of children than in adults due to their immature organ systems and less efficient metabolism of the metal WHO (2015). For Pb, the possible health risk of consumption of farmed and wild milkfish and mullet was calculated based on THQ and contribution of RfD for average and high consumers based on median and 95th percentile (Table S5). According to the EFSA report (EFSA, 2010), the

average dietary exposure of Pb to European high consumers is from 0.0004 to 0.0024 mg/kg body weight per day. This is far higher than corresponding levels found in the present study (mean, 4.04E-4). However, for calculations of THQ, the average and high per capita consumption was obtained from FAO (2018), which might not be representative for residents in the studied coastal areas where fish may be the main source of food. Therefore, THQ values for the populations in these areas might exceed the threshold value and, in that case, the consumption of the fish may pose potential health risks, especially for children. According to WHO (2015), children may, in addition to food, be exposed to Pb paint on children toys and in playground equipment in African countries, and calls for a programme of action to reduce Pb, including screening of vulnerable or high risk groups.

In addition, persistent organic pollutants (POPs), including organochlorine pesticides (OCPs) such as HCB, HCHs and DDTs, polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) were analysed earlier in the same fish (Mwakalapa et al., 2018). This implies that there is a potential of health effect to humans caused by synergistic impact from heavy metals and POPs (Singh et al., 2017) through fish consumption.

4.3.2. Fish health

Heavy metals may cause adverse effects on fish health, such as alteration in condition indices, biochemical disorders, and histopathology (Javed and Usmani, 2017; Zeitoun and Mehana, 2014). Pb in fish may cause inhibition of gonadal growth, increased level of cholesterol in the liver and may adversely affect reproduction at a level of 5 mg/kg in catfish (Clarias batrachus) (Katti and Sathyanesan, 1983). Pb concentrations >1 mg/kg were found to inhibit ALA-D activity in blood of tilapia (Dos Santos et al., 2016). The present maximum Pb concentrations in the wild milkfish muscles (1.96 mg/kg ww) and livers (47.3 mg/kg ww) and in wild mullet muscle (1.74 mg/kg ww) and liver (6.26 mg/kg ww) may thus pose health risk to fish itself and consequently threatens biodiversity for the respective wild species.

In 2008, The European Framework Directive identified 33 priority hazardous substances for which Environmental Quality Standards (EQS) were set, including Hg and Cd. When comparing with the EQS$_{Biota}$, 22% of the fish exceeded the threshold value (0.02 mg/kg ww) for Hg. This calls for further investigation of Hg in the Tanzanian aquatic environment, including fish. Increase of heavy metals in the future may pose serious threat to wild fish stock and aquaculture industry and thus regular monitoring of heavy metals in Tanzanian fish would be recommended.

Table 6
Assessment of risk of Pb exposure and % contribution of RfD from farmed and wild fish using (A) median and (B) 95 percentile Pb concentrations and THQ for average consumers and high consumers.

	Farmed milkfish				Wild milkfish	Wild mullet
	Jozani Pond	Shakani pond	Pemba pond	Mtwara pond	Mtwara	Pemba
(A)						
THQ average consumer	0.10	0.09	0.09	0.12	0.14	0.14
% contribution of RfD	10.27	9.12	8.97	12.24	13.96	14.35
THQ high consumer	0.16	0.14	0.14	0.19	0.22	0.22
% contribution of RfD	15.97	14.19	13.96	19.08	21.72	22.32
	Farmed milkfish				Wild milkfish	Wild Mullets
	Jozani Pond	Shakani pond	Pemba pond	Mtwara pond	Mtwara	Pemba
(B)						
THQ average consumer	0.14	0.11	0.14	0.17	0.20	0.17
% contribution of RfD	13.58	11.20	14.28	16.82	19.75	17.49
THQ high consumer	0.21	0.17	0.22	0.26	0.31	0.27
% contribution of RfD	21.12	17.43	22.22	26.16	30.72	27.21

Table 7
Mean concentrations of heavy metals in mg/kg ww in muscles of farmed and wild fish from this study compared to results from other studies and countries.

Country	Status	Fish specie	Pb	Cd	Hg	Fe	Zn	Cu	Ni	Co	Cr	Reference
Tanzania	FF	Milkfish	1.02	0.01	0.01	1.32	0.71	0.14	0.06	0.04	<LOD	*This study*
Tanzania	WF	Milkfish	1.41	0.01	N.A	0.85	0.36	<LOD	0.07	0.02	<LOD	*This study*
Tanzania	WF	Mullet	1.39	0.02	0.01	1.79	0.88	<LOD	0.05	0.03	0.01	*This study*
Tanzania	WF	Snapper	0.14	0.16		15.77		9.23				Saria (2016)
Tanzania	WF	Tilapia	0.03*	0.01*	0.03*							Mdegela et al. (2009)
Kenya	WF	Tilapia	6.11	1.90			17.10	5.80				Nyingi et al. (2016)
Zambia	WF	Tilapia	0.12	0.003			21.00	3.00	0.38		1.53	(Nakayama et al., 2010)
China	WF	Carp	0.03	0.01	0.04		14.50	0.48	0.02		0.25	Wei et al. (2014)
China	FF	Carp	0.17	0.01	0.01	6.71	7.90	0.30	0.12		0.12	Qin et al. (2015)
China	WF	Tilapia	8.62	0.03			29.50	1.38	3.50		0.51	Leung et al. (2014)
Turkey	WF	Mullet	0.68	0.35	70	125.00	86.20	2.14	2.74		1.30	Tuzen (2009)
Turkey	FF	Rainbow trout	0.05	0.001		7.14	3.40	0.38	1.04	0.74	0.53	Varol et al. (2017)
Taiwan	FF	Milkfish	3.63**	0.07**			24.08**	2.02**	0.22**			Chen et al. (2000)
Pakistan	FF	Carp	0.23	0.01								Chatta et al. (2016)
Palestine	WF	Mullet	0.17				12.78	0.907	0.98			Elnabris et al. (2013)

FF: Farmed fish; WF: Wild fish; N.A: Not Analysed; *: Maximum concentration; **: Calculated from the means of individual ponds.

5. Conclusion

The present study is the first to quantify the concentrations of heavy metals (Cu, Pb, Fe, Zn, Co, Cr, Cd, Ni, Hg, Al, As) in both farmed and wild fish in Tanzania. Except for Cr, Al and As the other analysed heavy metals were detected in at least 50% of the studied fish. The concentrations of heavy metals in the present study were lower, comparable and higher than those reported elsewhere in the literature. Pb and Ni were the dominant heavy metals in both the muscles and livers. The concentration of Pb in all the muscles of the analysed fish in all sites exceeded the ML set by WHO/FAO for human consumption and trade. Pb was detected in higher concentrations in wild fish than in farmed fish, suggesting that Pb contamination is more prevalent in wild fish than in farmed fish. This calls for the government to increase their efforts on fish farming. In addition, waste management and treatment should be encouraged in order to reduce pollution in the aquatic environment. Even though assessment of health risks (THQ and HI) indicated no appreciable health risk for humans from consumption of the studied fish, detection of Pb above MLs and detection of POPs in the same fish from previous study (Mwakalapa et al., 2018) is of concern. Possible synergistic impact by POPs and heavy metals needs further investigations. However, it is worth not to exclude the potential benefit of fish for human consumption as protein and vitamin source. The high Pb concentrations found in the present study may inhibit gonadal growth and affect reproduction. In addition, some of the analysed fish samples exceeded the EQS_{Biota} set for Hg. Based on the previous statement the high levels of Pb and Hg can affect fish health and that might threaten biodiversity and aquaculture development. Findings of this study calls for further investigation of sources of Pb, especially in wild fish from the ocean. Moreover, future investigations should include regular monitoring of heavy metals and POPs in farmed and wild fish in Tanzania for further development of aquaculture and the welfare of the wild fish stock in the coastal waters.

Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.02.063.

References

Ahmed, K.M., Baki, M.A., Kundu, G.K., Islam, S.M., Islam, M.M., Hossain, M.M., 2016. Human health risks from heavy metals in fish of Buriganga river, Bangladesh. SpringerPlus 5, 1697. https://doi.org/10.1186/s40064-016-3357-0.

AMAP, 2005. AMAP Assessment 2002 : Heavy Metals in the Arctic. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norwy.

Baki, M.A., Hossain, M.M., Akter, J., Quraishi, S.B., Shojib, M.F.H., Ullah, A.K.M.A., Khan, M.F., 2018. Concentration of heavy metals in seafood (fishes, shrimp, lobster and crabs) and human health assessment in Saint Martin Island, Bangladesh. Ecotoxicol. Environ. Saf. 159, 153–163. https://doi.org/10.1016/j.ecoenv.2018.04.035.

Biney, C., Amuzu, A.T., Calamari, D., Kaba, N., Mbome, I.L., Naeve, H., Ochumba, P.B.O., Osibanjo, O., Radegonde, V., Saad, M.A.H., 1994. Review of heavy metals in the African aquatic environment. Ecotoxicol. Environ. Saf. 28, 134–159. https://doi.org/10.1006/EESA.1994.1041.

Bosch, A.C., O'Neill, B., Sigge, G.O., Kerwath, S.E., Hoffman, L.C., 2016. Heavy metals in marine fish meat and consumer health: a review. J. Sci. Food Agric. 96, 32–48. https://doi.org/10.1002/jsfa.7360.

Burger, J., Gochfeld, M., Shukla, T., Jeitner, C., Donio, M., Shukla, S., Snigaroff, R., Snigaroff, D., Stamm, T., Volz, C., Burger, J., Gochfeld, M., Shukla, T., Jeitner, C., Donio, M., Shukla, S., Snigaroff, R., Snigaroff, D., Stamm, T., Burger, J., Jeitner, C., Burke, S., Donio, M., Shukla, S., Snigaroff, R., Snigaroff, D., Stamm, T., Volz, C., 2007. Heavy metals in pacific cod (*Gadus macrocephalus*) from the Aleutians : location , age , size , and risk. J. Toxicol. Environ. Health Part A 70, 1897–1911. https://doi.org/10.1080/15287390701551159.

Chatta, A.M., Khan, M.N., Mirza, Z.S., Ali, A., 2016. Heavy metal (Cadmium, lead, and chromium) contamination in farmed fish: a potential risk for consumers' health. Turk. J. Zool. 40, 248–256. https://doi.org/10.3906/zoo-1506-1.

Chen, C.M., Lee, S.Z., Wang, J.S., 2000. Metal contents of fish from cultureponds near scrap metal reclamation facilities. Chemosphere 40, 65–69. https://doi.org/10.1016/S0045-6535(99)00241-6.

Dos Santos, C.R., Cavalcante, A.L.M., Hauser-Davis, R.A., Lopes, R.M., Da Costa Mattos, R.D.C.O., 2016. Effects of sub-lethal and chronic lead concentrations on blood and liver ALA-D activity and hematological parameters in Nile tilapia. Ecotoxicol. Environ. Saf. 129, 250–256. https://doi.org/10.1016/j.ecoenv.2016.03.028.

Echegoyen, Y., Boyle, E.A., Lee, J.-M., Gamo, T., Obata, H., Norisuye, K., 2014. Recent distribution of lead in the Indian Ocean reflects the impact of regional emissions. Proc. Natl. Acad. Sci. Unit. States Am. 111, 15328–15331. https://doi.org/10.1073/pnas.1417370111.

EFSA, 2012. European Food Safety Authority. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA J 10, 1–241. https://doi.org/10.2903/j.efsa.2012.2985.

EFSA, 2010. European food safety authority. Scientific opinion on lead in food. EFSA J 8, 1570. https://doi.org/10.2903/j.efsa.2010.1570.

EFSA, 2009. European Food Safety Authority. Scientific opinion on cadmium in food: scientific opinion of the panel on contaminants in the food chain. EFSA J 980, 1–139.

Elnabris, K.J., Muzyed, S.K., El-Ashgar, N.M., 2013. Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip (Palestine). J. Assoc. Arab Univ. Basic Appl. Sci. 13, 44–51. https://doi.org/10.1016/j.jaubas.2012.06.001.

EMEP, 2015. Heavy Metals: Analysis of Long-Term Trends, Country-specific Research and Progress in Mercury Regional and Global Modelling. EMEP Status report, Oslo, Norway.

Fakhri, Y., Saha, N., Miri, A., Baghaei, M., Roomiani, L., Ghaderpoori, M., Taghavi, M., Keramati, H., Bahmani, Z., Moradi, B., Bay, A., Hosseini, R., 2018. Metal concentrations in fillet and gill of parrotfish (Scarus ghobban) from the Persian Gulf and implications for human health. Food Chem. Toxicol. 118, 348–354. https://doi.org/10.1016/j.fct.2018.05.041.

Fang, Y., Sun, X., Yang, W., Ma, N., Xin, Z., Fu, J., Liu, X., Liu, M., Mariga, A.M., Zhu, X., Hu, Q., 2014. Concentrations and health risks of lead, cadmium, arsenic, and mercury in rice and edible mushrooms in China. Food Chem. 147, 147–151. https://doi.org/10.1016/j.foodchem.2013.09.116.

FAO/WHO, 1995. General Standards for Contaminants and Toxins in Food and Feed. Codex Alimentarius Commission, Rome.

FAO, 2018. The State of the World Fisheries and Aquaculture; Meeting the Sustainable Development Goals. Rome.

FAO, 2015. FAO Aquaculture Newsletter, vol. 68. Food Agric. Organ. United Nations.

FAO, 2014. The State of World Fisheries and Aquaculture. Opportunities and Challenges. Food and Agriculture Organization of the United Nations, Rome.

Finley, B.L., Monnot, A.D., Paustenbach, D.J., Gaffney, S.H., 2012. Derivation of a chronic oral reference dose for cobalt. Regul. Toxicol. Pharmacol. 64, 491–503. https://doi.org/10.1016/j.yrtph.2012.08.022.

Goldhaber, S.B., 2003. Trace element risk assessment: essentiality vs. toxicity. Regul. Toxicol. Pharmacol. 38, 232–242. https://doi.org/10.1016/S0273-2300(02)00020-X.

Henry, R.K., Yongsheng, Z., Jun, D., 2006. Municipal solid waste management challenges in developing countries - Kenyan case study. Waste Manag. 26, 92–100. https://doi.org/10.1016/j.wasman.2005.03.007.

Hylland, K., Beyer, J., Berntssen, M., Klungsøyr, J., Lang, T., Balk, L., 2006. May organic pollutants affect fish populations in the North Sea? J. Toxicol. Environ. Health Part A 69, 125–138. https://doi.org/10.1080/15287390500259392.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscipl. Toxicol. 7, 60–72. https://doi.org/10.2478/intox-2014-0009.

Javed, M., Usmani, N., 2017. An overview of the adverse effects of heavy metal contamination on fish health. Proc. Natl. Acad. Sci. India B Biol. Sci. https://doi.org/10.1007/s40011-017-0875-7.

JECFA, 2017. Joint FAO/WHO Expert Committee on Food Additives. Food Contaminants [WWW Document]. URL (accessed 10.1.18). http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx.

JECFA Joint FAO/WHO Expert Committee on Food Additives, 2011. Evaluation of Certain Food Additives and Contaminants. Seventy-Third Report of the Joint FAO/WHO Expert Commitee on Food Additives. WHO Technical Report Series. No 960. Geneva.

Katti, S.R., Sathyanesan, A.G., 1983. Lead nitrate induced changes in lipid and cholesterol levels in the freshwater fish Clarias batrachus. Toxicol. Lett. 19, 93–96. https://doi.org/10.1016/0378-4274(83)90267-9.

Khan, M.Z.H., Hasan, M.R., Khan, M., Aktar, S., Fatema, K., 2017. Distribution of heavy metals in surface sediments of the Bay of Bengal coast. J. Toxicol. 7 https://doi.org/10.1155/2017/9235764.

Leung, H.M., Leung, A.O.W., Wang, H.S., Ma, K.K., Liang, Y., Ho, K.C., Cheung, K.C., Tohidi, F., Yung, K.K.L., 2014. Assessment of heavy metals/metalloid (As, Pb, Cd, Ni, Zn, Cr, Cu, Mn) concentrations in edible fish species tissue in the Pearl River Delta (PRD), China. Mar. Pollut. Bull. 78, 235–245. https://doi.org/10.1016/j.marpolbul.2013.10.028.

Lin, H., Wong, S., Li, G., 2004. Heavy Metal Content of Rice and Shellfish in Taiwan, vol. 12, pp. 167–174.

MALF, 2016. Ministry of Agriculture Livestock and Fisheries. The Tanzania Fisheries Sector: Challenges and Opportunities. Dar es salaam.

Mdegela, R.H., Braathen, M., Pereka, A.E., Mosha, R.D., Sandvik, M., Skaare, J.U., 2009. Heavy metals and organochlorine residues in water, sediments, and fish in aquatic ecosystems in urban and peri-urban areas in Tanzania. Water Air Soil Pollut. 1–11. https://doi.org/10.1007/s11270-009-0019-7.

Mwakalapa, E.B., Mmochi, A.J., Müller, M.H., Mdegela, R.H., Lyche, J.L., Polder, A., 2018. Occurrence and levels of persistent organic pollutants (POPs) in farmed and wild marine fish from Tanzania. A pilot study. Chemosphere 191, 438–449. https://doi.org/10.1016/j.chemosphere.2017.09.121.

Mziray, P., Kimirei, I.A., 2016. Bioaccumulation of heavy metals in marine fishes (Siganus sutor, Lethrinus harak, and Rastrelliger kanagurta) from Dar es Salaam Tanzania. Reg. Stud. Mar. Sci. 7, 72–80. https://doi.org/10.1016/j.rsma.2016.05.014.

Nakayama, S.M.M., Ikenaka, Y., Hamada, K., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N., Ishizuka, M., 2010. Metal and metalloid contamination in roadside soil and wild rats around a Pb-Zn mine in Kabwe, Zambia. Environ. Pollut. 159, 175–181. https://doi.org/10.1016/j.envpol.2010.09.007.

Nchima, G., 2014. Standard Operating Procedure (S.O.P) for Heavy Metals Analysis Using the Atomic Absorption Spectrophotometer. Lusaka, Zambia.

Nyingi, Gitahi, B., Kiptoo, J.M., Jackson, K., 2016. Heavy metas concentrations in water and selected fish species (tilapia, catfish and lungfish) from Lake Baringo, Kenya. Int. J. Sci. Environ. 5, 4288–4295.

OSPAR, 2017. Inputs of Mercury, Cadmium and Lead via Water and Air to the Greater North Sea. OSPAR Intermed. Assess [WWW Document]. www.ospar.org/assessments (accessed 10.26.18).

Qin, D., Jiang, H., Bai, S., Tang, S., Mou, Z., 2015. Determination of 28 trace elements in three farmed cyprinid fish species from Northeast China. Food Control 50, 1–8. https://doi.org/10.1016/j.foodcont.2014.08.016.

Rothuis, A., Turenhout, M., Duijn, A., Roem, A., Rurangwa, E., Katunzi, E., Kabagambe, J.B., Shoko, A., 2014. Aquaculture in East Africa; A Regional Approach. Wageningen, Netherlands.

Rumisha, C., Elskens, M., Leermakers, M., Kochzius, M., 2012. Trace metal pollution and its influence on the community structure of soft bottom molluscs in intertidal areas of the Dar es Salaam coast, Tanzania. Mar. Pollut. Bull. 64, 521–531. https://doi.org/10.1016/j.marpolbul.2011.12.025.

Rumisha, C., Leermakers, M., Mdegela, R.H., Kochzius, M., Elskens, M., 2017. Bioaccumulation and public health implications of trace metals in edible tissues of the crustaceans Scylla serrata and Penaeus monodon from the Tanzanian coast. Environ. Monit. Assess. 189, 13. https://doi.org/10.1007/s10661-017-6248-0.

Saria, J.A.M., 2016. Assessment of health risks associated with concentrations of heavy metals in fish from the coast of Tanzania. J. Multidiscip. Eng. Sci. Stud. 2, 7. https://doi.org/10.4314/ejesm.v10i4.12.

Shilla, D.A., 2016. Distribution of Pb, Cr, Cu and Zn in the marine-coastal region of Zanzibar (Tanzanian archipelago, East Africa). Chem. Ecol. 32, 774–785. https://doi.org/10.1080/02757540.2016.1178727.

Singh, N., Gupta, V.K., Kumar, A., Sharma, B., 2017. Synergistic effects of heavy metals and pesticides in living systems. Front. Chem. 5, 1–9. https://doi.org/10.3389/fchem.2017.00070.

Steinnes, E., Hanssen, J.E., Rambæk, J.P., Vogt, N.S.B., 1994. Atmospheric deposition of trace elements in Norway: temporal and spatial trends studied by moss analysis. Water Air Soil Pollut. 74, 121–140. https://doi.org/10.1007/BF01257151.

Su, C., Jiang, L., Zhang, W., 2014. A review on heavy metal contamination in the soil worldwide: situation, impact and remediation techniques. Environ. Skept. Critics 3, 24–38. https://doi.org/10.1037/a0036071.

Tuzen, M., 2009. Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. Food Chem. Toxicol. 47, 1785–1790. https://doi.org/10.1016/j.fct.2009.04.029.

USEPA, 1989. Risk Assessment Guidance for Superfund, Volume 1 Human Health Evaluation Manual. EPA/540/1– 89/002. Office of Emergency and Remedial Response, Washington, DC [WWW Document]. URL. https://nepis.epa.gov/ (accessed 2.27.18).

USEPA, 2018. Regional Screening Levels (RSL). Superfund Risk Assessment [WWW Document]. URL. https://epa-prgs.ornl.gov/cgi-bin/chemicals/csl_search (accessed 6.22.18).

Varol, M., Kaya, G.K., Alp, A., 2017. Heavy metal and arsenic concentrations in rainbow trout (Oncorhynchus mykiss) farmed in a dam reservoir on the Firat (Euphrates) River: risk-based consumption advisories. Sci. Total Environ. 599–600, 1288–1296. https://doi.org/10.1016/j.scitotenv.2017.05.052.

Vigneri, R., Malandrino, P., Gianì, F., Russo, M., Vigneri, P., 2017. Heavy metals in the volcanic environment and thyroid cancer. Mol. Cell. Endocrinol. 457, 73–80. https://doi.org/10.1016/j.mce.2016.10.027.

Wei, Y.H., Zhang, J.Y., Zhang, D.W., Tu, T.H., Luo, L.G., 2014. Metal concentrations in various fish organs of different fish species from Poyang Lake, China. Ecotoxicol. Environ. Saf. 104, 182–188. https://doi.org/10.1016/j.ecoenv.2014.03.001.

WHO, 2015. Lead Exposure in African Children: Conteporry Sources and Concern. South Africa.

WHO, 1995. Environmental Health Criteria 165. Inorganic Lead. Geneva, Switzerland.

Yi, Y., Yang, Z., Zhang, S., 2011. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. Environ. Pollut. 159, 2575–2585. https://doi.org/10.1016/j.envpol.2011.06.011.

Zeitoun, M.M., Mehana, E.S.E., 2014. Impact of water pollution with heavy metals on fish health: overview and updates. Glob. Vet. 12, 219–231. https://doi.org/10.5829/idosi.gv.2014.12.02.82219.

Zhang, H., Cui, B., Xiao, R., Zhao, H., 2010. Heavy metals in water, soils and plants in riparian wetlands in the Pearl River Estuary, South China. Procedia Environ. Sci. 2, 1344–1354. https://doi.org/10.1016/j.proenv.2010.10.145.