Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Department of Food Safety and Infection Biology

Philosophiae Doctor (PhD)
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Persistent Organic Pollutants (POPs) and heavy metals in fish from Tanzania – levels and associated health risks to humans and fish

Persistente organiske forbindelser og tungmetaller i fisk fra Tanzania – nivåer og relatert helse risiko for mennesker og fisk

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ABBREVIATIONS

As	Arsenic
AhR	Aryl hydrocarbon receptor
Al	Aluminium
ATSDR	Agency for Toxic Substances and Disease Registry
BFR	Brominated flame retardants
BW	Body weight
Cd	Cadmium
CHLs	Chlordanes
CIESM	Mediterranean Science Commission
CLRTAP	Convention on Long-Range Transboundary Air Pollution
Co	Cobalt
CR	Carcinogenic Risk
Cr	Chromium
CSF	Cancer Slope Factor
Cu	Copper
CVRI	Central Veterinary Research Institute
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DL	Dioxin-like
DPTE	Dibromopropyltribromophenyl ether
EC	European Commission
ECDG	European Commission DG
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EQS	Environmental Quality Standard
EU	European Union
FAO	Food and Agricultural Organization
Fe	Iron
HBB	Hexabromobiphenyl
HBCD	Hexabromocyclododecane
HCB	Hexachrolobenzene
HCHs	Hexachrolocyclohexanes
Hg	Mercury
HLPE	High level panel of expert
HRGC	High resolution gas chromatograph
HRGC-	High resolution gas chromatograph
IMS	Institute of Marine Sciences
IRS	Indoor residue spray
IPEN	International POPs Elimination Network
ITN	Insecticide treated nets
Li	Lithium
LOD	Limit of detection

LRMS	Low resolution mass spectrophotometry
LRT	Long range transport
LRAT	Long range atmospheric transport
lw	Lipid weight
MALF	Ministry of Agriculture Livestock and Fisheries
mg	Milligram
MINA	Faculty of Environmental Sciences and Natural Resource Management
MLs	Maximum limits
MRLs	Maximum residue levels
MS	Mass spectrophotometry
MUST	Mbeya University of Science and Technology
NAOT	National Audit Office of Tanzania
NBS	National Bureau of Statistics
NDL	Nondioxin-like
ng	Nanogram
NMBU	Norwegian University of Life Sciences
Ni	Nickel
NORAD	Norwegian Agency for Development Cooperation
OCGS	Office of Chief Government Stitistician
OCPs	Organochlorine pesticides
Pb	Lead
PBDE	Polybrominated diphenyl ethers
PBEB	Pentabromoethylbenzene
PCB	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzodioxins
PCDFs	Polychlorinated dibenzofurans
PFASs	Polyfluorinated alkyl substances
PFOS	Perfluorooctanesulfonic acid
pg	Picogram
POPs	Persistent organic pollutants
PTB	Pentabromotoluene
RfD	Reference doses
SADCAS	Southern African Development Community Accreditation Services
Se	Selenium
TDI	Tolerable daily intake
TEF	toxic equivalent factor
TEQ	Toxic equivalent
THQ	Target hazard quotient
TRAHESA	Training and Research in Aquatic and Environmental Health in Eastern and Southern Africa
TWI	Tolerable weekly intake
UiO	University of Oslo
UNECE	United Nations Economic Commission for Europe
UNEP	United Nation Environmental Protection

UNIDO	United Nations Industrial Development Organization
USDA	United States Development Agency
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Authority
V	Vanadium
WFD	Water Framework Directive
WHO	World Health Organization
WIO	Western Indian Ocean
WTO	World Trade Organization
Zn	Zinc

SUMMARY

Diet is considered as the main route of exposure to pollutants such as persistent organic pollutants (POPs) and heavy metals to humans during life time. POPs and heavy metals are both persistent and can bioaccumulate in the tissues of organisms and biomagnify up in food chains. POPs are lipophilic and hence they can accumulate in lipid tissues of animal food stuffs, such as fish, eggs and meat. Therefore, they can accumulate considerable amount of POPs and heavy metals.

POPs and heavy metals are potentially toxic to living organisms, such as humans and fish. Heavy metals can be grouped as essential and non-essential, yet both can be toxic depending on dose. POPs and heavy metals can cause adverse health effect such as endocrine disruption, impaired reproduction and immune functions, and teratogenicity. Fish is an important source of protein, vitamins, and fatty acids. Fish and other seafood contribute to the transfer of POPs and heavy metals to humans through consumption. It plays an important role in food security as a commodity for livelihood and income generation to many communities worldwide. Tanzania is rapidly developing, which leads to pollution of aquatic system through industrial and agricultural discharges, and improper disposal of waste. Due to the rapid population growth, the demand for fish production has increased. Hence aquaculture and fish import in Tanzania are growing. However, the information on the distribution of POPs and heavy metals, and the human health risks related to consumption of wild fish, fish from aquaculture and imported fish are lacking. Therefore, the main aims of this study were to identify the occurrence and levels of persistent organic pollutants (POPs) and heavy metals in farmed, wild and imported fish in Tanzania and to increase knowledge on potential health risks related to consuming fish contaminated by POPs and heavy metals in Tanzania.

In paper I, the occurrence and levels of POPs in farmed and wild fish in Tanzania were assessed. The study revealed geographic variation in the occurrence and levels of POPs suggesting various sources of POPs in the Tanzanian environment. In Tanzania, the ratio between *p,p'*-DDE and *p,p'*-DDTs in the fish revealed both historic and recent use of DDT. The study also revealed higher levels of DDTs in wild fish than in farmed fish. DDT was banned

globally in 2001, but since 2006 it is allowed as indoor spraying by the WHO in fighting malaria such as in Tanzania. However, some illegal use might contribute to the recent exposure. The finding of PCBs and PBDEs in the present study were suggested to relate to emissions from the airport and improper management of waste.

In paper II, the same fish samples as in paper I were assessed for occurrence and levels of heavy metals. In this study, geographic variation of heavy metals was found. Pb was the dominant heavy metal in the livers and muscles. The levels in the fish muscles exceeded the Maximum limits (MLs) indicating that the fish are not safe for consumption and don't meet international guidelines for fish trade. The study revealed higher levels of Pb in wild fish than in farmed fish and suggested that wild fish are more exposed to pollutants than farmed fish.

In paper III the variation of POPs and heavy metals in imported tilapia from China were assessed and significant variations between fish from the same package were found. Tilapia from China were dominated by DDTs and the ratio of *p,p'*-DDE to *p,p'*-DDT suggested both historic and recent use in China. The study also revealed high levels of PCB-209 and HBCDD in the Chinese fish, suggesting that import of these fish maybe a pathway of pollution of these compounds to the Tanzanian environment. The toxic As was the second most dominating heavy metals after Al.

In the assessments of human health risks (paper III), the estimated weekly intake for DL-PCB-118 and -105 exceeded the current tolerable weekly intake (TWI) set by EFSA, suggesting potential adverse health effects from consumption of the studied fish from China. Moreover, the cancer risk levels for As exceeded the acceptable cancer risk safe level range ($1x10^{-6}$ to $1x10^{-4}$), indicating potential carcinogenic effect from consumption of the studied fish.

In assessment of the health risk for fish, the levels of PBDE (not published) and Hg (paper II) exceeded the environmental quality standard for biota $EQS_{Biota,}$ suggesting potential adverse health effect in fish from the study areas.

Overall, the findings reported in this thesis calls for authorities in Tanzania to establish proper monitoring plans and measures to reduce human and fish exposure to pollutants.

Furthermore, the findings urge the fish inspectors to include monitoring of individual fish samples rather than pooled samples.

SAMMENDRAG (SUMMARY IN NORWEGIAN)

Mat er betraktet som den største eksponeringsveien for forurensningsstoffer som persistente organiske forurensningsstoffer (POPs) og tungmetaller for mennesker gjennom livet. POPs og tungmetaller er begge tungt nedbrytbare og de kan bioakkumulere i biota og biomagnifisere i næringskjeden. De fleste POPs er fettløselige og kan derfor hope seg opp i fettrikt materiale i animalske matvarer, slik som fisk, egg og kjøtt.

POPs og tungmetaller er potensielt giftige for levende organismer slik som mennesker og fisk. Tungmetaller kan deles i essensielle og ikke essensielle, men begge kan være giftige avhengig av dosen. POPs og tungmetaller kan forårsake negative helseeffekter slik som hormonforstyrrelser, dysfunksjon av reproduksjons – og immun systemet, og medfødte misdannelser. Fisk er en viktig kilde for proteiner, vitaminer og fettsyrer. Gjennom konsum overføres POPs og tungmetaller fra fisk og annen sjømat til mennesker. Fisk er et viktig levebrød og handelsvare for inntekt generering for mange lokalsamfunn i verden. Tanzania er under rask utvikling som fører til forurensing av det akvatiske miljø gjennom utslipp fra industrien og landbruket og dårlig avfallshåndtering pga manglende infrastruktur. På grunn av den hurtige befolkningsveksten øker etterspørselen etter fisk. Derfor øker lokalt oppdrett av fisk og import av oppdrettsfisk fra andre land. Dessverre mangler informasjon om forekomst av POPs og tungmetaller i fisk og helserisiko forbundet med å spise villfisk, oppdrettsfisk eller importert fisk i Tanzania. Derfor var de viktigste målene for denne studien å identifisere forekomst og nivå av POPs og tungmetaller i villfisk, oppdrettsfisk og importert fisk i Tanzania og øke kunnskap om potensiell helsefare relatert til konsum av fisk forurenset med POPs og tungmetaller i Tanzania.

I artikkel I ble forekomst og nivå av POPs studert i villfisk og oppdrettsfisk fra Tanzania. Studien viste geografiske forskjeller i forekomst og nivå av POPs som antyder forskjellige kilder. Ratio mellom *p,p'*-DDE og *p,p'*-DDT i fisken tydet på både historisk og pågående bruk av DDT. Studien viste også at villfisk inneholdt høyere DDT nivå enn oppdrettsfisk. Bruk av DDT ble forbudt i 2001, men ble igjen tillatt av WHO i 2006, men kun for innendørs malaria kontroll, som i Tanzania. Ulovlig bruk kan derfor ha bidratt til nylig eksponering av villfisken.

Funn av PCB'er og PBDE'er ble i denne studien relatert til utslipp fra flyplassen og dårlig avfallshåndtering.

I artikkel II ble de samme fiskene som i artikkel I analysert for tungmetaller. Også i denne studien fant man geografiske forskjeller. Bly (Pb) var det dominerende metallet i fiskelever og muskel. Nivået av Pb i fiskemuskel overskred grenseverdien (Maximum Limits (MLs) som antydet at fisken ikke er trygg for konsum og ikke møter internasjonale retningslinjer som handelsvare. Studien fant høyere nivå av Pb i villfisk enn i oppdrettsfisk, noe som tyder på at villfisk er mer utsatt for forurensing av Pb enn oppdrettsfisk.

I artikkel III ble forekomst og nivå av POPs og tungmetaller undersøkt i importert tilapia fra Kina. Studien fant signifikante forskjeller i både mønster og nivåer av POPs og tungmetaller mellom de individuelle fiskene som var pakket i en og samme pakke. Videre fant studien at DDT dominerte POP mønster og at ratio mellom *p,p'*-DDE and *p,p'*-DDT i fisken tyder på både historisk og pågående bruk av DDT i Kina. Studien fant høye nivåer av PCB 209 og HBCDD i den kinesiske fisken, og det kan antyde at import av fisk fra Kina kan være en mulig kilde for disse forurensningsstoffer til miljøet i Tanzania. Den toksiske arsen (As) var det mest dominerende tungmetallet etter aluminium (Al) i fiskene.

I vurdering av helserisiko for mennesker (artikkel III) fant studien at det estimerte ukentlige inntak for dioksinlignende PCB'er (PCB-118 og -105) overskred det gjeldende tolerable ukentlige inntak, etablert av EFSA 2018, og antyder dermed en potensiell helsefare forbundet med konsum av denne fisken.

I vurdering av helserisiko for fisk (artikkel III) fant studien at nivåer av PBDE og kvikksølv (Hg) overskred miljøkvalitetsstandarden for biota (EQS_{Biota}) som antyder en potensiell negativ helseeffekt i fiskene fra Kina.

Tilsammen viser funn i denne studien at myndighetene i Tanzania bør igangsette gode overvåkingsprosjekter, gjøre tiltak for å begrense eksponering av forurensningsstoffer for både mennesker og fisk. Videre viser funn i denne studien at det i kontrolluttak i overvåkningsprogram er nødvendig å utføre individuelle analyser fremfor å bruke samleprøver fra flere fisk i en analyse.

LIST OF PAPERS

The thesis is based on the three papers listed below, and referred to in the text by their Roman numerals:

Paper I
Occurrence and levels of persistent organic pollutants (POPs) in farmed and wild marine fish from Tanzania. A pilot study.
Mwakalapa EB, Mmochi AJ, Müller MHB, Lyche JL, Mdegela RH, Polder A.
Chemosphere 2018; 191:438-449. DOI:10.1016/j.chemosphere.2017.09.121

Paper II
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, Simkoko CK, Mmochi AJ, Mdegela RH, Berg V, Müller MHB, Lyche JL, Polder A.
Chemosphere, submitted 16 November 2018.

Paper III
Concentration and patterns of persistent organic pollutants (POPs) and heavy metal residues in imported tilapia (*Oreochromis niloticus*) in Tanzania. A potential human health risk assessment.
Mwakalapa EB, Müller MHB, Mmochi AJ, Fundi JJ, Karimi M, Berg V, Lyche JL, Polder. A Manuscript.

1. INTRODUCTION

1.1 Persistent Organic Pollutants (POPs)

Persistent Organic Pollutants (POPs) are synthetic (man-made) halogenated hydrocarbons which are stable and resistant to degradation, and hence persistent in the environment with long half-lives. Because of their lipophilicity, POPs can bioaccumulate in the fatty tissues of living organisms, and biomagnify in food chains and are toxic to living species (Lohmann et al., 2007; Polder et al., 2008a). POPs are volatile or semi-volatile and have the possibility to undergo long-range atmospheric transport (LRAT) (Breivik et al., 2016; Wania, 2003) and be found far away from where they were manufactured and/or used. The mechanism by which long distance transport occur is referred to as the "grasshopper effect" where chemicals repeatedly evaporate and condense while they move away from the source. However, POPs undergo a higher degree of accumulation in the North because the cold climate causes low evaporation rates, thereby decreasing the grasshopper effect (Simonich and Hites, 1995; Wania and Mackay, 1993). POPs can also be distributed by ocean currents and rivers (Breivik et al., 2016; Ilyina et al., 2006; Stemmler and Lammel, 2010). In addition, POPs can be distributed by migrating birds, fish and sea mammals and trade.

POPs have been manufactured since 1900s and used in a wide range of products such as pesticides, transformer oils, building materials, flame retardants, antifoulings, coolants and a wide variety of other products. Some POPs, like dioxins, are unintentionally produced as by-products in processes involving combustion such as in waste incinerators, metal processing industries, chemical manufacturing and oil waste refining (UNIDO, 2003). Because of their toxicity, persistence, ability to bioaccumulate and biomagnify and atmospheric transport, POPs are included in The Stockholm Convention, which is a global treaty to protect human health and the environment against the exposure and effects caused by chemical contaminants. The Stockholm Convention was adopted in 2001 and implemented in 2004 with an initial listing of 12 POPs. It is administered by the United Nations Environmental Programme (UNEP). Until now, more than 20 POPs are listed for elimination, restrictions or reduction of unintentional release (Stockholm Convention, 2018)

(Table 1), and 152 countries have ratified the Convention. In addition, other conventions concerning POPs such as Basel Convention (1992) a treaty on the control of transboundary movement of hazardous waste, and Rotterdam Convention (1998) on the prior informed consent procedure for certain hazardous chemicals and pesticides in international trade, were implemented earlier. The three conventions share the common goal to protect human health and environment from hazardous chemicals (Stockholm Convention, 2018).

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[1]	α-HCH[1]	Endosulfan[1]	Dicofol[1]
Chlordane[1]	β-HCH[1]	HBCDD[2]	PFOA and related compounds[2]
Dieldrin[1]	Lindane (γ-HCH)[1]	HCBD[2,3]	PFHxS and related compounds[2]
Endrin[1]	Chlordecone[1]	PCP[1]	
Heptachlor[1]	HBB[2]	PCN[2,3]	
HCB[1,2,3]	PeCB[1,2,3]	Deca-PBDE[2*]	
Mirex[1]	Penta-PBDE[2]	Hexa- and Hepta-PBDE[2]	
Toxaphene[1]	PFOS[1,2]	SCCP[2]	
DDT[1]		Tetra-PBDE[2]	
PCB[2,3]			
PCDD[3]			
PCDF[3]			

[1]Pesticides; [2]Industrial chemicals; [3]By-products. The acronyms are listed in Abbreviations. *Deca-PBDE is including octa-and nona-BDE.

The list of studied POPs include organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and perfluoroalkyl substances (PFASs). Chemical structures and description of the compounds can be found in the Appendix.

1.2 Heavy metals

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Although heavy metals are considered as pollutants it is important to recognize that they are naturally occurring components of the earth's crust (Jaishankar et al., 2014; Landrigan et al., 2017; Walker et al., 2012). Therefore heavy metal pollution may result from natural phenomena such as weathering of rocks and volcanic eruption but also

from anthropogenic activities such as industrial processing, mining, improper waste disposal, pesticide use and atmospheric deposition (Walker et al., 2012).

Heavy metals cannot be biologically degraded, hence they are persistent and have the ability to bioaccumulate in living organisms and biomagnify in food chains (Hoyle, 2015; Walker et al., 2012). Heavy metals can be classified as essential and non-essential metals. Essential heavy metals are biologically or metabolically significant to living organisms and include iron (Fe), cobalt (Co), copper (Cu), chromium (Cr), zinc (Zn), nickel (Ni), lithium (Li), selenium (Se) and vanadium (V). Non-essential heavy metals do not have any known biological or metabolic function but can be toxic to living organisms such as lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As) and aluminium (Al). Essential heavy metals can also be toxic to living organism when their concentrations are higher than the recommended dose and may cause health effects in case of deficiency. The list and description of the heavy metals can be found in appendix 1. Because of potential harmful effects of heavy metals in humans and biota, international bodies aim to reduce their emission (Convention of the United Nations Economic Commission for Europe (UNECE) and Long-range Transboundary Air Pollution (CLRTAP)). The Convention focuses on mercury (Hg), lead (Pb) and cadmium (Cd) (UNECE, 1979).

1.3 Pollution in Aquatic Environment

Due to human population growth there is an increasing risk of marine environmental pollution worldwide. In the Indian Ocean region, anthropogenic activities such as industrial and urban developments, agricultural activities, improper waste disposal and dismantling of ships are major sources of pollution of the marine environment (Machiwa, 2010; UNEP et al., 2009, 1998). River and rain runoffs from agricultural areas containing pesticides and fertilizers, effluents with industrial wastes and sewage, may carry pollutants including POPs and heavy metals, into the marine environment (EC, 2002). Oil and gas exploration and exploitation have just started in the coastal waters of the Western Indian Ocean (WIO) region, especially in Tanzania, and may also increase regional pollution.

1.4 Human exposure to POPs and heavy metals

1.4.1 POPs

Humans and other living organisms can be exposed to POPs through different routes. However, diet is supposed to be the major route of exposure to POPs for humans (Darnerud et al., 2006; Kannan et al., 1997; Liem et al., 2010; Polder et al., 2010; Zheng et al., 2007) and due to the lipophilic nature of POPs, consumption of fatty foods contributes most to the human exposure. Other routes of exposure include occupation in chemical, textile and agricultural industries (Manyilizu et al., 2017, 2015; Nonga et al., 2011; Thomsen et al., 2007). People living in the vicinity of industries or near agricultural areas are also more exposed to POPs than the populations (Koopman-esseboom et al., 1994; Müller et al., 2016; Nieuwoudt et al., 2009). Moreover, people can be exposed to POPs, such as BFRs from indoor air and dust (Harrad et al., 2006; Lorber, 2008).

1.4.2 Heavy metals

The exposure routes of heavy metals to humans are in general similar to those of POPs and for the heavy metals dietary exposure is suggested to contribute >90% of the human exposure (Morais et al., 2012; Zheng et al., 2007). Food items documented as significant sources of heavy metals include cereals, vegetables, fruits, milk, bean, egg and sea products including fish (Zheng et al., 2007). Various food items contribute to different degree to human exposure from heavy metals. For example, cereals and seafood contribute 48% and 42% to Hg exposure while cereals and vegetables contribute 54% and 30% to Pb exposure. For Cd, vegetables contribute 57% (Zheng et al., 2007). The use of ground water is another potential source of exposure to heavy metals especially for As and Li. In Tanzania, ground water is contaminated with As probably from natural weathering of rocks and from mining activities (Kassenga and Mato, 2008). People working and/or living nearby mining, metal smelting and chemical industries can also be exposed to considerable amounts of heavy metals. Several studies have documented the contamination of the environments in the vicinity of active or abandoned mining areas in Tanzania (Banzi et al., 2015; Bitala et al., 2009; Gomezulu et al., 2018; Mtui et al., 2006). Additionally, humans can also get exposed to

Pb and Hg through inhalation (Wani et al., 2015; WHO, 2015). Pb has been used in gasoline for many years before its ban in 1990s (Landrigan, 2002), however through emission from automobiles and due to its persistence, it is still found in the environment (Bornman et al., 2017; Kacholi and Sahu, 2018). Painting and toys containing Pb is still assumed as an important source for human exposure to Pb in Africa (WHO, 2015).

1.5 Health effects of POPs and heavy metals

1.5.1 Human health

1.5.1.1 POPs

Human exposure to POPs are of concern due to their potential to cause adverse health effects (Gore et al., 2015; Longnecker et al., 1997; WHO, 2010). Health effects caused by POPs have been documented from experimental and epidemiological studies (Gore et al., 2015; Hansen et al., 2019; Mohammed Ahmed et al., 2018), from improper use and accidents (Pesatori et al., 2009; Tsukimori et al., 2008). POPs have been associated with health effects in children such as endocrine disruption, neuropsychological and behavioural problems, impairment of psychomotor development, immunological, haematological and asthma (Fimm et al., 2017; Leijs et al., 2009; Linares et al., 2015; WHO, 2010; Xu et al., 2015, 2014). Otherwise, POPs have been associated with intestinal tumour, endocrine disruption, testicular, prostate and thyroid cancer, diabetes, and neurological effects (Crinnion, 2009; Gore et al., 2015; Marushka et al., 2018; Rylander et al., 2015). In Tanzania, few studies were done on health effect on humans from POPs. Two studies were on self-reported symptoms and health effects of farmers from use of OCPs such as chest pain, memory loss, eye and face irritation, skin rash, respiratory disorder, impotence and loss of libido and miscarriages (Manyilizu et al., 2017, 2015). In addition, acute pesticide poisoning in children were reviewed by (Lekei et al., 2017). Generally, there is little knowledge on possible health effects related to use of POPs especially pesticides in Tanzania

1.5.1.2 Heavy metals

The adverse health effects of heavy metals depend on the route of exposure and the heavy metal. Adverse health effects of the non-essential heavy metals Hg, Pb, Cd and As in humans have been given priority because of their toxicity (WHO, 2007). Hg has been associated with adverse effect on heart function and the thyroid system and impairment of neurodevelopment (Jaishankar et al., 2014; WHO, 2007). Pb has been associated with impairment of neurodevelopment, decreased cognitive development and intellectual performance in children, and elevated blood pressure in adults (ATSDR, 2007a; Bosch et al., 2016; Mahurpawar, 2015; WHO, 2007). Cd and As are suggested to be potential carcinogens causing lung, kidney and skin cancer (ATSDR, 2007a, 2005; Järup, 2003; Morais et al., 2012; WHO, 2007, 2001). Al has been associated to Alzheimer disease (WHO, 1997). Extremely high or low levels of essential metals can have adverse health effects such as anaemia, eczema, cancer; effect on haematological, neurological, immunological, reproductive, cardiovascular and endocrine responses, and DNA damage (ATSDR, 2011; Jaishankar et al., 2014; Mahurpawar, 2015; Plum et al., 2010; Saria, 2016).

1.5.2 Fish health

1.5.2.1 POPs

Fish can be exposed to POPs directly from water or indirectly by feeding on other organisms or sediments. Fish can bioaccumulate POPs in their tissues and the concentration increases (biomagnification) each step up in the food chain , which means that the predator fish have the highest concentrations (Authman, 2015). Thus, predatory fish are often used as bioindicators of pollution in aquatic systems (Authman, 2015; Oost et al., 2003). The accumulation of POPs can cause adverse health effect to fish. Experimental studies documented an increased mortality and disruption of endocrine system in fish induced by mixtures of POPs (Berg et al., 2016; Khezri et al., 2017; Xu et al., 2002). Coimbra and Reis-Henriques, (2007) showed that POPs can cause alteration in reproductive tissues; and affect thyroid hormones in adult fish. Berg et al., (2016) showed that fish from a Norwegian lake contaminated with high levels of POPs had significant higher prevalence of tuberculosis and

parasitic infections compared to reference fish, suggesting immune system suppression. Burkina et al., (2018) showed that fish from rivers in the Czech Republic with high levels of POPs had reduced gonad size, and changes in biomarkers used for assessing estrogenic- (vitellogenin) and dioxin activity (EROD, P450 content). POPs can affect reproduction of adult fish through impairing maturation, fecundity, secondary sexual characterises and egg and larval size (King-Heiden et al., 2012). Furthermore, POPs are documented to cause developmental health effects such as opercular defect, reduced heart size, and bone malformations (King-Heiden et al., 2012).

1.5.2.2 Heavy metals

At toxic concentrations, all heavy metals have the potential to exert various health effects in animals including fish (Tchounwou et al., 2012). Adverse health effects induced by non-essential heavy metals in fish, as suggested by various studies, are briefly summarized. Hg is reported to cause neurological impairment and reproductive effects such as reduction of spermatozoa viability and egg production and further affect survival rate of developing eggs and fry (Clarkson et al., 1985; Raldúa et al., 2007). Pb is documented to affect gonadal growth, damage in the liver and reproductive organs, impairment of embryonic and larval development and inhibit ALA-D activity in blood (Authman, 2015; Dos Santos et al., 2016), and Cd induces histopathological changes in liver (Omer et al., 2012). A study by Al-sawafi et al., (2017) showed that zebrafish exhibited brain cells degeneration when exposed to Cd while Low and Higgs, (2015) showed effect on structure and function of auditory system. Hallauer et al., (2016) showed that the exposure of zebrafish to As caused a dysfunction in the neurological system and this might induce genetic or epigenetic changes over generations. Authman, (2015) documented that As interferes with the fish immune system. Correia et al., (2010) suggested aluminum as an endocrine disrupting metal in mature tilapia. Essential heavy metals can induce various adverse health effects to fish including, respiratory problems, hyperplasia and necrosis, histopathological and histological changes in tissues, impairment and alteration of reproductive and immune functions, disruption of calcium uptake (hypocalcemia), fecundity, growth and hatching (Authman, 2015; Zeitoun

and Mehana, 2014). Furthermore, essential heavy metals can result in inhibition of growth, decreasing in body weight, haemoglobin and protein levels, and increase in cholesterol (Authman, 2015; Javed and Usmani, 2017).

1.6 Assessment of human health risks related to POPs and heavy metals

Risk assessment of human health is the process of estimating the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future (USEPA, 2017). It includes, hazard identification, hazard characterization, exposure assessment and risk characterization. It involves identification, compilation and integration of information on the health hazards of chemicals, human exposure to the chemical and relationships among exposure, dose and adverse effects. International bodies such as WHO, FAO and EU have established maximum residue limits (MRLs) for POPs, and maximum levels (MLs) for heavy metals. MRLs and MLs are identified as safe levels for human consumption and trading food commodities such as fish between countries. Moreover, USEPA, (2000) has developed human health risk assessment methods for most of the POPs and heavy metals as follows in the next subchapters.

1.6.1 Estimated Daily Intake (EDI)

EDI is an estimate of the human daily intake of a chemical substance. EDI is calculated and compared with the established tolerable daily intake (TDI), which is the daily dose humans can be exposed to over a lifetime without presenting appreciable risk to human health.

$$EDI = \frac{MC * IRd}{BW}$$

Where, MC is the chemical substance concentration in fish muscles (mg/kg ww), IRd is the daily average fish ingestion by an adult person (24.66 g/day) (MALF., 2016) and BW is an average body weight for an adult individual (70 kg). FAO, (2018) assumes 38.36 g/day for the high consumer in East Africa.

1.6.2 Target Hazard Quotient (THQ)

A THQ is the ratio of the potential exposure to a chemical substance, i.e. estimated daily intake (EDI) of a chemical substance, and the level at which no adverse effects are expected, i.e. Reference doses (RfD) set by United States Environmental Protection Agency (USEPA). 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

$$THQ = \frac{E_F * E_D * F_{IR} * C}{R_{fD} * W_{AB} * T_A} * 10^{-3}$$

EF is the exposure frequency to chemical substance (365 days/yr.); E_D is the exposure duration (61.8 years) equivalent to life expectancy; F_{IR} is the fish ingestion rate (x g/day); C is the metal concentration in fish muscle (mg/kg ww); R_{fD} 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)

The HI is the sum of all the THQ from individual metal.

$$HI = \sum_{i=1}^{n} THQ_i$$

THQi is the THQ of the individual chemical substance and n is the number of chemical substances analysed included in the health risk assessment. THQ and HI are then compared to the threshold value of 1. If the THQ or HI is calculated to be less than 1 then no adverse health effects are expected as a result of exposure. If the THQ or HI is greater than 1, then adverse health effects are possible.

1.6.3 Carcinogenic risk (CR)

CR is estimated as the probability of an individual to develop cancer over a lifetime as a result of exposure to a potential carcinogen using the formula below (USEPA, 1989). USEPA, (2005), has established acceptable lifetime cancer risk levels range ($1x10^{-6}$ to $1x10^{-4}$). Above and below this range the risks are unacceptable and negligible, respectively. Due to

the toxicity, carcinogenicity of As it is among the major chemicals of concern (WHO, 2010). USEPA, (2005) established the cancer slope factor (CSF) of 1.5 mg/kg ww day for assessment of cancer risk from As over a life time exposure.

$$CR = \frac{E_F * E_D * F_{IR} * C * CSF}{W_{AB} * T_A} * 10^{-3}$$

1.6.4 Toxic Equivalent Factor (TEF) and Toxic Equivalent (TEQ)

For assessment of human health risk for dioxin-like PCBs, WHO has assigned toxic equivalent factor (TEF) which are the estimates of compound-specific toxicity relative to the toxicity of the most toxic dioxin (Berg et al., 2006). TEFs are used to calculate toxic equivalent (TEQ) of the individual DL-PCBs. TEQs are then used to estimate the dietary intake of the DL-PCBs. TEQ values are calculated as follows;

$$TEQ_{total} = \sum (C_i * TEF_i)$$

Where TEQ_{total} is the summation of individual dioxin-like PCB, C_i and TEF_i are the concentration and toxic equivalent factor.

1.7 Fish health risk: EQS

European Union has set Environmental Quality Standards (EQS) for various chemicals of concern including POPs and mercury in biota, water and sediment (EC, 2000). This standard was implemented through the EU Water Framework Directive (WFD) (Directive, 2000/60/EC), which aims at the protection and improvement of the aquatic environment (EC, 2000). Priority substances were identified that require action at the European Union (EU) level. The EQSs serve as benchmark concentrations for harmful effects to wildlife and humans. Monitoring in fish is advised for the following eight substances (EC, 2013, 2008).: HCB[1], PBDEs[1], HBCDD[1], PFOS[1], dicofol, HCBD, and heptachlor þ heptachlor epoxide (HC þ

[1] Compounds included in this study

HCE) and Hg[1]. In this study, we assessed the compliance of the levels of Hg with the established EQS_{BIOTA} in farmed and wild fish from Tanzania.

1.8 Food safety and food security

Food safety and food security are interrelated concepts with a profound impact on quality of human life (Hanning et al., 2012). Food safety includes many factors from biological contamination such as microbial aspect to chemical contamination, such as POPs and toxic heavy metals. FAO defined the concept of food security as; "A situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life" (FAO, 2006). Fish is a very important nutritious food. It is rich in essential nutrients such as high quality protein, long chain fatty acids, omega-3 fatty acids, calcium and vitamins (HLPE, 2014). It is a major source of livelihood and income, especially in developing countries. It is estimated that about 60 million people were engaged in capture fisheries and aquaculture in 2016 (FAO, 2018). Capture fisheries and aquaculture continue to play a crucial role in food security (FAO, 2016). However, aquaculture is increasingly important for a secure supply of fish for human consumption since capture fisheries have stagnated the last two decades (FAO, 2016). In addition, fish is among the most traded food item worldwide. It is estimated that 35% of the global fish production entered international trade with China being the leading country (FAO, 2018). Due to the stagnation of capture fisheries and reduction in wild fish stocks, which are signs of food insecurity (FAO et al., 2018), aquaculture and fish trade are encouraged.

1.9 Aquaculture in Tanzania

Because of the rapid population growth, there has been an increasing demand for food including fish in East Africa. Although aquaculture in Tanzania started more than 60 years ago (Shoko et al., 2011), it has still a huge unexploited potential. Farming of rainbow trout and tilapia started in 1946 (FAO, 2003). Due to various reasons such as donor withdraw and improper management many aquaculture projects failed (Rothuis et al., 2014). However,

while still practiced in small scale, Tanzania has picked up, with the production of 4000 tonnes of fish in 2015 (MALF, 2016). Currently aquaculture is mainly dominated by tilapia in freshwater and milkfish in marine waters (MALF, 2016). Other farmed species include catfish, rainbow trout, mud crab and seaweed. Currently the government has implemented means to increase aquaculture production through establishing hatcheries for both fresh and marine water fish species (MALF, 2016). Moreover, importation of fish for consumption is growing in Tanzania with tilapia being the second contributor after mackerel (MALF, 2016). Coastal aquaculture in Tanzania started after 1980s with the introduction of seaweed, shrimps and different herbivorous and detrivorous fishes, molluscs and algae. Attempts to culture tilapia in controlled marine waters were made and have been adopted by different coastal communities (Msuya et al., 2007; Torell and Mmochi, 2006). Milkfish farming has been introduced to most coastal districts in Tanzania and it has become an important commercial activity along the coast (Lio and Gilda, 1984). Milkfish are cultured in wide range of salinities including brackish, oceanic as well as hyper saline waters (Martinez et al., 2006). However, the development and sustainability of coastal aquaculture depend on its surrounding marine environment (Paquotte and Bailly, 1994), including coastal lands and marine waters.

1.10 Possible sources of POPs and heavy metals in Tanzania

The pollution of aquatic environment in Tanzania originate from various sources. Among others, improper management of waste, rain runoffs from agriculture and urban areas, discharges from the industries and residence are suggested (Machiwa, 2010; Mohammed, 2002; UNEP et al., 1998). The use of pesticides such as DDTs in agriculture has been a source of DDT compounds in food and marine environment. The ratios of *p,p'*-DDE to *p,p'*-DDT in fish from Tanzanian lakes suggested both historic and recent use of DDT (Polder et al., 2014). Tanzania has never produced PCBs, BFRs and PFASs but leakage from imported consumer products containing these POPs, have probably contaminated the Tanzanian environment (IPEN, 2005; Trading and Ecomonics, 2018). PCBs, BFRs and PFASs were earlier detected in chicken eggs, human milk, fish and vegetables from Tanzania (Kiwango et al., 2018; Müller et al., 2017, 2016; Mziray and Kimirei, 2016; Polder et al., 2016, 2014; Saria, 2016) showing

their presence in the Tanzanian environment. Tanzania is among the 152 countries that has ratified the Stockholm Convention in 2004 and has a National Implementation Plan. Despite the ratification of the convention there may be still ongoing unregistered use of the banned chemicals (NAOT, 2018). Earlier studies in fish from Tanzania have reported levels of POPs (Machiwa, 2010; Mdegela et al., 2009; Mwevura et al., 2002; Polder et al., 2014) and heavy metals (Bungala et al., 2017; Mdegela et al., 2009; Mziray and Kimirei, 2016; Saria, 2016). However, data on the levels and distribution of POPs and heavy metals in fish from aquaculture and their associated health risk is lacking. Additionally, Tanzania imports fish and despite the ongoing monitoring, there is a lack of information on the levels of POPs and heavy metals in imported fish. There is also a lack of information on the potential human health risk from consumption of farmed fish and imported fish in Tanzania. Therefore, this study aimed at investigating the levels and distribution of POPs and heavy metals in farmed and wild marine farmed fish and imported tilapia and assess potential health risk from their consumption.

2 AIMS AND OBJECTIVES

The overall goal of this PhD is to identify the occurrence and levels of persistent organic pollutants (POPs) and inorganic chemical contaminants (heavy metals) in the farmed, wild and imported fish in Tanzania. To increase knowledge on potential health risks related to consuming fish contaminated by POPs and heavy metals in Tanzanian.

In order to achieve the main goal of this project five objectives were formulated:

1. To determine the occurrence and levels of POPs in selected farmed and wild marine fish in Tanzania (paper I)

2. To determine the occurrence and levels of heavy metals in selected farmed and wild marine fish in Tanzania (paper II)

3. To determine the levels of POPs and heavy metals in imported tilapia (*Oreochromis niloticus*) (paper III)

4. To assess the potential health risks to humans from consumption of fish contaminated with POPs and heavy metals (papers II and III)

5. To assess the possible fish health risk from the exposure to pollutants (paper II)

3 METHODOLOGY

3.1 Study locations and sampling sites

This study was conducted in three locations, on Unguja and Pemba of Zanzibar islands and Mtwara on the coast of mainland Tanzania (*Fig 1*). In both locations the fish were obtained from the fish farms and from the ocean.

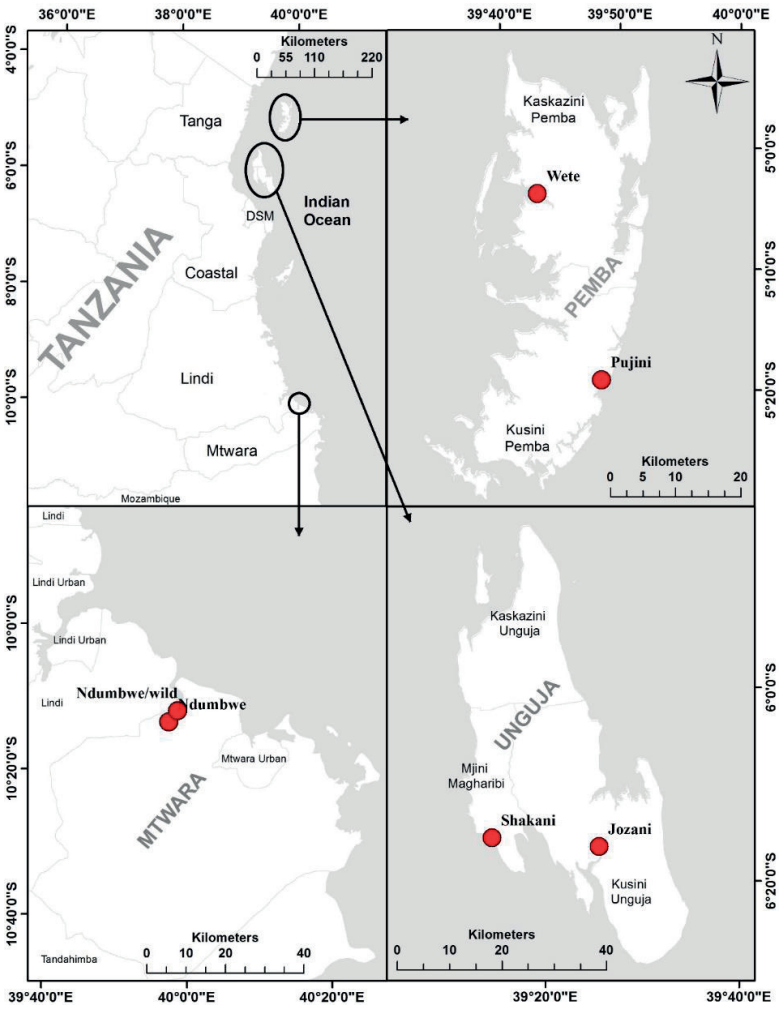

Figure 1: Map of Coast of Tanzania showing the sampling sites. ***Made by the author***.

The description of the locations is presented in the paragraph below.

Unguja Island

Unguja Island is one of the two main Zanzibar islands situated in the Tanzanian part of the Western Indian Ocean. Unguja is known for its touristic destination and the production of seaweed and spices. It has a population of 896,455 and farming is the main activity (NBS, 2015). It has a small-scale rice production. The samples were collected from two locations in Unguja; Shakani situated 6°16'30'' south of the Zanzibar Stone Town and Jozani situated at 6°15'15'' south east of the Zanzibar Stone Town.

Shakani is a vacated quarry area which was used for extraction of stones for building purpose. The holes left after quarry digging are filled with seawater seeping from the ocean, and are now used as ponds. They are stocked with different types of marine organisms including sea turtles, barracudas and milkfish for recreational purposes. The surrounding area has tree shrubs on the landward side and mangrove stand on the seaside. A total of 11 milkfish were collected from one of the ponds (*Fig 2A*).

Jozani is located within Jozani Forest reserve, which is famous for its red colobus monkeys. Jozani ponds are owned by the group of villagers for tourist activities. The ponds are concrete bordered by mangrove forest on the south and terrestrial bushes on the north. The main economic activity is tourism but there are some ongoing agricultural activities outside the reserve. The ponds are connected to the ocean by a channel which is used for filling the ponds with seawater during spring tides. A total of 20 milkfish were collected from this site (*Fig 2B*).

Fig 2: Fish sample collection. A: From Shakani ponds. B: From Jozani pond

Pemba Island

Pemba Island is situated north of Unguja and is the second biggest island in Tanzania (Fig. 1). The island is well known for clove production and rice farming. It has a population of 406,848 (NBS, 2015). In Pemba, milkfish were collected from ponds in Pujini situated at 5°19'11.08''S, 39°48'28.85''E about 10 km from Chake Chake town. The ponds are located on salt flats behind the mangroves near to salt pans (*Fig 3A*). The economic activities in the vicinity of the area include salt extraction activities, small scale agriculture and animal husbandry. Samples of mullets, collected from the Indian Ocean, were bought from the fisherfolks at the landing site in Wete town situated at 5°03'44.25''S, 39°43'6.33''E, 30 km from Chake Chake town. Wete is one of the highly populated and busiest regions in Pemba with a population estimate of 97,246 (NBS-OCGS, 2013). A total of 30 milkfish and 20 mullets were collected from Pemba

Mtwara

Mtwara is located on mainland in southern Tanzania, bordering Mozambique. It is famous for cashew nuts production, oil/gas industries and a recently built and operating cement industry. It has a population of 1,270,854 with the majority being farmers (NBS, 2015). Sampling was conducted in Mtwara rural district at Ndumbwe village from the Tujifunze fish farmers group, which is among the 27 groups of farmers managed by Mtwara fish farmers' association (UWASA). The ponds are situated on salt flats behind the mangrove forest (*Fig*

3B). The ponds are located at 10°13'25.99''S, 39°57'32.66''E about 2 km from the main road and 30 km from Mtwara town. The main economic activity around the ponds is agriculture. A total of 30 milkfish were collected from the ponds and 10 fish (eight milkfish and two mullets) were bought from the fisherfolks who fished in the nearby Ocean.

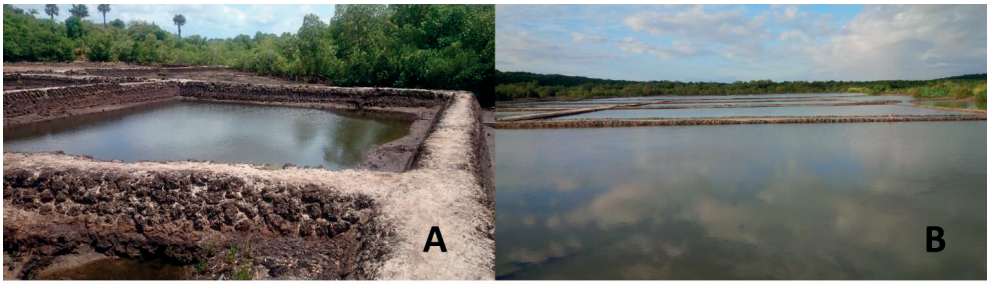

Fig 3: Milkfish ponds behind the mangrove forest. A: In Pemba. B: In Mtwara

3.2 Species description

Milkfish (*Chanos chanos*, Forsskål 1775)

Milkfish is one of the most important cultured brackish water food fish in Southeast Asia with Philippines and Indonesia producing more than 1 million tonnes annually (FAO, 2014; Garcia, 1990). It is the only member of the family Chanidae in the order Gonorychiformes. Milkfish occur near the coast and islands in the Indo-Pacific region and usually their distribution coincides with clear, shallow coral reef and mangrove areas. Milkfish are omnivorous fishes that feed on a range of foods from copepods, diatoms, detritus and zooplankton, (Bagarinao, 1991; Garcia, 1990; Lee et al., 1986). They are highly tolerant species, can thrive and grow well in temperature between 8.5 to 42.7^{o}C and salinity between 0 to 70 depending on their growth stage (Bagarinao, 1991; Garcia, 1990). Due to the tolerance of milkfish to environmental changes, they have been reared in earthen ponds in commercial fish farms. The market size in most farms is between six months to one year when they attain approximate 1 kg. In Tanzania, milkfish was caught as a bycatch in salt production sites (Sobo, 2013). It became introduced in experimental farms by Institute of Marine Sciences (IMS) from 1996-2003 and introduced to the farmers in 2004 (Mmochi and

Mwandya, 2003). Milkfish production in Tanzania has increased since 2007 (Sobo, 2013). It is now a potential marine aquaculture species in the coasts of Tanzania with more than 1300 farmers engaged in its production, and an annual production estimate of 231 tons (MALF, 2016).

Mullet (*Mugil cephalus*, Linnaeus 1758)

Mullet is an important food fish species in the Mugillidae family. It is found in coastal tropical and subtropical waters, it is a diurnal coastal specie that enters estuaries and rivers. It usually schools over sand and mud areas and feed on zooplanktons, detritus, diatoms and small vertebrates and invertebrates (Saleh, 2008). It is a euryhaline specie, can acclimatize to different levels of salinity and can tolerate temperatures between 8 to 24^{o}C. Mullet grows up to the maximum of 100 cm and attain maturity at the age of 2 years and above. It is similar to milkfish in term of feeding, habitat and they are on the same trophic level in the food chain (Saleh, 2008; Schmittou, 1973). Due to the similarity with milkfish, mullet can be cultured in polyculture with milkfish to increase profit in the fish farms (Mirera, 2011).

Tilapia (*Oreochromis niloticus*, Linnaeus 1758)

Nile tilapia (*Oreochromis niloticus*) is a cichlid fish belonging to a cichlidae family in the order cichliformes. It is a tropical fresh water fish that prefers to live in shallow water but can survive to a depth up to 20m (FAO, 2010). Tilapia migrates within fresh water but can exist in a variety of freshwater and brackish habitats. Its optimal living temperature is from 31-36 °C but can survive in temperatures between 12-42 °C (FAO, 2010). It is omnivorous, feeding on phytoplankton, periphyton mats, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus (FAO, 2010). They reach sexual maturity in the pond at the age of 5-6 months, can live longer than 10 years and reach weights beyond 5kg (FAO, 2010). Tilapia is an important fish in aquaculture. It is the second most intensively farmed species in the world. The most produced tilapia is Nile tilapia (FAO, 2018). Nile tilapia, originally from African fresh waters, was exported and distributed to other countries in the 1940s and 1950s for fish farming purposes (FAO, 2010; Modadugu and O. Acosta, 2004). Today China is the leading country in the production of tilapia worldwide, its production in 2017 stands at about 1.8 million metric tons (USDA, 2017).

3.3 Sample Collection

Sampling for papers I and II was done from dry season in January to dry-wet transitional period in April 2016. A total of 121 fish were collected. Fish were obtained from the fish farms and from the ocean. Samples of fish from the fish farms were obtained by using fishing nets and scoop nets. Samples from the ocean were purchased from the fisherfolks. Subsample collection from the fish was conducted on site except for one site (Shakani). Due to the lack of the suitable shade from the sun at Shakani, fish had to be collected and transported live in the plastic containers to the laboratory at IMS for subsample collection.

Subsample collection was done after examining and taking biometric information of the fish, which include weight, length and girth. After measurements every fish was dissected on its own aluminium foil for subsample collection. The livers were sampled and put in 10mL labelled plastic vials; muscles were wrapped in labelled aluminium foil and immediately transferred into the cool box with ice. To avoid cross contamination cleaning of the dissecting and measurement tools with ethanol was done between each fish subsampling. The collected samples were transferred to IMS and kept in a freezer at -20°C. During transport to Norway the samples were kept frozen in the icebox with ice packs, and after the arrival to Norway the samples were kept in a freezer at -20°C until analysis.

Sampling of fish for paper III was done in March 2017. Samples were purchased frozen in a paper box from the fish trading industry. The box was brought to a subsampling station and the fish was thawed before subsampling. Before subsampling the biometric information including weight and length were measured. A portion of the dorsal muscle was sampled from every fish after removing the skin. The muscle samples were wrapped in a labelled aluminium foil and immediately frozen at -20°C until transportation to Norway for analysis. During transportation the samples were kept frozen and upon arrival they were stored at -20°C until analysis.

3.4 Sample analysis

Analysis of samples for contaminants was conducted in three different laboratories. The analysis of POPs for papers I and III was done at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences, Campus Oslo, Norway (MT-lab). The MT-lab is accredited for analysing chemicals in biological samples by the Norwegian accreditation according to the requirement of the NS-EN ISO/IEC 17025 (TEST 137). The laboratory has therefore strict routines of ensuring the quality control and quality assurance of the analysis undertaken by including one blind sample of non-spiked sample, two recoveries of spiked sample and three procedural blanks of solvents in the analytical series. The analytical quality of the laboratory is approved by participating in several inter-laboratory and international ring test and by including Certified Reference Material and the laboratory's own reference material in the analytical series.

The analysis for heavy metals in paper II were done partly at the laboratory of the Central Veterinary Research Institute in Zambia (CVRI) and partly 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. The analysis of heavy metals in paper III were done at Laboratory at MINA, NMBU, Norway. The CVRI laboratory is accredited by the Southern African Development Community Accreditation Services (SADCAS). The laboratory has an established standard operating procedure (S.O.P) for heavy metals analysis in solid and liquid samples of animal and plant origin. The laboratory quality control and assurance include intra-analyst comparisons (IAC) at least twice per year, inclusion of standard controls each time an analysis is made (2 nitric acid blank and 1 reagent blank), participating in Proficiency Testing and Inter-Laboratory Comparisons at least once per year and undertake verification of instrumental performance using certified reference material. The MINA laboratory used the DORM-3 (Fish Protein Certified Reference Material for Trace Metals) from National Research Council Canada for quality assurance. Schematic overviews of the methods used in this study are given in Fig. 3 and 4. Details are described in papers I, II and III.

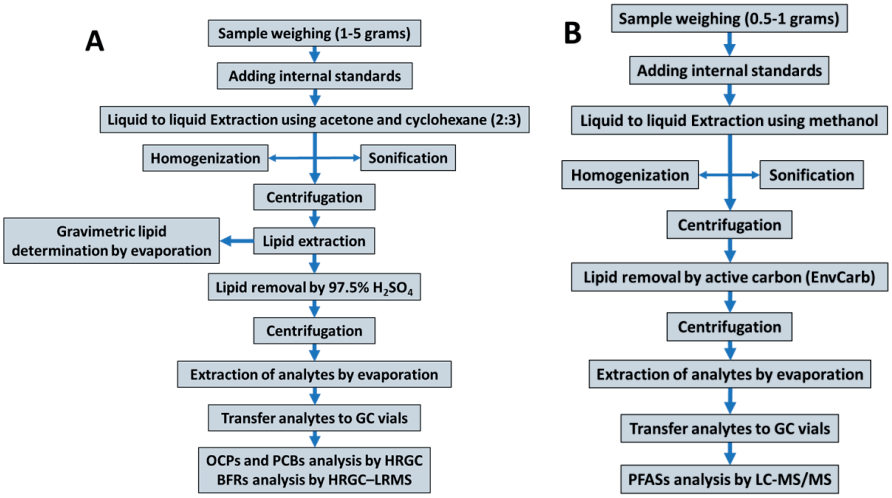

Fig.4: Schematic overview of the method for A: OCPs, PCBs, BFRs. B: PFASs analyses. Details are described in paper I and III. ***Adopted from Müller, (2017) modified by the author of this study.***

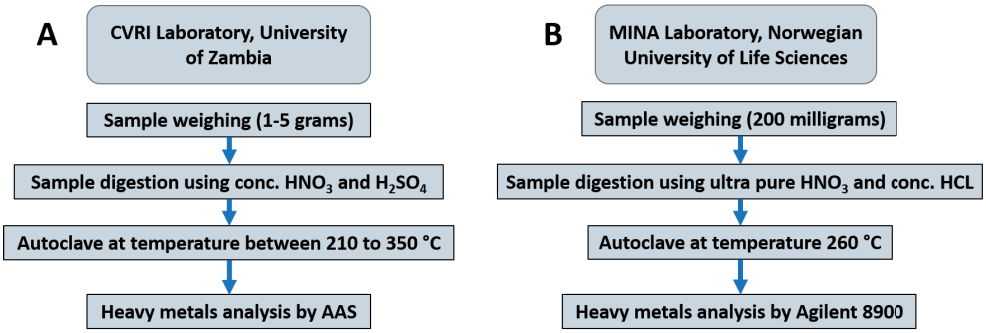

Fig.5: Schematic overview of the method for heavy metals analyses in A: Zambia and B: Norway ***Details are described in paper II and III. Figure made by the author of this study***

3.5 Ethical requirements

Fish farmers and local authorities were informed about the aim of this study, and acquisition of fish samples from the farmers was done upon their agreement and presence. 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 in Tanzania and The Norwegian Food Safety Authority

3.6 Statistical analysis

After obtaining data from the field and laboratory, all dataset for all studies were organized in Microsoft Excel® 2016 for windows. All statistical analyses were performed using JMP 11 (SAS Institute Inc., Cary, NC, USA) and Stata 14 for windows (Stata Corp® LLC, College Station, TX). The variables were subjected to normality test by using Shapiro-Wilk test, as all data were far from normal distribution even after log transformation, the non-parametric Kruskal Wallis Test (paper I) and Mann Whitney Test (paper II) were used for studying significance differences. Comparison between means was done by using Tukey test. Spearman rank correlation was used to assess the correlation between variables (papers I and II). Due to no replicate in paper III only the descriptive statistics were used. The uncertainties for replacing <LOD with either ½ LOD or 0 were tested. Due to higher uncertainties when replacing <LOD with ½ LOD, we chose 0 instead. The analytes with the detection >LOD in more than 60% of the analysed fish were reported in mean, median and range (paper I) and median, range and percentile (paper III) and included further in statistical analysis. The analytes with detection <LOD in less than 60% of the analysed fish were reported in range only. For statistical differences the *p-value* was set at 0.05.

4 SUMMARY OF THE PAPERS

Paper I

Occurrence and levels of persistent organic pollutants (POPs) in farmed and wild marine fish from Tanzania. A pilot study.

Eliezer Brown Mwakalapa, Aviti John Mmochi, Mette Helen Bjorge Müller, Robinson Hammerthon Mdegela, Jan Ludvig Lyche, Anuschka Polder

Chemosphere. 2018 191:438-449. doi.org/10.1016/j.chemosphere.2017.09.121

In 2016, farmed and wild milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) from Tanzania mainland (Mtwara) and Zanzibar islands (Pemba and Unguja) were collected for analyses of persistent organic pollutants (POPs). Fish livers were analysed for organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs). Muscle tissue was used for analyses of perfluoroalkyl substances (PFASs). The major contaminant was *p,p'*-DDE. The highest *p,p'*-DDE concentration was found in wild milkfish from Mtwara (715.27 ng/g lipid weight (lw)). This was 572 times higher than the maximum level detected in farmed milkfish from the same area. The ratios of *p,p'*-DDE/*p,p'*-DDT in wild milkfish and mullet from Mtwara and Pemba indicate historical use of DDT. In contrast, ratios in farmed milkfish from Unguja and Mtwara, suggest recent use. The levels of HCB, HCHs and *trans*-nonachlor were low. $\sum_{10}$PCBs levels were low, ranging from <LOD to 8.13 ng/g lw with the highest mean level found in farmed milkfish from Shakani, Unguja (3.94 ng/g lw). The PCB pattern was dominated by PCB -153 > -180> -138. PBDEs were detected in low and varying levels in all locations. BDE-47 was the dominating congener, and the highest level was found in farmed milkfish from Jozani (1.55 ng/g lw). HBCDD was only detected in wild mullet from Pemba at a level of 16.93 ng/g lw. PFAS was not detected in any of the samples. POP levels differed between geographic areas and between farmed and wild fish. Human activities seem to influence levels on PCBs and PBDEs on Unguja.

Paper II

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.

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Chemosphere, submitted 16 November 2018.

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 the maximum levels (ML) set by FAO/WHO (0. 3 mg/kg ww) in 100% of the analysed fish. Other metals were below the respective MLs. Concentrations of Pb were higher in wild fish than in farmed fish, implying different sources. Cd concentrations were generally low, but highest in wild mullet muscle from Pemba (0.02 mg/kg ww). 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 were 40 to 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. However, the detection of Pb in concentrations above ML in all fish suggest that Pb may affect the health of fish and other water living organisms in the area. Future investigations should include regularly monitoring of heavy metals and POPs 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.

Paper III

Concentration and patterns of persistent organic pollutants (POPs) and heavy metals residues in imported tilapia (*Oreochromis niloticus)* in Tanzania. A potential human health risk assessment.

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Manuscript

Fish is an important part in the diet for people in Tanzania. The high price of Tanzanian tilapia and insufficient production has led to increased import from China. In 2017, imported tilapia from China were collected and analysed for POPs and heavy metals. Generally, the concentrations of POPs and heavy metals were relatively low. However, the levels and patterns of compounds in individual fish were varying, suggesting that tilapia from the same package were from different locations in China. This was further confirmed by the differences in percent contribution of OCPs, PCBs and PBDEs to the sum of POPs between individual fish. The dominating POP was *p,p'*-DDE and its highest concentration was 5.99 ng/g wet weight. The ratio of *p,p'*-DDE to *p,p'*-DDT indicated both historic and recent use of DDT in the Chinese environment. HCB, HCHs and mirex were detected in more than 85% of the samples. The PCB pattern was dominated by PCB-153> -138> -209. Finding of PCB-209 may suggest that imported goods can introduce PCB-209 in the Tanzanian food web. HBCDD was the dominating BFR. For lifetime exposure, no potential health risks were observed for any POPs or heavy metals. However, the 95th percentile EWI for DL-PCBs: PCB-118 and -105 exceeded the recent set TWI (2 pgTEQ/kg bw/week), suggesting potential health risk to high fish consumers in Tanzanian adults. In addition, this study found the high contribution of As to the hazard index of concern. In general, it is important to take into consideration the positive effects of a varied diet that includes fish. For traceability it is recommended that future monitoring studies include individual samples.

5 DISCUSSION

5.1 Methodological consideration

5.1.1 Choice of samples and sampling locations

Samples were collected from Mtwara in Tanzania mainland and Unguja and Pemba from Zanzibar islands. The sites were chosen due to their potential in marine aquaculture operation and the availability of active commercial farms. Sampling was done from dry season in January to dry-wet transitional period in April 2016. Obtaining fish samples from the farms was easy because of the accessibility and availability. Fish were caught by using fishing nets. The difficulty was in obtaining fish from the ocean. Milkfish and mullets have migratory behaviour and therefore it was not easy to catch the specific species. Arrangements were made in advance with fisherfolks in the intended area and to give a call when they got milkfish and mullets caught. These arrangements made it easier to obtain fish rather that wasting time fishing the migratory fishes.

5.1.2 Choice of fish species

In this study fish were sampled from farms as well as from the nearby ocean. Milkfish and mullet were chosen because they are the most cultured marine fish species in the coast of Tanzania. The two species are omnivores and they have been cultured together in an integrated system to maximize profit since they are in the same trophic levels and technically have similar behaviours. They are mainly cultured in earthen ponds behind the mangroves in mudflats.

5.2 Analytical methods

5.2.1 Extraction and detection of analytes

5.2.1.1 POPs

POPs were extracted by liquid- liquid extraction using cyclohexane and acetone. The method was based on Brevik, (1972) modified by Polder et al., (2008b). Adding of standards and

solvents before homogenizing secures an efficient mixing-up of the standards and extraction of the analytes. Concentrating the extracts using the Zymark® evaporator with the blow of nitrogen gas saved time and increased the analyte concentration. Clean-up was performed two times using high concentrated sulphuric acid for removing all the fats to ensure no impurities. The top layer with the clean extracts is then transferred to a tube for final concentration. The use of sulphuric acid is challenging because if acids are injected in the GC, the GC-column and the detector will be destroyed. A small volume of the extract is therefore checked on a pH paper to detect possible acid content. Detection of the analytes were performed using HRGC-MS for OCPs and PCBs, HRGC-LRMS for BFRs and GC MS/MS for the PFASs. Good recoveries were obtained, few analytes had very high recoveries, but they were corrected prior to data analysis.

5.2.2.2 Heavy metals

Heavy metals were analysed in different laboratories. The idea of choosing different laboratories was to fulfil the concept of capacity building for laboratories in developing countries. For paper II, some of the heavy metals were analysed at the laboratory in Zambia and Hg was analysed in Norway. The choice of the laboratory was based on their competence but also their established method.

5.3 Ethical consideration

In all aspects of conducting research in Tanzania and exporting samples, permission is required to proceed. Therefore, a request must be submitted to the responsible body for granting permission and introducing a researcher to the local areas where the research will be carried out. This study was given permission to undertake research in the selected sites by the management of the Institute of Marine Sciences of the University of Dar es Salaam. For avoiding conflicts of interest, local authorities and fish farm owners were informed about the intention of the research and sampling was conducted upon farmers' consent and in their presence.

5.4 Levels and distribution of POPs and heavy metals farmed and wild fish

This study revealed the variation of POPs and heavy metals between sampling location indicating various exposure sources in Tanzania. DDTs and Pb were the dominating compounds in farmed and wild milkfish and mullets (paper I and II). The results further revealed that wild fish are more contaminated than farmed fish (paper I and II). This is important for food security in the country like Tanzania where there is insufficient fish for consumption. Food production through aquaculture can therefore be encouraged. DDT can be degraded to its metabolites, DDD and DDE. DDE is very stable, toxic and has longer half-life than DDT and DDD. Thus, we used the ratio between *p,p'*-DDE and *p,p'*-DDT levels to assess whether the fish were contaminated from recent or historical of use of DDT. The variation of *p,p'*-DDE/ *p,p'*-DDT ratios from low to high reported in paper I indicated that marine fish from Tanzania are exposed to both historic and recent use of DDT. This estimate is similar to what has been reported in the previous study undertaken in fish from Tanzanian lakes (Polder et al., 2014). This study showed widespread distribution of HCB and HCHs in coastal areas (paper I). However, due to significant higher levels in Unguja, airport, industries and human activities were suggested as the possible active point source of HCB. This calls for further monitoring of OCP in the country. Other OCPs in reported in paper I were in low levels indicating background levels and positive results of their ban and measures taken.

PCB levels were relatively low but were detected in fish from all study sites, indicating widespread distribution in the Tanzanian environment (paper I). However, the higher levels of PCBs in Unguja and different pattern of PCBs in this study compared to previous studies (Polder et al., 2014) suggest different sources of PCB such as airport emission and human activities. Tanzania has never produced PCBs but has imported products containing PCBs such as transformers, capacitors, lubricants paints, glue and carbonless paper (IPEN, 2005). The present study showed that PCB-153 was the dominating PCB congener.

BFRs were detected in all study sites indicating their ubiquitously spread in the Tanzanian environment (Paper I). The varying level and pattern of PBDEs in the present study and their

detection in fish from lakes, free range chicken and human breast milk in Tanzania (Müller et al., 2016; Polder et al., 2016, 2014), suggested various BFR sources such as burning of consumer products and e-waste, emissions from industries and airport. The domination of BDE-47 found in the present study may be a result of using consumer products with penta-mixtures of BDEs (Asante et al., 2013) and via debromination of higher BDE (La Guardia et al., 2007; Stapleton et al., 2006). HBCDD was detected in only four fish from one study site. Similar individual and scarce detecting frequency were reported earlier in fish and human milk (Müller et al., 2016; Polder et al., 2014) indicating low contamination of HBCDD in the Tanzanian environment. However future monitoring of this compound is advised due to increasing importation of consumer products.

PFASs are not well documented in African countries such as Tanzania. Their low levels <LOD in fish in the present study (paper I) suggest low levels in the environment and less active potential sources. However, the detection of PFASs in maternal blood (Müller et al., 2018) indicate the presence in the Tanzanian environment and the levels may increase in the future due to expected increased use of consumer products containing PFAS compounds.

The detection of Pb in all fish and all study sites reported in paper II suggests a wide distribution of this heavy metal in the Tanzanian environment. Different exposure routes of Pb are suggested in the present study, including ocean currents, historic use of leaded gasoline, and leakage from industrial products such as leaded paint.

5.5 Pattern and varying levels of POPs and heavy metals imported fish

This study is the first to investigate the variation in the levels of POPs and heavy metals in imported tilapia from China (paper III), purchased in Tanzania. Although the analysed fish were packed in one box, the level and pattern of POPs and heavy metals differed between individual fish. This finding suggested that the fish in the pack were not from the same location, thus showing to have poor traceability. Traceability is recommended in food safety, it enables proper management of contamination (FAO/WTO, 2017; Moretti et al., 2003).

DDTs were the dominant compounds and the *p,p'*-DDE/*p,p'*-DDT indicated both the recent and historic use of DDT in the Chinese environment. The study also found residues of mirex and HCH and were suggested from historic use.

To our knowledge, this is the first study to report PCB-209 in import fish in Tanzania. PCB-209 has been detected in China as a by-product from industries and waste water treatment (Huo et al., 2017). The detected of PCB-209 (paper III) in the Chinese imported fish in Tanzania may be a pathway for this compound to the Tanzanian food web. Additionally, this study found that HBCDD was the most abundant BFR in Chinese imported fish. These results call for proper monitoring of imported goods. In Tanzania, BDE-47 dominated the PBDE pattern in the analysed fish (paper I). In contrary BDE-209 was dominant in the fish from China where it is still produced and used (Zhang et al., 2017). The study suggest that the importation of Chinese tilapia and other commodities is a connecting pathway for BDE-209 and can increase the levels of lower brominated BDEs in the Tanzanian environment due to debromination.

The toxic As was the second dominant heavy metal after Al in the imported tilapia (paper III). Tanzanians are exposed to As mostly through drinking water (Kassenga and Mato, 2008). Therefore, imported fish from China may act as additional exposure route of As to the Tanzania general population.

5.6 Health risk related to levels of POPs and heavy metals

Exposure of fish to persistent organic pollutants and heavy metals can have adverse health effects to humans and fish. The present study assessed the potential health risk to humans and fish using the established guidance.

5.6.1 Comparison to maximum residue limits (MRLs) and maximum limits (MLs)

In order to verify the safety of fish for trade and consumption, MRLs for POPs and MLs for heavy metals have been established by various organisation such as FAO, WHO and EU. Food items containing POPs and heavy metals, which are lower than the threshold levels, are

regarded as safe for trade and consumption. In the present study all the POP concentrations were below the recommended MRLs set by FAO/WHO and EU (papers I and III). The heavy metal concentration from the imported tilapia from China were all below the MLs set by FAO/WHO (paper III). In paper II, Pb concentrations in fish muscles of farmed and wild Tanzanian were all above the ML (0.3 mg/kg ww) in all sites, whereas the other metals were below the MLs. These findings suggest that the farmed and wild milkfish from Tanzania may not be safe for human consumption and not fulfilling international standards.

5.6.2 Dietary intake of POPs and heavy metals

Diet is considered the main route of exposure and contributor to body burden of POPs and heavy metals in humans. In the present study, the dietary intake of POPs and heavy metals from consumption of fish were assessed. The assessment revealed that consumption of farmed and wild milkfish and mullet (paper II) and imported tilapia (paper III) will not pose non-carcinogenic health risk from heavy metals over a lifetime. However, the 53% contribution of As from Chinese imported tilapia (paper III) to the hazard quotient is of concern. USEPA, (1986) established an acceptable lifetime cancer risk level range ($1x10^{-6}$ to $1x10^{-4}$) for As. The estimated cancer risk for As (paper III) was 2 times higher than the acceptable range for both the average and higher consumers, indicating the potential carcinogenic effect from consuming the studied fish from China.

Dietary intake for all POPs revealed no health risk to human except for DL-PCBs in paper III. The dietary intake is calculated on weekly basis and compared to TEQs set by EFSA. Recently, in November 2018, EFSA established new values for the tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs (2 pgTEQ/kg bw week) which is seven times lower than the previous one (14 pgTEQ/kg bw week). The study indicated that consumption of imported fish from China can pose health risk from DL-PCB-118 and -105 for both average and higher consumer (paper III) when compared to current TWI. However, when comparing to the previous TWI no health risk will be posed to the consumers.

5.6.3 Possible health risk of POPs and heavy metals to fish

The EU Water Framework Directive (WFD) has established environmental quality standards (EQSs) for priority substances in biota including fish aiming to protect fish, human and environment from priority hazardous substances (EC, 2013). The present study compared the levels of Hg in fish muscle in paper II and HCB, PCB-118, PBDE, and HBCDD (not published) to threshold EQS_{Biota} (*Fig 6*). The concentration of Hg exceeded the EQS_{Biota} in 22% of the farmed fish from Jozani. This suggest that the fish from Jozani may be polluted by Hg in levels that can affect the fish health (paper II). However, data showed that not all fish exceeded the EQS_{Biota}, this could be due to different fish size and lipid percentage. The analysed fish from the other sites complied with the EQS_{Biota}. The concentrations of HCB, DL-PBD-118 and HBCDD were far below the EQS_{Biota} in fish from all sites and indicated no adverse health effect for the fish from these compounds at recent levels. However, the concentration of PBDE exceeded the EQS_{Biota} in 100% of the farmed milkfish (Jozani, Shakani, Mtwara), wild milkfish and mullet (Mtwara and Pemba) and 63% farmed milkfish from Pemba. This finding suggests the environment in Jozani, Shakani, Mtwara and Pemba ocean are significantly polluted with PBDEs in levels which can cause adverse health effect in fish.

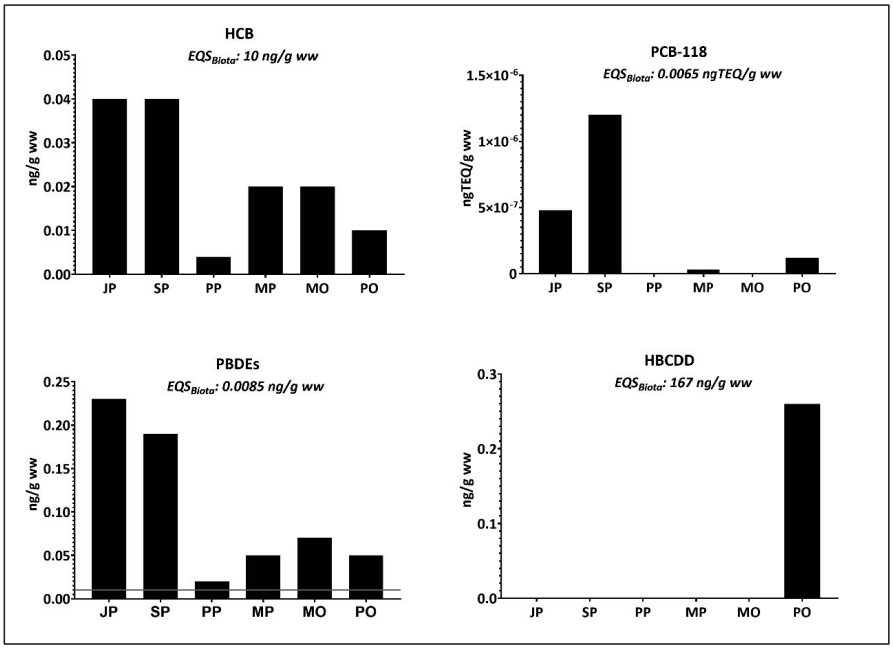

Fig 6: EQS$_{Biota}$ compliance for priority substances HCB, PCB-118, PBDE and HBCDD in farmed milkfish from Jozani (JP), Shakani (SP), Pemba (PP) and Mtwara (MP) and wild milkfish from Mtwara (MO) and wild mullet from Pemba (PO). The red colours indicate that the substance exceeded the threshold value indicated by a line.

5.7 Strength and limitations of the study

The strengths of this study are the collection of samples in locations that would not be accessible without local contact. This communication made it easier to arrange fieldwork and to rely on the obtained samples. Having knowledge of the locations also contributed to the success of this work.

The analysis of samples in this study were performed at high capacity and competent laboratories with skilful lab scientist. In addition, the large number of compounds analysed for gave a better background for a thorough assessment of health risks. The inclusion of the wide suite of contaminants also contributed to the knowledge of contaminant exposure in Tanzania.

Moreover, performing contaminant analysis especially for POPs at the same accredited laboratory, reduced the uncertainty of the results presented in this study. Furthermore, the North-South collaboration contributed much in the capacity building and transfer and sharing of knowledge.

The limitations of this study include the political disturbances which delayed sampling in the beginning of the study and the ability to access some sites and reduced the possibility to sample a large number of fish. Low number of sites and uneven number of fish species that were analysed, made it complex to make comparison between species and sites. Low sample number and the low amount of sample matrix were limiting analyses of other compounds. Moreover, limitations from the timing of sampling sometimes made it difficult to obtain the samples from the chosen sites. An additional problem was that fish farmers sometimes had other arrangements for delivering of the fish at the time when it was agreed on for the project to come and harvest.

Furthermore, some other chemicals of interest were not included in the analysis because of financial limitations, such as dioxins, but also PAHs, organophosphates and pyrethroids. There is an uncertainty about the diets of the Tanzanian population due to lack of diet surveys and reports especially on per capita consumption of fish for different population groups. This resulted to uncertainty on estimated dietary intake of contaminants and on health risk assessment. In the present study we used the higher per capita fish consumption estimated by FAO for East African populations, which might not be representative for the general population.

The assessment of risk of POPs and heavy metals to Tanzanians in this study did not include the contributions from other food types and environment such as indoor dust. Therefore, the level of exposure to POPs and heavy metals for a Tanzanian is likely to be higher than estimated. Thus, the potential health risks to Tanzanians may be higher than reported in this study.

6 MAIN CONCLUSIONS AND RECOMMENDATIONS

The main findings from this study were that levels of POPs and heavy metals in farmed and wild fish from Tanzania varied widely between locations indicating exposure from various sources, such as discharges from agricultural activities and sewage, emission from airports and improper disposal of consumer products. The levels of DDTs and Pb were higher in wild fish compared to farmed fish in Tanzania, suggesting that wild fish is more exposed to pollution that farmed fish. Hence aquaculture may be more emphasized. However, PCB levels were higher in farmed fish than in wild fish, possibly related to a local source. *p,p'*-DDE was the dominant contaminant in the Tanzanian fish and the ratio of *p,p'*-DDE to *p,p'*-DDT suggested both recent and historical use of DDT. HCB and γ-HCH were found in 100% of the samples of farmed and wild fish from all sites showing a wide distribution of the compounds in the Tanzanian environment. The study revealed Pb levels above MLs in 100% of the studied fish from Tanzania. The wide distribution of Pb in the Tanzanian environment may be caused due to historical use of leaded gasoline and its presence in various consumer products such as paint and batteries. In addition, the Indian Ocean current may contribute to high Pb levels in wild fish. The assessment of human health risk from the study of Tanzanian fish, suggest no adverse health effect from consuming farmed and wild milkfish and mullets from Tanzania.

In the imported fish from China, significant variation in levels and patterns of POPs and heavy metals were found between the individual fish, suggesting that tilapia from the same package were from different locations in China. The ratio of *p,p'*-DDE to *p,p'*-DDT suggested both recent and historical use of DDT in China. The study found low levels of mirex in the 94% of the fish samples and that the pattern of HCHs most likely reflected use of technical mixture of HCH in the past. The study also suggests that importing commodities from China may be a pathway of introducing compounds like PCB-209 and HBCDD to the Tanzanian environment. Assessment of human health risk suggested possible adverse health effect due to DL-PCB-118 and -105 and As from consumption of tilapia from China. Although the levels of As were relatively low in the tilapia, it is a highly toxic metal and showed a high contribution to the hazard index and the 95th percentile cancer risk levels were two times higher than the upper end of the cancer risk level range. The importation of tilapia from

China may be an additional pathway of exposure to As for humans in Tanzania, in addition to exposure from ground water sources. The detection of wide varying levels and patterns in individual fish from a package of imported tilapia from China, calls for inspectors to occasionally include monitoring of pollutants in individual fish rather that pooled samples.

The assessment of fish health risk in this study revealed that PBDEs and Hg may pose possible adverse health effects in fish and may pose ecological health risk and threatens biodiversity. Further monitoring studies are recommended. In addition, the study calls for regular monitoring of POPs and heavy metals in farmed and wild fish in Tanzania for further development of aquaculture and the welfare of the wild fish stock in the coastal waters.

Although this study revealed some potential concerns on levels of POPs and heavy metals in Tanzanian and Chinese fish, the benefits of fish consumption should be put in mind.

7 FUTURE PERSPECTIVE AND REFELECTIONS

Future perspectives and reflections are described below.

- The low ratio of *p,p'*-DDE/*p,p'*-DDT in some of the fish samples, indicated recent use of DDT in Tanzania which may suggest illegal use in agriculture besides combating malaria. Farmers should be given an opportunity for returning old pesticide stocks. In order to eliminate the illegal sources, existing regulations for use of DDT by the government and the Stockholm Convention should be followed up more strictly. This needs proper policy on pollutant management.

- Monitoring programs of key POPs and heavy metals should be conducted in the Tanzanian environment and in food products to trace sources and to implement effective measures for their management and assess health risks.

- For traceability purpose, the origin of imported fish should be known in detail and the inspection for harmful chemical contaminants in imported fish should include assessment of individual fish sample rather than pooled samples.

- Future investigations should include analyses of emerging contaminants such as HBCDD, substitutes for brominated flame retardants (BFRs) and fluorinated repellents (PFASs) and pesticides, like pyrethroids, carbamates and other toxicants like plasticizers (phenols, phthalates) not included in this study.

- A database should be developed to monitor levels and trends of POPs in Tanzania to understand the dynamics and implementations of restrictions and bans.

- National diet surveys should be performed to identify high-risk population groups together with monitoring programs on long-term health effects of pollutants to humans.

- Increase of public awareness on the possible health risks related to exposure to POPs, heavy metals and other toxic compounds in Tanzanian should be done through scientific workshops and symposiums, media coverage and education at schools.

- North-South collaboration should be strengthened in order to improve the scientific knowledge and building capacity on studies on pollutants, their distributions and associated health risks.

- Multidisciplinary research such as collaboration between public health, anthropology and natural science should be established in order to understand the social aspects of pollutants exposure to the population.

- Future studies should take into consideration the impact of climate change on the global and local distribution of POPs and heavy metals and direct focus on this area, because the influence of climate change on these pollutants is not fully understood.

- For fish, further studies on the levels and effects of pollutants in fish and their trophic transfer in the marine ecosystem should be encouraged.

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APPENDIX

Description of POPs and heavy meals

POPs

DDTs: Dichlorodiphenyltrichloroethane was synthesized in 1870s and its insecticidal properties were discovered in 1930s by Swiss biochemist Paul Hermann Müller (Walker et al., 2012). It was widely used during world war II as a vector control and thereafter extensively used for combatting malaria, pests in agriculture and ectoparasites in livestock worldwide (Stockholm Convention, 2008; Walker et al., 2012). Due to its potential toxic effects to wildlife and humans, DDT was banned in 1970s and included in the Stockholm Convention in 2004. Due to absence of equally effective and economic alternatives DDT is still allowed to be used as vector control as indoor residue spray (IRS) and insecticide treated nets (ITN) in malaria endemic regions especially in Africa (WHO, 2011). Nonetheless, illegal ongoing use in agriculture is suggested in some Asian and African countries such as Tanzania and China. DDT has five metabolites *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD and *p,p′*-DDT. *p,p′*-DDE is the principle DDT metabolite, it is more toxic and persistent than the parent DDT with the half-life of 10 years.

HCHs: Hexachlorocyclohexanes is one of the most widely used insecticide worldwide in soil application, treatment of seed, wood for preservation and household insects. HCH comes in two formulations; as a technical grade mixture containing α-HCH (55-80%), γ-HCH (8-15%), β-HCH (5-14%), δ-HCH (2-16%) isomers and as pure γ-HCH also known as lindane. Lindane was initially used in the USA and western Europe while technical HCH was used in Russia, Romania India and China. China still produces technical HCH for use as insecticide in agriculture, wood preservations and homes. β-HCH isomer is the most persistent isomer and more toxic than lindane, its half-life is approximated to be 7 years in human.

HCBs: Hexachlorobenzene is an organochlorine pesticide, which was introduced in agriculture as a fungicide for seed treatment in 1945. It is also a by-product in the production of large number of chlorinated compounds such as carbon tetrachloride, trichloroethylene and pentachlorobenzene and exists as an impurity in several pesticide formulations. It was

also used in the manufacture of fireworks, ammunition, and synthetic rubber. It is potentially bio-accumulative with the half-life of more than one year in biota.

CHLs: Chlordanes was produced as in insecticides in 1945 and used in agriculture and for the control of termites, ants and cockroaches. It consists of more than 120 components but the most common and important are *cis*-chlordane, *trans*-chlordane, *trans*-nonachlor, oxychlordane and heptachlor. CHL is highly persistent with the half-life of about 4 years.

Mirex: Mirex was introduced in 1959 and used as an insecticide against ants and termites and fire retardant for plastic rubber, paints, paper and electrical equipments. Mirex is highly persistent with the half-life of approximately 10 years.

PCBs: Polychlorinated biphenyls are synthetic compounds consisting two benzene rings linked by a single carbon to carbon bond. The benzene rings have some or all the hydrogen atoms substituted by the chlorine atoms. Theoretically there are 209 individual PCB congeners that can be formed depending on the number and position of chlorine atoms (ATSDR, 2014). PCBs can be categorized by degree of chlorination. PCBs with the same number of chlorines are termed as 'homolog' such as a group of penta- hexa- and hepta-PCBs. Depending on the chlorine content, production process and origin, PCBs were used as complex, technical mixtures such as Aroclor, Clophen, Phenochlor, Kanechlor, Pyralene, Fenclor, Delor. PCBs were first manufactured commercially in 1929 and due to its stability and heat resistant nature was used in various products including dielectric, transformers, heat exchange fluids and building materials. Some PCB congeners with either one or no Cl atom in the *ortho* position are similar to dioxins in terms of physicochemical properties. They can bind the AhR receptor (EFSA, 2015) and are therefore referred to as dioxin-like PCBs (DL-PCBs), and hence their toxicity is expressed in toxic equivalency factors (TEFs). The toxicity of other PCB congeners is related to other mechanisms (USEPA, 2000).

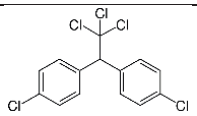
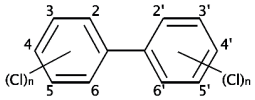
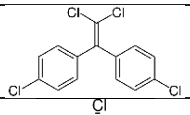
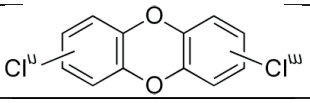
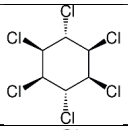
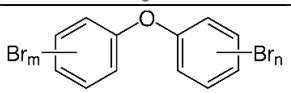
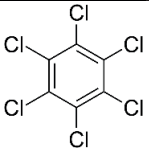
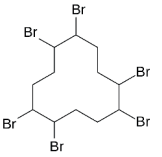
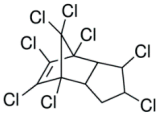
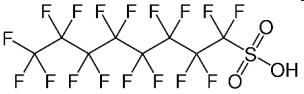
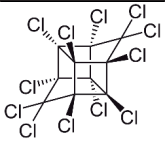
PCCDs/PCDFs: Dioxins are not manufactured but produced as a by-product of various industrial processes (Walker et al., 2012). PCCDs/PCDFs are extremely toxic to humans. The mechanism of toxicity of dioxins is associated with the interaction with a cytosolic protein, the aryl hydrocarbon receptor (AhR), which regulates the synthesis of a variety of proteins. The AhR receptor is present in many human tissues, including the lung, liver, placenta, and

lymphocytes. Because of their toxicity, individual dioxins have been assigned a TEF. The factor is related to the most toxic dioxin compound, the 2,3,7,8-tetrachlorodibenzo-p-dioxin with the TEF of 1 (one).

BFRs: Brominated flame retardants have been commercially manufactured since 1960s and added in consumer products for fire safety to enhance the flame-retardancy properties. Poly brominated diphenyl ether (PBDE), is a largest group of BFRs. Like PCBs, PBDEs have 209 congeners and are compounds of two benzene rings but in contrary linked by an ether and some or all of its hydrogen atoms are substituted by bromine. The commercial PBDEs were produced in three mixtures penta-BDE (BDE-28, -47, -99, -100), octa-BDE (BDE-153, -154, -183) and deca-BDE (BDE-206, -207, -208, -209). Hexabromocyclododecane (HBCDD) is another BFR most widely used as aliphatic cyclic additive, it is mainly as polystyrene in construction materials such as thermal insulation or molded foam packing, and textiles, such as household furniture and appliances. Other BFRs include pentabromotoluene (PBT), pentabromoethylbenzene (PBEB), Dibromopropyl tribromophenyl ether (DPTE) and hexabromobiphenyl (HBB).

PFASs: Perfluoroalkyl substances have been produced since 1949, they are compounds of carbon chains with at least one carbon fully bonded to fluorine atoms. They are highly persistent like other POPs, however they are less lipophilic. They have been used as repellent in various consumer products, food boxes, outdoor clothing, Gore-Tex, ski wax, furniture.

Chemical structures of POPs included in this study

DDT		PCB	
DDE		PCDD	
y-HCH		PBDE	
HCB		HBCDD	
CHL		PFAS	
Mirex		HBB	

Heavy metals

Fe: Iron is essential for haemoglobin formation (Yasmeen et al., 2016) responsible for oxygen transportation in the bodies of living organism (Saria, 2016) and important for many vital proteins and enzymes in living organisms (Jaishankar et al., 2014; Valko et al., 2005).

Co: Cobalt is essential in human as part of vitamin B_{12}. It has been used in technology industries such as rechargeable batteries, alloy and catalyst (Pourret and Faucon, 2016).

Cu: Copper is essential for metabolic activities in the body of living organisms. It is used in the enzymatic process in the production of haemoglobin (Magu et al., 2016). It is supplemented in the diet of farmed fish (CIESM, 2007).

Cr: Chromium is essential for the metabolism of carbohydrate (Tchounwou et al., 2012). The levels of Cr in the environments have been increased by various anthropogenic activities such as leather and textile, dying and printing industries and chromate production (Authman, 2015; Tchounwou et al., 2012). Cr has been used as wood preservatives and fungicides (Mdegela et al., 2009) in electroplating and fertilizers and used in pulp and paper production (Jaishankar et al., 2014).

Zn: Zinc is an important trace element and micronutrient that has a significant role in enzymatic actions, immune system and neurotransmission (Authman, 2015).

Ni: Nickel is released in to the environment by both natural and man-made sources (Authman, 2015). Ni is essential to many organisms at low concentration and becomes toxic when its concentration exceeds threshold levels.

Li: Lithium is an alkali metal, which exist naturally as mixture of isotopes. Lithium is used in various industrial application such as lithium batteries and air conditioners (Lenntech, 1998a). It has also been used in various medication for psychiatric conditions such as bipolar disorders (Sirois, 2003).

Se: Selenium is among the rare elements on earths and is mostly found in ground and surface waters. It is an essential element for normal growth and physiological functions of animals such as thyroid enzyme metabolism in spermatogenesis (Authman, 2015).

V: Vanadium is found naturally and is released by industrial emissions through coal and fuel burning (Authman, 2015). It is essential in regulating various enzymatic activities for metabolic changes.

Pb: Lead has no important biological role in the body of living organisms. It is among the most toxic heavy metals for living organisms including fish and human (Tchounwou et al., 2012). Its levels has been increased by various anthropogenic activities including burning of

fuel, mining, and manufacture of different commodities such as paints, batteries, ammunitions (Tchounwou et al., 2012) and use of Pb containing fuel (Luilo and Othman, 2009).

Hg: Mercury is one of the most toxic metals that is widespread in the environment. Hg levels are increasing in the environment due to use in fungicides chemicals and various industrial activities (ATSDR, 1999; Authman, 2015; Tchounwou et al., 2012) and mining (Bosch et al., 2016). Burning of fossil fuel and smelting or metal ores contributes significantly to atmospheric Hg.

Cd: Cadmium is a naturally occurring metal. It is used in various industrial productions such as production of alloys, pigments and batteries (Authman, 2015; Tchounwou et al., 2012).

As: Arsenic is a naturally occurring element widely distributed in soil and ground water. It is toxic element to animals including humans (Lenntech, 1998b). People can get exposed to arsenic via drinking water, air and food (ATSDR, 2007b). Arsenic compounds have been manufactured and used for several purposed including wood preservative, veterinary drugs in the treatment of worms and in human to treat bacterial infection such as amoebic dysentery (Tchounwou et al., 2012). It is still being used in herbicides, pesticides, fungicides and antimicrobial additives (ATSDR, 2007b; Bosch et al., 2016).

Al: Aluminium is among the most abundant heavy metal on the earths' surface. It has no known biological role in living organisms, hence considered as toxic. The elevated levels of Al in the environment is caused by its mining and processing (ATSDR, 2008).

ERRATA

Errata

Page	Line	Original text	Corrected text
7	19-20	A study by (Al-sawafi et al., 2017)	A study by Al-sawafi et al., (2017)
7	21	(Low and Higgs, 2015)	Low and Higgs, (2015)
8	17	paragraph	subchapters
9	11	EF	E_F
		ED	E_D
9	12	FIR	F_{IR}
9	13	RFD	R_{fD}
9	14	WAB	W_{AB}
9	14	TA	T_A
9	15	EF*ED	E_F*E_D
11	2	EQS	EQS_{BIOTA}
26	22	95^{th} EWI for DL-PCBs PCB-118	95^{th} percentile EWI for DL-PCBs: PCB-118
27	11	to give when	to give a call when
28	4	concerted	concentrated
29	4	in Tanzania DDTs	in Tanzania. DDTs
32	5	in all sites (paper II).	in all sites.
32	16	(USEPA, 1986)	USEPA, (1986)
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Appendix Heavy metals As		and inhuman	and in human

PAPERS I-III

I

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Occurrence and levels of persistent organic pollutants (POPs) in farmed and wild marine fish from Tanzania. A pilot study

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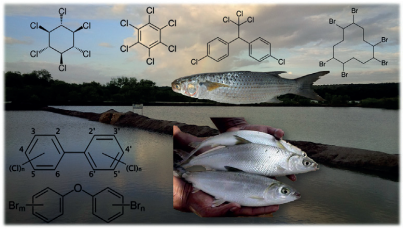
HIGHLIGHTS

- DDTs were the major POPs in farmed and wild milkfish and mullets.
- *p,p′*-DDE was 572 times higher in wild milkfish than in farmed milkfish from Mtwara.
- PCBs and PBDEs were low and in varying ranges in milkfish and mullets.
- HBCDD in mullet from Pemba warrant further research on its occurrence in the region.

GRAPHICAL ABSTRACT



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ABSTRACT

In 2016, farmed and wild milkfish (Chanos chanos) and mullet (Mugil cephalus) from Tanzania mainland (Mtwara) and Zanzibar islands (Pemba and Unguja) were collected for analyses of persistent organic pollutants (POPs). Fish livers were analysed for organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs). Muscle tissue was used for analyses of perfluoroalkyl substances (PFASs). The major contaminant was *p,p′*-DDE. The highest *p,p′*-DDE concentration was found in wild milkfish from Mtwara (715.27 ng/g lipid weight (lw)). This was 572 times higher than the maximum level detected in farmed milkfish from the same area. The ratios of *p,p′*-DDE/*p,p′*-DDT in wild milkfish and mullet from Mtwara and Pemba indicate historical use of DDT. In contrast, ratios in farmed milkfish from Unguja and Mtwara, suggest recent use. The levels of HCB, HCHs and *trans*-nonachlor were low. $\sum_{10}$PCBs levels were low, ranging from <LOD to 8.13 ng/g lw with the highest mean level found in farmed milkfish from Shakani, Unguja (3.94 ng/g lw). The PCB pattern was dominated by PCB -153 > -180> -138. PBDEs were detected in low and varying levels in all locations. BDE-47 was the dominating congener, and the highest level was found in farmed milkfish from Jozani (1.55 ng/g lw). HBCDD was only detected in wild mullet from Pemba at a level of 16.93 ng/g lw. PFAS was not detected in any of the samples. POP levels differed between geographic areas and between farmed and wild fish. Human activities seem to influence levels on PCBs and PBDEs on Unguja.

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1. Introduction

Persistent Organic Pollutants (POPs) are halogenated chemical substances that are characterised by high lipophilicity and chemical persistence. POPs accumulate in fatty tissues and biomagnify in the food chain (Lohmann et al., 2007). They are volatile and may undergo long range atmospheric transport and be found far from where they were used or manufactured (Polder et al., 2014; Wania and Mackay, 1993). Since 1940s POPs have been manufactured and used in a wide range of products such as pesticides, transformer oil, building materials, flame retardants, antifouling agents and coolants. They have also been unintentionally released as by-products in combustion processes (UNIDO, 2003). In aquatic ecosystems, POPs bioaccumulate in organic matter and aquatic organisms including fish (Walker et al., 2012). POPs, such as dichlorodiphenyltrichloroethane and metabolites (DDTs), polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs), have been documented to cause adverse health effects in animals and humans (Trollerud, 2013; Walker et al., 2012), such as egg shell thinning in marine birds (Bouwman et al., 2008; Ratcliffe, 1970), reproductive impairment in seals (Bergman, 2007) in whales (Béland et al., 1993) and endocrine disruption in fish (Berg et al., 2016). The Stockholm Convention, a global treaty for protecting humans and the environment against POPs contamination, has listed more than 20 POPs so far (Stockholm Convention, 2016). Tanzania ratified the Convention in 2004 and has a national implementation plan. Due to the hot and humid tropical climate in Tanzania, many pests and weeds threaten food production in agriculture. Thus, use of pesticides is inevitable. Several studies have documented the presence of POPs in the Tanzanian environment, including food and humans, due to discharges from agricultural activities, malaria control, waste disposals and obsolete stockpiles (Kariathi et al., 2016; Kishimba et al., 2004; Lema et al., 2014; Machiwa, 2010; Mtashobya and Nyambo, 2014; Müller et al., 2016, 2017; Mwevura, 2014; Nonga et al., 2011; Polder et al., 2014, 2016).

Fish represents a valuable source of proteins and nutrients for the general population and is of high importance for food security and economy in many countries (FAO, 2016). Therefore, aquaculture is rapidly expanding worldwide (FAO, 2014) including Tanzania, (Watengere et al., 2008). The main farmed fish species in Tanzania are Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarius gariepinus*), rainbow trout (*Oncorhynchus mykiss*) in fresh water, and milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) in marine waters (Rothuis et al., 2014). Whereas a few studies on POPs have been done in fresh water and marine fishes in Tanzania (Machiwa, 2010; Mdegela et al., 2009; Mwevura et al., 2002; Polder et al., 2014), no studies have been performed on POPs levels in farmed fish in Tanzania in comparison to POP levels in corresponding wild species.

The main objective of the present study was to assess the levels and occurrence of POPs in farmed and wild milkfish and mullet from Jozani and Shankani at Unguja, Mtwara, and Pemba Islands in Tanzania.

2. Materials and methods

2.1. Description of species and study sites

The samples of farmed fish were collected from fish farms on two Zanzibar Islands (Unguja and Pemba) and Mtwara region in Tanzania mainland (Fig. 1). Wild fish were collected in the ocean close to the respective fish farm sites. A full description of sampling sites and species is presented in Supplementary materials (Suppl) S 1. In short, milkfish (*Chanos chanos*, Forsskål, 1775) and mullet (*Mugil cephalus*, Linnaeus, 1758) are omnivorous fish species which are tolerant to wide ranges of temperatures and salinity and therefore suitable for culture. They have become important marine aquaculture species in the coast of Tanzania. Milkfish from Unguja Island were obtained from Jozani and Shakani ponds (Fig. 1). Jozani ponds are located near Jozani forest famous for colobus monkeys, while Shakani ponds are situated in quarry areas about 3 km from the airport. Milkfish from Pemba island were collected from ponds in Pujini village in Chake Chake town (Fig. 1). Wild mullet were bought from fisherman at Wete (capital of Pemba island). Farmed and wild milkfish from Mtwara were collected from the ponds and nearby ocean in Ndubwe village. Mtwara region in the southern part of Tanzania, is well-known for the production of cashew nuts, oil and gas, and cement (Fig. 1). The study intended to include analyses of fish feed from the fish farms for elucidating contaminant pathways, however, this was not possible.

2.2. Ethical clearance and permissions to conduct research

Permission to conduct this research in the selected sites was given by the management of Institute of Marine Sciences, University of Dar es Salaam. Local authorities and fish farm owners were informed about the research aims, and sampling was conducted upon farmers' consent. 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. Sampling and sample treatment

Sampling was done in Unguja, Pemba and Mtwara in January, March and April 2016, respectively. A total of 121 fish were obtained from both fish farms and the ocean. The fish were euthanized with a blow to the head. Dissection and collection of tissue samples were conducted on site except for Shakani site. Due to the high temperature (up to 40 °C) and absence of suitable shade at Shakani, live fish were transported in plastic containers to the laboratory at the Institute of Marine Sciences (IMS) for collection of tissue samples. Samples were immediately put on dry ice or in liquid nitrogen, depending on the following analysis, as described below.

Overview of the analysed samples and fish characteristics is presented in Table 1. Dissection took place on aluminium foil cleaned with ethanol. Liver samples were put in 10 mL labelled plastic vials; muscles were wrapped in labelled aluminium foil and put in a zipper bag, then immediately transferred into the cool box with ice. To avoid cross contamination, tools were cleaned with ethanol between each fish sampling. Samples were transferred to IMS and kept in a freezer at -20^{O}C. For sites that required over a day of transportation, samples were stored in a freezer until the traveling time; then transported in a cool box with ice to IMS and then stored in the freezer at -20^{O}C until transportation to Norway for analysis. During transportation to Norway the samples were kept frozen, and after the arrival to Norway the samples were stored in a freezer at -20^{O}C until analysis.

2.4. 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. Chemical analysis of the samples was conducted 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

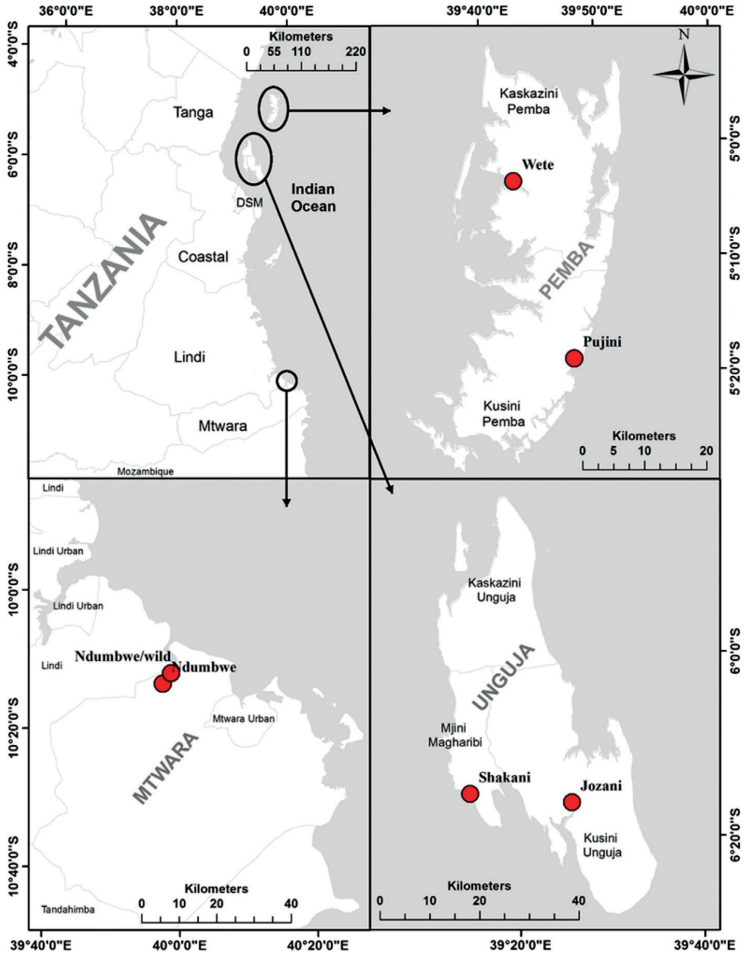

Fig. 1. A map of Tanzanian coasts showing the location of sampling sites.

Table 1
Sites and fish characteristics: Sampling time, salinity, mean and range of individual weight and length and number of individual samples of liver from farmed milkfish from Jozani and Shakani (Unguja), Mtwara and Pemba, wild milkfish from Mtwara and wild mullets from Pemba, Tanzania.

Site	Fish type	Sampling time	Salinity (ppt)	Mean weight (g)	Weight range (g)	Mean length (cm)	No of sample
Jozani ponds	Milkfish	Jan-16	36	662	413–826	44.0625	8
Shakani ponds	Milkfish	Jan-16	40	683.375	533–936	43.5	8
Pemba Ponds	Milkfish	Mar-16	25	211.875	196–226	29.4375	8
Pemba wild	Mullets	Mar-16	30	611.75	542–711	39.6357	8
Mtwara ponds	Milkfish	Apr-16	22	189.05	83.8–308.6	25.8125	8
Mtwara wild	Milkfish	Apr-16	29	107.55	59.2–185.2	21.957	7

the requirement of the NS-EN ISO/IEC 17025 (TEST 137). The fish liver samples were analysed for OCPs, PCBs and BFRs. Due to small sample seize of liver tissue, perfluoroalkyl substances (PFAS) were analysed in fish muscles.

2.4.1. Chemical analyses; sample extraction and clean up

2.4.1.1. OCPs, PCB, BFRs. From 121 fish, 48 fish liver samples were chosen based on their weights and the amount of subsample available. One sample was lost during sample preparation, leaving 47 fish liver samples for analyses for OCPs: hexachlorobenzene (HCB), α, β- and γ-hexachlorocyclohexanes (∑HCHs), oxychlordane, *trans*-chlordane, *cis*-chlordane and *trans*-nonachlor (∑CHLs), mirex, bis-2,2-(4-chlorophenyl)-1,1,1- trichloroethane (*p,p′*-DDT) and its metabolites *p,p′*-DDE, *p,p ′*-DDD and *o,p′*-DDT (∑DDTs); PCBs: PCB-28, -52, −74, −99, −101, −118, −138, −153, and −180 (Σ_{10}PCBs); BFRs such as polybrominated diphenyl ethers:

BDE-28, -47, −99, −100, −153, −154, −183, −206, −207, −208 and −209 ($\sum_{11}$PBDEs) and hexabromocyclododecane (HBCDD).

Before weighing, fish liver samples were macerated with scalpel and thoroughly mixed to obtain homogeneous samples. Approximately 0.5–1 g of homogenized fish liver was weighed into pre-cleaned glass centrifuge tubes. After weighing, 25 μL of internal standards PCB -29, −112 and −207 (1000 μg/mL) (Ultra-Scientific, RI, USA); 20 μL of BDE -77, −119, −181, and $^{13}C_{12}$-209, $^{13}C_{12}$-TBBP-A (500 μg/mL) (Cambridge Isotope Laboratories, Inc., MA, USA) were added in all the samples. Furthermore, 10 mL distilled water, 2 mL 6% sodium chloride (NaCl), 15 mL acetone and 20 mL cyclohexane were added. The mixture of sample tissue, standards and fluids were homogenized using Ultra Turax homogenizer (IKA Ultra-Turrax T25, IKA Laboratory Technology, Staufen, Germany). Thereafter followed by lipid extraction using ultrasonic homogenizer (Cole Parmer CPX 750, Vernon Hills IL, USA) The second extraction was done with 5 mL acetone and 10 mL cyclohexane. Separation of the lipid extract from the sample was done by centrifuging at 2095 × g for 10 min using Allegra X-12R Centrifuge (Beckman Coulter, Fullerton, CA, USA). The method for analysis used is based on Brevik (1978) with modification as described in Polder et al. (2008b). The lipid extract was concentrated by evaporation using the Zymark Turbo Vap II evaporator (Zymark Cooperation, Hopkinton, MA, USA) at 40 °C and thereafter lipid determination was done gravimetrically using 1 mL aliquot of fat extract. The clean-up of the remaining lipid extract was performed using 96% H_2SO_4 (Fluka Analytika, Sigma-Aldrich, St. Louis, USA). The final extracts were evaporated on a sand bath at 40 °C with the blow of N_2 and concentrated to a final volume of 0.5 mL before transferred into the 2 mL amber vials for GC analysis.

2.4.1.2. PFAS. Due to lack of liver tissue after analyses of OCPs, PCBs and BFRs, muscle was used as matrix for analysing PFASs. Out of 121 fishes, muscle of the same 47 fish were analysed for 2 PFSAs groups: perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS) and 6 PFCAs: perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), and perfluorotridecanoic acid (PFTrDA), summarized as Σ_8PFAS.

The muscle samples were finely chopped with a scalpel before weighing. Approximately 1 g was weighed into the plastic centrifuge tubes. 40 μL internal standards I.S PFAS Syrene (500 ng/mL) and 40 μL I.S PFAS Sulfonate (500 ng/mL) (Wellington Laboratories Inc, CANADA) were added in all the samples followed by the addition of 5 mL of methanol. After adding methanol, the mixture was homogenized using Ultra Turax T25 Homogenizer then further sonicated using Ultra Sonic processor. Extraction was done twice with methanol by shaking the sonicated samples in Vibrax VXR (IKA Werke GmbH & Co. KG, Germany) machine for 30 min followed by centrifugation at 2095 × g for 10 min. The extracts were concentrated by evaporation in a Turbo Vap LV evaporator at 40 °C before cleaning up the lipids with active carbon (Env Carb). After lipid clean-up, the extracts were concentrated again by evaporation to a final volume of 1 mL and then transferred into the LC-MS vials. During the analytical procedure sample extracts were not in contact with any glassware to avoid the possible adsorption of the analytes.

2.4.2. Instrumental analysis

2.4.2.1. Separation and detection of the POPs. OCPs, PCBs and BFRs were separated and detected using GC-MS methods, PFASs were separated and detected using LC-MS/MS. For all components, five- to eight-point linear calibration curves were used and calculations were done within the linear range for the component. OCPs and BFRs were monitored using negative chemical ionization (NCI) in selected ion monitoring (SIM), see list with target ions in Suppl S 1. 3.

2.4.2.2. OCPs and PCBs. Separation and detection of chlorinated compounds were performed on a HRGC (Agilent 6890 Series) coupled to a MS detector (Agilent 5975C Agilent Technologies) which was operated in negative chemical ionization (NCI) mode with selected ion monitoring (SIM) as described in Polder et al. (2014). The OC compounds were separated on a DB-5 MS column (J&W Scientific, Agilent Technologies) (60 m, 0.25 mm i.d., 0.25 μm film thickness). The temperature program was: 90 °C (3 min hold); 25 °C/min increase to 180 °C (2 min hold); 1.5 °C/min increase to 220 °C (2 min hold); and 3 °C/min increase to 275 °C (15 min hold) and 25 °C/min increase to 300 °C (1 min hold). The total run time was 70.6 min. The carrier gas was hydrogen (H_2) at a 1.3 mL/min constant flow. The injection volume was 2 μL.

2.4.2.3. BFRs. Detection of PBDEs (except from BDE-209) and HBCDD was performed on a HRGC–LRMS (Agilent 6890 Series; Agilent Technologies), equipped with an auto-sampler (Agilent 7683 Series; Agilent Technologies) and coupled to a MS detector (Agilent 5973 Network; Agilent Technologies) (Polder et al., 2014). In short, the separation and identification of the compounds were performed on a DB-5 MS column (30 m, 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific). The injection volume was 2 μL. For detection of BDE-209, extracts (10 μL) were injected on a GC–MS (Agilent 6890 Series/5973Network) configured with a programmable temperature vaporization (PTV) injector (Agilent Technologies). The separation and identification of BDE-209 were performed on a DB-5-MS column (10 m, 0.25 mm i.d., 0.10 μm film thickness; J&W Scientific, Agilent Technologies).

2.4.2.4. PFASs. Separation and detection of PFASs were conducted using the LC-MS/MS (API 3000; Applied bioscience, MDX SCIEX) composing of API 3000 triple-quadrupole mass spectrometer coupled to Agilent 1100 HPLC (Agilent Technologies), as described by Grønnestad et al. (2017). The injection volume was 10 μL.In short, the column characteristics include 2.1 mm inner diameter and 150 mm long, with a particle size of 3.5 μm (Agilent Eclipse Plus C18). The operation software was the Analyst version 1.6.

2.5. QA/QC

2.5.1. OCPs, PCBs, BFRs

Every analytical series included one blind sample of non-spiked salmon trout (*Salmo trutta*), two samples of spiked salmon trout for recovery, three procedural blanks of solvents and the laboratory's own reference material of the blubber of a harp seal (*Pagophilus groenlandicus*).The analytical quality was successfully approved by routinely analysing different Certified Reference Materials (CRMs). In addition, the laboratory successfully participated in Arctic Monitoring and Assessment Program (AMAP) ring test for PCBs, OCPs and PBDEs in human serum 2016, and Quasimeme 2016, round 1: QOR126BT, QOR127BT, QBC046BT, QBC047BT for OCs in fish muscle, fish liver and shellfish tissue inter-laboratory studies. The limits of detections (LOD) for individual analytes were defined as 3 times the noise level of each analyte. The LODs (ng/g ww) ranged from 0.001 to 0.061 for OCPs, 0.001 to 0.117 for PCBs and 0.001 to 0.196 for BFRs. The relative recoveries for OCPs were between 84 and 118%, for PCBs between 81 and 117% and for BFRs between 87 and 112%. The results above and below the limit (80–120%) were corrected for recovery.

2.5.2. PFASs

Every analytical series included one blind sample of non-spiked

Atlantic cod (*Gadus morhua*), two recoveries of spiked Atlantic cod and three blanks of solvent. The analytical quality of the method was assessed by including an inter-laboratory test (AMAP) in the analysis of samples. The LOD for PFAS ranged between 0.08 ng/g ww to 0.43 ng/g ww and the recoveries were between 97 and 125%.

2.5.3. Statistical data analysis

Data were organised in spread sheets (MS Excel, 2016). JMP 11 statistical software was used for further analysis. Compounds detected in less than 60% of the samples were only reported in range and not included in statistical analysis. The compounds which were detected in more than 60% of the samples were reported in mean, median and range and were included in further statistical analysis. The non-detects (nd) were treated as zero during analysis as the concentrations of POPs in general were very low. Shapiro-Wilk Test W was used to test for the distribution of the data. Since the data were not normally distributed even after transformation, a non-parametric Kruskal-Wallis test was used to test for differences among sites and Tukey test was used to identify the means that were different from each other. Spearman rank correlation was used to assess the correlation between variables. The difference between sites were considered statistically significant when $p<0.05$.

3. Results

3.1. Site and fish characteristics

Salinity varied among locations and is presented in Table 1. The mean weights of farmed milkfish from Jozani and Shakani were higher than the farmed and wild milkfish from Mtwara and farmed milkfish from Pemba (Table 1). The mean lipid percentage in fish liver ranged between 3.1% in farmed milkfish from Pemba and 11.4% in farmed milkfish from Shakani (Table 2).

3.2. Levels of OCPs

DDTs, HCB and γ-HCH were detected in more than 90% of the samples (Suppl S 3.2). DDTs were the dominating OCPs in milkfish and mullet with the highest mean concentrations in all sites (Table 2). The mean percent contributions to the ∑DDTs were 84.4% for *p,p′*-DDE, 8.4% for *p,p′*-DDD, 7.1% for *p,p′*-DDT, and 0.2% for *o,p′*-DDT (Suppl S 3.1). *p,p′*-DDE was the dominating compound in all sites with highest maximum concentrations of 715 ng/g lw in wild milkfish from Mtwara (Suppl S 2.1). The ratios of *p,p′*-DDE/*p,p′*-DDT (Suppl S 2.1) ranged from 3.4 to 6.6 (mean 4.6) in farmed milkfish from Jozani, 6.4–9.3 (mean 7.6) in farmed milkfish from Shakani, 5.3–10.2 (mean 7.1) in farmed milkfish from Mtwara, 40.9 (only one ratio available) in wild milkfish from Mtwara and 15.1–95.9 (mean 34.6) in wild mullet from Pemba. No ratio was available for farmed milkfish from Pemba because *p,p′*-DDE was the only DDT metabolite detected. The highest concentration of HCB was 0.52 ng/g lw found in farmed milkfish from Shakani (Table 2; Fig. 2). γ-HCH contributed 97% to ∑HCHs. The highest level of γ-HCH was detected in wild milkfish from Mtwara (0.19 ng/g lw) (Suppl S 2.1.). CHLs were detected in only 42% of the samples with *trans*-Nonachlor contributing more than 70% to ∑CHLs (Suppl 3.1). Mirex was detected in low levels in only two farmed milkfish from Shakani (Table 2). Levels of CHLs and mirex were < LOD in mullets.

3.3. Levels of PCBs

PCBs were detected in all sites. The highest mean concentration of $\sum_{10}$PCBs was found in farmed milkfish from Shakani (3.9 ng/g lw) (Table 2). The pattern of PCBs was dominated by PCB-153>PCB-180>PCB-138>PCB-170>PCB-118>PCB-101>PCB-99. PCB-153 and PCB-180 were detected in more than 70% and 60% of the samples, respectively (Suppl S 3.2), and contributed 39% and 22% to the $\sum_{10}$PCBs concentrations, respectively (Suppl 3.1). The levels of PCB-153 and PCB-180 were ranging from <LOD to 2.92 ng/g lw and <LOD to 2.19 ng/g lw, respectively. PCB-28, -52 and -74 were detected in levels < LOD. The mean concentration of $\sum_{10}$PCBs in wild mullet from Pemba was 0.59 ng/g lw (Table 2).

3.4. Levels of PBDEs and HBCDD

The highest mean concentration of $\sum_{11}$PBDEs was found in farmed milkfish from Jozani (3.52 ng/g lw) (Table 2). BDE-47, BDE-209 and BDE-100 were detected in >80%, >60% and >27% of the samples, respectively. BDE-47, -100 and -209 contributed most to $\sum_{11}$PBDEs (Suppl 3.1) and highest maximum concentrations of BDE-47, BDE-100 and BDE-209 were found in farmed milkfish from Jozani in concentrations of 1.55 ng/g lw, 2.78 ng/g lw and 7.03 ng/g lw (Suppl S 2.1), respectively. BDE -28, -153, -154, and -183 were detected in less than 30% of the samples (Suppl S 3.1). The mean concentration of $\sum_{11}$PBDEs in wild mullet from Pemba was 1.10 ng/g lw (Table 2). HBCDD was only detected in four wild mullet samples from Pemba with concentrations ranging from <LOD to 16.93 ng/g lw (Table 2).

3.5. Levels of PFAS

PFAS was not detected in any of the analysed fish muscle samples in levels above the LOD.

3.6. Associations between POP compounds and sites

3.6.1. Correlations

Spearman rank correlations for lipid percentage and dominant contaminants are presented in Table 3. A strong correlation was observed between HCB and *p,p′*-DDT, PCB-153, PCB-180 and BDE-47; between *p,p′*-DDE and *p,p′*-DDT; between PCB-153 and PCB-180 and between BDE-47 and BDE-209. HBCDD and γHCH had only significant correlation with *p,p′*-DDE and *p,p′*-DDT, respectively.

3.6.2. Comparisons between sites

The mean levels of HCB in farmed milkfish from Jozani and Shakani were significantly higher than in the other sites ($p < 0.0001$) (Table 2, Fig. 2). The mean *p,p′*-DDT level in farmed milkfish from Jozani was significantly higher than in the other sites ($p < 0.0001$). The mean level of $\sum_{10}$PCBs in farmed milkfish from Shakani was significantly higher from the other sites ($p < 0.0001$). The mean level of $\sum_{11}$PBDEs was significantly higher in farmed milkfish from Jozani than in the other sites ($p < 0.0001$) (Table 2).

4. Discussion

The variation in salinity between sampling sites may have been caused by seasonal differences. Shakani and Jozani ponds were sampled in January during the warm/dry season. While Pemba and Mtwara sites were sampled during the cool/rainy season.

4.1. Fish characteristics

The weight differences in farmed milkfish between the sites may be caused by differences in harvesting time, catch method and feeding regime. The range of lipid percentages in the present study

Table 2
Lipid percentage and concentration levels of persistent organic pollutants in (1) ng/g lipid weight (lw) (2) ng/g wet weight (ww) in farmed milkfish from Jozani and Shakani (Unguja), Pemba and Mtwara, wild milkfish from Mtwara and wild mullets from Pemba, Tanzania.

	Farmed fish																Wild fish							
	Jozani milkfish				Shakani milkfish				Pemba milkfish				Mtwara milkfish				Mtwara milkfish				Pemba mullets			
	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)
Lipid%	8/8	9.4	9.53	3.42–15.15	8/8	11.35	7.97	6.30–22.96	8/8	3.06	2.90	2.46–4.38	8/8	8.10	8.22	5.09–11.52	7/7	7.81	6.91	4.60–12.92	8/8	4.85	4.68	3.22–6.70
1.ng/g lw																								
HCB	8/8	0.42	0.42	0.37–0.48	8/8	0.41	0.41	0.32–0.52	8/8	0.11	0.09	0.07–0.21	7/8	0.19	0.21	<LOD-0.25	7/7	0.21	0.22	0.14–0.28	8/8	0.23	0.23	0.15–0.28
∑HCHs[a]	8/8	0.04	0.02	0.01–0.09	8/8	0.06	0.05	0.03–0.12	7/8	0.08	0.088	<LOD-0.12	8/8	0.05	0.03	0.02–0.15	7/7	0.12	0.14	0.02–0.19	8/8	0.13	0.13	0.05–0.20
∑DDTs[b]	8/8	42.22	45.01	23.54–51.16	8/8	17.54	17.66	11.56–22.91	8/8	5.73	5.91	3.08–7.92	7/8	1.01	1.08	<LOD-1.41	5/7	116.82	1.33	<LOD-808.25	8/8	73.31	70.83	25.34–137.51
∑CHLs[c]	7/8	0.06	0.05	<LOD-0.10	7/8	0.15	0.17	<LOD-0.24	0/8	<LOD	<LOD	<LOD	6/8	0.05	0.05	<LOD-0.10	0/7	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD
Mirex	0/8	<LOD	<LOD	<LOD	2/8	0.07	<LOD	<LOD-0.27	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/7	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD
∑PCBs[d]	8/8	1.57	1.6	0.67–2.41	8/8	3.94	3.66	1.32–8.13	3/8	0.09	<LOD	<LOD-0.54	7/8	0.27	0.09	<LOD-1.11	4/7	0.15	0.04	0–0.63	8/8	0.59	0.57	0.03–1.46
∑PBDEs[e]	8/8	3.52	3.20	1.67–7.03	8/8	1.78	1.49	1.03–2.87	5/8	0.44	0.48	<LOD-1.45	8/8	0.80	0.27	0.10–4.70	7/7	1.30	0.86	0.09–5.30	8/8	1.10	1.15	0.69–1.40
HBCDD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/7	<LOD	<LOD	<LOD	4/8	5.23	2.87	<LOD-16.93
2.ng/g ww																								
HCB	8/8	0.04	0.04	0.02–0.06	8/8	0.05	0.04	0.02–0.10	8/8	0.004	0.003	0.002–0.008	7/8	0.02	0.02	<LOD-0.03	7/7	0.017	0.01	0.007–0.03	8/8	0.01	0.01	0.005–0.02
∑HCHs[a]	8/8	0.003	0.002	0.001–0.007	8/8	0.006	0.007	0.002–0.01	7/8	0.002	0.003	<LOD-0.003	8/8	0.004	0.003	0.001–0.10	7/7	0.009	0.009	0.003–0.02	8/8	0.006	0.007	0.002–0.01
∑DDTs[b]	8/8	4.13	3.80	0.81–7.45	8/8	2.07	1.61	0.89–5.11	8/8	0.18	0.18	0.09–0.29	7/8	0.08	0.09	<LOD-0.13	5/7	9.22	0.16	<LOD-63.93	8/8	3.72	2.965	0.82–6.83
∑CHLs[c]	7/8	0.005	0.005	<LOD-0.01	7/8	0.019	0.018	<LOD-0.04	0/8	<LOD	<LOD	<LOD	6/8	0.004	0.005	<LOD-0.01	0/7	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD
Mirex	0/8	<LOD	<LOD	<LOD	2/8	0.01	<LOD	<LOD-0.06	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/7	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD
∑PCBs[d]	8/8	0.16	0.15	0.02–0.37	8/8	0.49	0.40	0.11–1.22	3/8	0.003	<LOD	<LOD-0.02	7/8	0.02	0.008	<LOD-0.08	4/7	0.01	0.004	<LOD-0.04	8/8	0.03	0.03	0.001–0.07
∑PBDEs[e]	8/8	0.28	0.28	0.16–0.39	8/8	0.19	0.21	0.08–0.35	5/8	0.02	0.01	<LOD-0.05	8/8	0.05	0.022	0.01–0.24	7/7	0.074	0.05	0.01–0.24	8/8	0.05	0.05	0.04–0.07
HBCDD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/8	<LOD	<LOD	<LOD	0/7	<LOD	<LOD	<LOD	4/8	0.26	0.13	<LOD-0.86

[a] ∑HCHs: sums of αHCH, βHCH, γHCH.
[b] ∑DDTs: sums of *p,p′*-DDT, *p,p′*-DDE, *o,p′*-DDT and *p,p′*-DDD.
[c] ∑CHLs: sum of oxychlordane, *trans*-chlordane, *cis*-chlordane and *trans*-nonachlor.
[d] ∑PCBs: sum of PCB-28, -52, −74, −99, −101, −118, −138, −153, −170, and −180.
[e] ∑PBDEs: sum of BDE-28, -47, −99, −100, −153, −154, −183, −206, −207, −208, and −209.

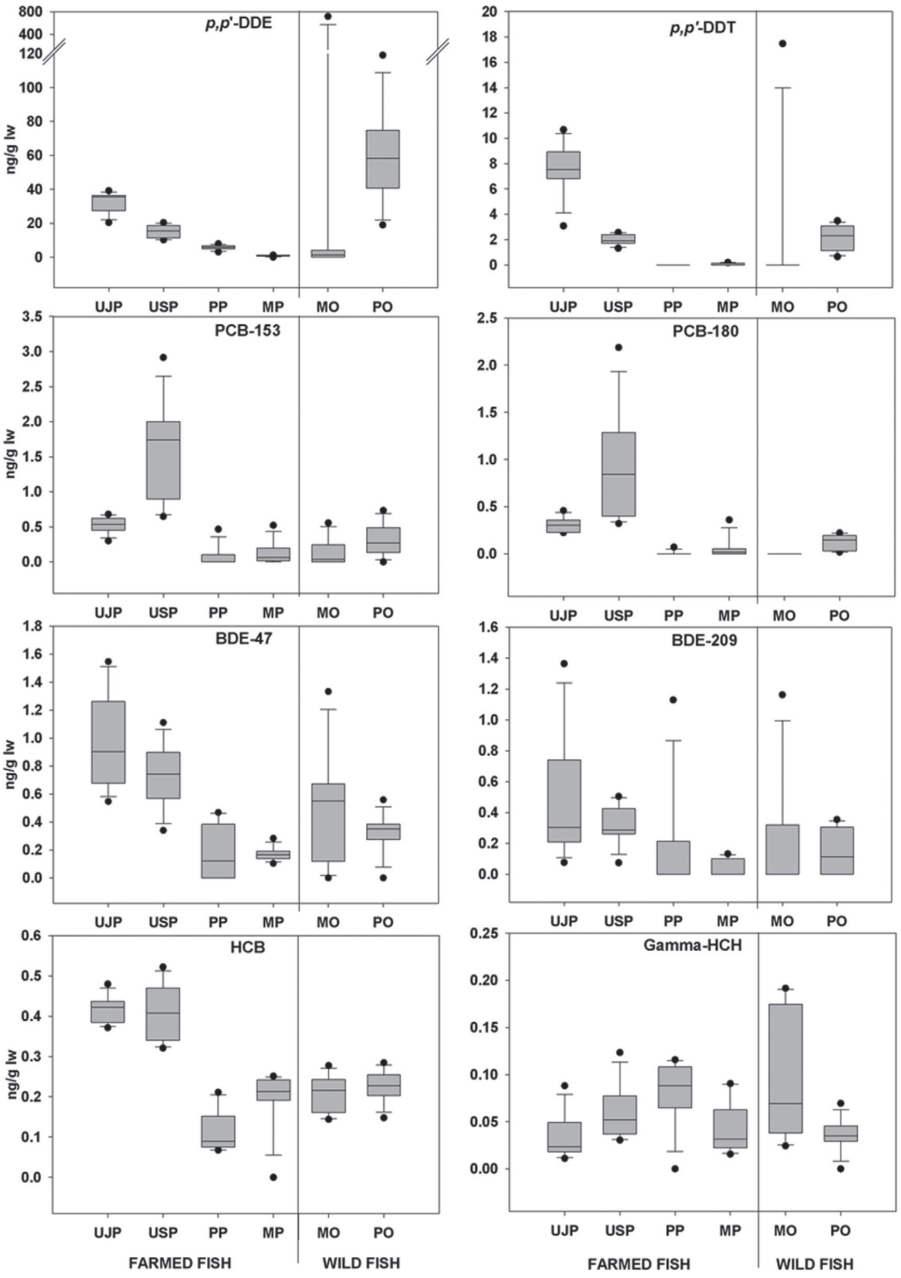

Fig. 2. Levels of *p,p′*-DDE, *p,p′*-DDT, PCB-153, PCB-180, BDE-47, BDE-209, HCB and γ-HCH in farmed milkfish from Jozani (UJP), Shakani (USP), Pemba (PP) and Mtwara (MP) and wild milkfish from Mtwara (MO) and wild mullet from Pemba (PO) illustrated using a box-plot with median line, the 50% box and inter-quartile range (whiskers).

(2.5%–23%) corresponds with the lipid percentage reported by Schlechtriem et al. (2009). Lipid percentage may vary depending on species, age, season and location (Schlechtriem et al. (2009). Although age was not determined, the farmed fishes in Shakani and Jozani at Unguja are harvested for consumption after approximately two years, while those in Mtwara and Pemba are harvested at less than a year of farming. The time of harvesting, and thus difference in age, may have contributed to the differences in lipid percentage.

Table 3
Correlations between dominant contaminants.

	Lipid%	HCB	γ-HCH	*p,p′*-DDE	*p,p′*-DDT	PCB-153	PCB-180	BDE-47	BDE-209	HBCDD
Lipid%	1									
HCB	0.52**	1								
γ-HCH	−0.34*	−0.27	1							
p,p′-DDE	0.01	0.08*	0.2	1						
p,p′-DDT	0.29*	0.53**	−0.09*	0.69**	1					
PCB-153	0.44*	0.66**	−0.1	−0.05*	0.14**	1				
PCB-180	0.49*	0.65**	−0.19	−0.06*	0.15**	0.92**	1			
BDE-47	0.09	0.65**	0.2	0.06*	0.43**	0.41**	0.38**	1		
BDE-209	−0.13	0.29*	0.29	0.05*	0.25*	0.23**	0.21*	0.54**	1	
HBCDD	−0.17	−0.08	−0.19	0.04*	−0.07	−0.11	−0.13	−0.09	−0.11	1

*$p < 0.05$; **$p < 0.0001$.

4.2. Levels and congener profile of OCPs in milkfish and mullet

DDTs were the dominating group of compounds detected in fish liver in farmed and wild milkfish and mullet (Table 2). The highest maximum ∑DDT levels were found in wild milkfish from Mtwara (808 ng/g lw) and in wild mullet from Pemba (138 ng/g lw). However, only one of the seven wild milkfish from Mtwara showed an elevated level of ∑DDTs, while the other six samples showed low or moderate levels of ∑DDTs. This was illustrated by the substantial difference between the mean and median level of ∑DDTs for Mtwara wild milkfish (Table 2). ∑DDT levels in farmed milkfish and wild mullet from the other sites showed little variation (Table 2). High DDT levels are usually related to former or ongoing use of DDT. Especially in countries where DDT has been used (or still is in use) for vector control, higher variation between samples of same species is expected due to possible local sources. The ratio of *p,p′*-DDE to *p,p′*-DDT is used to explain the time of input and degradation of DDT to metabolites in the environment.(Marco and Kishimba, 2005; Polder et al., 2016; Ssebugere et al., 2009). The high level *p,p′*-DDE together with the high ratio of *p,p′*-DDE/*p,p′*-DDT in wild milkfish from Mtwara (40.9) and wild mullet from Pemba (34.6) compared to levels and ratios in the farmed fish indicate that wild fish from the Indian Ocean coast line is more likely to be exposed to high *p,p′*-DDE levels than farmed fish from Tanzanian aquacultures. The dominance of *p,p′*-DDE in the fish liver in the present study corresponds with the findings of Mwevura et al. (2002) in marine biota from the Tanzanian coast, in farmed salmon muscle from Norway (Jacobs et al., 2002), in catfish and tilapia muscle from Uganda (Bagumire et al., 2008), in tilapia muscle from Tanzanian lakes (Polder et al., 2014), in fish muscle from Ethiopian dam (Teklit, 2016) and in whole fish from Indonesia (Sudaryanto et al., 2007) (Table 4). The lipid adjusted levels of ∑DDTs in the present study were lower than ∑DDT levels in tilapia muscle from Lake Victoria (Henry and Kishimba, 2006; Polder et al., 2014) and bonga fish muscle from Nigeria (Williams and Unyimadu, 2013) (Table 4), and were comparable to the levels reported by Mdegela et al. (2009) in tilapia muscle and catfish muscle from Mindu dam, Tanzania. The mean levels of ∑DDTs in wild milkfish and mullet in the present study were comparable to the levels in whole body of wild milkfish and mullet (Sudaryanto et al., 2007) in Indonesia.

HCB was the second dominating contaminant, detected in 98% of the samples. The levels were relatively low and in the same range in all sites. However, the significantly higher levels of HCB in Jozani and Shakani than in the other sites, suggest a low but active source of HCB at Unguja. The concentrations of HCB in the present study were comparable to those reported by Polder et al. (2014) in tilapia from different lakes in Tanzania but lower than the levels reported by Sudaryanto et al. (2007) in fish from Indonesia and Shi et al. (2013) in butterfish from Shantou harbour, China (Table 4). HCB was used as a fungicide, but also produced as a by-product in the manufacture of other industrial chemicals and incineration of chemical waste. HCB is no longer used as fungicide in Tanzania. Possible relevant sources for HCB at Unguja may be the vicinity of a nearby airport, and industrial and human activities.

The γ-HCH (lindane) was detected in low concentration ranges in all sites, but showed widespread distribution in the region. The lipid adjusted mean levels of ∑HCHs detected in the present study were lower than the levels in muscle from bonga fish from Nigeria (Williams and Unyimadu, 2013) and in muscle of butterfish from China (Shi et al., 2013). Compared to studies in Tanzania the levels of ∑HCHs were lower than in tilapia muscle from Lake Tanganyika and Lake Nyasa by Polder et al. (2014). Lindane was banned in 2009 for use as a pesticide in many countries including Tanzania and the low γ-HCH concentrations found in the present study reflect exposure to historic use.

Other detected OCPs were mirex and chlordanes, although in very low levels (Suppl S 2.1). These OCPs were used as insecticides and have been banned for decades (ATSDR, 1994). Lipid adjusted levels of ∑CHLs were 64 fold lower than the highest levels (15.5 ng/g lw) in muscle of catfish from Mindu dam (Mdegela et al., 2009), and much lower than levels in fish muscle from China (Liu et al., 2010; Shi et al., 2013), in whole body milkfish and mullet from Indonesia (Sudaryanto et al., 2007) and in muscle of bonga fish from Nigeria (Williams and Unyimadu, 2013) (Table 4).

4.3. Levels and congener profile of PCBs

Tanzania has never produced PCBs but has been using imported products containing PCBs such as transformers, capacitors, lubricants paints, glue and carbonless paper (IPEN, 2005). Although PCB levels were relatively low, they were detected in fish from all studied sites. The highest mean concentrations of $\sum_{10}$PCBs were found in farmed milkfish from Shakani (3.94 ng/g lw) and Jozani (1.57 ng/g lw) (Table 2). The mean lipid adjusted level of $\sum_{10}$PCBs in farmed milkfish liver from Shakani was fourfold lower than the mean $\sum_{7}$PCB level in tilapia muscle from Southern Lake Victoria (Polder et al., 2014), but higher than in tilapia muscle from Napoleon Gulf, Northern Lake Victoria, Uganda, (Ssebugere et al., 2014a) (Table 4). The PCB concentrations in present study were much lower than levels of ∑PCB in muscle of butterfish from Shantou harbour, China (Shi et al., 2013). The detection of PCBs in marine and farmed fishes in the present study and in freshwater fishes in Tanzanian lakes and Uganda further indicate the spread of PCBs contamination in aquatic environment in this region and consequently human exposure due to fish consumption. The dominance of PCB-153 to PCB-138 and PCB-180 has also been reported in other studies (Asante et al., 2013; Hayward et al., 2007; Polder et al., 2008a, 2008b, 2016), and this is related to high persistence of PCB-153 to other PCB congeners. However, PCB-138 dominated in

Table 4
Mean concentrations of persistent organic pollutants in ng/g lipid weight (ng/g lw) in fish from this study: farmed milkfish (mean of Jozani, Shakani, Pemba and Mtwara ponds), wild milkfish and wild mullet compared to results from other studies and countries.

Country	Location *Specie*	Sampling year	Fish Tissue	Lipid %	HCB	∑HCH	*p,p'*-DDE	*p,p'*-DDT	∑DDTs	∑CHLs	∑PCBs	∑PBDEs	HBCDD	Reference
Tanzania	All ponds *Chanos chanos*	2016	Liver	7.98	0.22	0.05	13.5	3.2	16.6		1.47	1.64	<LOD	This study
Tanzania	Indian Ocean *Chanos chanos*	2016	Liver	7.81	0.2	0.1	103	2.5	117		0.2	1.3	<LOD	This study
Tanzania	Indian Ocean *Mugil cephalus*	2016	Liver	4.85	0.2	0.04	60.7	2,2	73.3		0.6	1.1	5.2	This study
Tanzania	Lake Tanganyika *O. niloticus*	2011	Muscle	3.3	1.2	1.1	116	41.8	273	0.9	17.2	4.1		Polder et al. (2014)
Tanzania	Lake Victoria *O. niloticus*	2006	Muscle	1.5					500					Henry and Kishimba (2006)
Tanzania	Mindu dam *O. niloticus*	2009	Muscle	8	2.5		25	21.25	46.3	2.5				Mdegela et al. (2009)
Tanzania	Mindu dam *C. gariepinus*	2009	Muscle	11	2.7		54.6	29.1	90.9	15.5				Mdegela et al. (2009)
Uganda	Napolean Gulf *O. niloticus*	2011	Muscle								0.073			Ssebugere et al. (2014a)
Uganda	Murchison bay *O. niloticus*	2011	Muscle	1.7								5		Ssebugere et al. (2014b)
Ghana	Benya lagoon *S. melanotheron*	2010	Muscle	3.1							150	19	2.2	Asante et al. (2013)
Ghana	Lagos lagoon *E. fimbriata*	2008–9	Muscle	*2.3*		*15438*	*2280*	*7736*	*13175*					Williams and Unyimadu (2013)
Indonesia	Jakarta Bay *Chanos chanos*	2003	Whole body	3.9	28	3.7			120		400			Sudaryanto et al. (2007)
Indonesia	Jakarta Bay *V. buchanani*	2003	Whole body	7.3	7.4	1.1			110		260			Sudaryanto et al. (2007)
Chile	Southern Chile *Salmo salar*	2005	Muscle									15.9		Montory and Barra (2006)
China	Shantou harbour *P. argenteus*	2010	Muscle	2.61	15.3	122		1958	731	228	1413			Shi et al. (2013)
China	Lioaning province *C. carpio*	2007	Muscle	1.9	26.3	50.5	258.4	245.8	1379	109				Liu et al. (2010)
Norway	frozen *Salmo salar*	1999	Muscle	13.7		10.9	2.8	1.3	5.2		145	1.1		Jacobs et al. (2002)

Numbers written in Italic: value was calculated using data from the referred literature.

tilapia from Tanzanian Lakes rather than PCB-153 (Polder et al., 2014) suggesting that PCB contamination in Tanzania may originate from different PCB sources. The higher PCB levels in the farmed milkfish from Shakani and Jozani in Unguja compared to Pemba and Mtwara, suggest contamination from airport's emissions and human urban activities. Shakani is situated closer to the airport (3 km) than Jozani (23 km), and this may explain the higher PCBs levels in the Shakani milkfish compared to Jozani.

4.4. Levels and congener profile of BFRs

BFRs are ubiquitously present in the global environment (Lyche et al., 2015) and were detected in fish from all sites in the present study, in free-range chicken eggs and in human milk from Tanzania (Müller et al., 2016; Polder et al., 2016). The BDE-47 and BDE-209 levels in farmed milkfish from Jozani were significantly higher than in all the other sites ($p<0.0001$), except from Shakani (Fig. 2). The BDE pattern was different between the sites (Fig. 3). The lipid adjusted mean concentrations of ∑PBDEs in fish in the present study were comparable to levels in tilapia muscle from Northern Lake Victoria, Uganda (Ssebugere et al., 2014b) and from lakes Tanganyika, Nyasa and Babati in Tanzania, (Polder et al., 2014), in the same range as in farmed salmon muscle from Norway (Jacobs et al., 2002), but lower than the levels in tilapia muscle from Benya lagoon in Ghana, Asante et al. (2013) and in farmed salmom muscle from Southern Chile (Montory and Barra, 2006) (Table 4). BDE-47, BDE-100 and BDE-209 dominated the PBDEs detected in the present study (Fig. 3; Suppl S 3.1). The dominance of BDE-47 and its high contribution to the ∑PBDEs is in agreement with other studies in fish and environment (Asante et al., 2013; Montory and Barra, 2006; Parolini et al., 2013; Ssebugere et al., 2014b). BDE-47 is resistant to microbial degradation in the environment (Hale et al., 2006) and it is one of the major components in the penta mixture of PBDEs, included in the Stockholm Convention (Stockholm Convention, 2016). Furthermore, the high concentration of BDE-47 in the present study could also be due to debromination of more highly brominated congeners in the fish tissues (Stapleton et al., 2004). BDE-100 was only detected in farmed milkfish from Jozani and Shakani. In Jozani, the BDE-100 was significantly higher than BDE-47 (Fig. 3). PBDEs are not manufactured in Tanzania, but they are used as flame-retardants in commercial products such as electronics and furniture imported from developed countries. Dismantling and burning of e-waste may be the most important sources for PBDE contamination in the Tanzanian environment. The higher levels of BDEs and pattern difference in the farmed fish from Jozani and Shakani, compared to the other locations, may be related to emissions from urban, industrial and airport activities (Fig. 3).

HBCDD was only detected in four samples of wild mullet from Pemba in concentrations up to 10 times higher than other BFRs. HBCDD was not detected in farmed and wild milkfish (all sites). The mean level of HBCDD in the present study was two times higher than in muscle of tilapia from Lake Babati (Polder et al., 2014) (Table 4). The presence of high HBCDD levels in wild mullets from Pemba could not be explained and argues for further investigations.

4.5. Levels of PFASs

This was the first study to analyse PFASs in fish from Tanzania. Liver tissue is preferred for PFAS analyses in fish because PFASs is

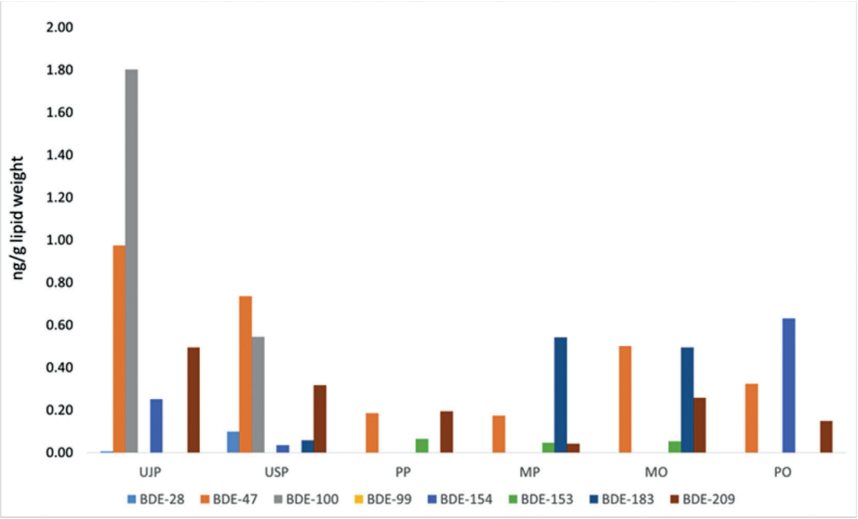

Fig. 3. Pattern of individual PBDEs in fish muscle from farmed milkfish from Jozani (UJP), Shakani (USP), Pemba (PP) and Mtwara (MP) and wild milkfish from Mtwara (MO) and wild mullet from Pemba (PO).

expected to be higher in liver than blood or other organs (Giesy and Kannan, 2001). In the present study, however, most of the liver tissue was used for analyses of OCPs, PCBs and BFRs, leaving too little tissue for analyses of PFAS. Therefore muscle tissue was used. No PFAS levels above LOD were detected in the studied fish muscle. The detection levels of the different PFASs were in the same range as used for cod liver in a Norwegian study (Valdersnes et al., 2017), supporting the analytical method used. However, low sample amount and high blank levels may have contributed to the absence of measurable levels of PFAS. Nevertheless, PFASs in low levels were detected in blood from delivering mothers from northern Tanzania, showing their presence in the Tanzanian environment (Müller et al., 2017 in prep). As PFASs are added to various consumer products, the increasing modernization and importation of consumer products in Tanzania in the future may lead to an increase of the PFAS levels in the environment and humans. For future studies fish liver should be used for detecting PFASs.

4.6. Association among contaminants

The strong positive correlation observed between HCB, *p,p′*-DDE, *p,p′*-DDT, PCB-153, PCB-180, BDE-47, BDE-209 suggests that the exposure of these contaminants is from the same source. The negative and weak correlations of HBCDD, and γ-HCH to other contaminants indicates that HBCDD and γ-HCH levels are from different source as compared to other contaminants (Table 3).

4.7. Possible implications for human and fish health with regard to POPs

The present study intended to serve as a pilot study for monitoring occurrence and levels of POPs in farmed fish. Liver was chosen as matrix for this pilot study because the concentration of lipophilic POPs is considered to be highest in this lipid rich organ, increasing the possibility for finding low level POPs. However, fish fillets are used for consumption. Risk assessment of POPs in fish is thus done in fish fillets. Since lipid content in muscle for milkfish and mullet (0.19–1.8% and 0.56–6.3%, respectively), (Hung et al., 1980)), are lower than in corresponding liver in the present study (2.5–23% and 3.2–6.7%, respectively) we assume that the POP levels in the studied fish liver are higher than in the muscles. Therefore, we hypothesize that POP levels in the muscle of the studied fish are lower than the maximum residue limits (MRLs) set by European Union (EU) (EU Food Safety, 2017) and the United States Food and Drug Authority (USFDA) (USFDA, 2011) for different chemical contaminants in fish fillet for human consumption (Suppl S 2.2). This needs to be confirmed by further studies of POPs in fish fillets from the same species.

Increased contaminant levels in the future may pose risk to the development of aquaculture in Tanzania. Experimental studies have shown that different contaminants induce health effects in fish (Berg et al., 2016; Mills and Chichester, 2005). Nevertheless, it has been difficult to associate the effect of contaminant to fish in the wild due to mobility and recruitment of fish (Pait and Nelson, 2002). However, the levels detected may cause adverse health effects in farmed and wild fish and may threaten sustainability of aquaculture and biodiversity.

4.8. Comparison between farmed versus wild-caught fish

The present study revealed little difference in levels of HCB and γ-HCH between farmed and wild milkfish (Table 2), but ∑DDTs were detected in higher levels in wild than in farmed milkfish. This means that fish feed is an insignificant DDT source for aquaculture fish in Tanzania. In contrast, higher levels of ∑PCBs were detected in farmed fish than in wild fish, maybe related to airport and human activities, although influence of fish feed as a source is not excluded. In terms of human health, it is important to monitor PCB levels in fish feed used in the different fish farms, for excluding that this is an active PCB source for farmed fish.

5. Conclusion

This is the first study to investigate the occurrence and levels of

OCPs, PCBs, BFRs and PFASs in aquaculture in Tanzania. OCPs, BFRs and PCBs were detected in all the studied fish in Jozani, Shakani, Pemba and Mtwara in Tanzania. The concentrations of the contaminants were lower, comparable and higher than those reported from elsewhere in the literature. *p,p'*-DDE, PCB-153 and BDE-47 were the dominant POPs. Wild milkfish from Mtwara contained higher levels of DDTs than the farmed fish from other sites. The low levels of HCBs, HCHs, mirex and chlordanes detected in the present study in comparison to other countries may be due to positive measures taken after the ban of these pesticides. Significantly higher levels of PCBs and BFRs were detected in farmed milkfish from Jozani and Shakani, Unguja, compared to milkfish in Pemba and Mtwara suggesting influence of various human activities, such as emissions from airports and improper disposal of wastes. The occurrence of high levels of HBCDD in wild mullet from Pemba, calls for further investigations to elucidate sources of HBCDD in the area. PFASs were not detected in any of the samples in the present study, however they may increase in the future due an ongoing importation of PFASs imbedded in consumer products in the region. Aquaculture is a fast growing activity in Tanzania contributing both to income and food security. Therefore, if POPs are not regulated and monitored properly, increased levels of contaminants in farmed fish may pose human health risks and unsustainable aquaculture production in the future. Future studies in fish from aquaculture should include fish fillets for human health risk assessment and fish feed for investigation of exposure route. However, the reported POP levels may pose ecological and fish health risk and threaten biodiversity. Therefore, the findings of the present study warrant further research and monitoring of POPs in aquaculture and in the environment in Tanzania.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2017.09.121.

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SUPPLEMENTARY DATA

S1.1: The minimum and maximum levels of contaminants in ng/g lw in livers of farmed and wild milkfish and mullets from Tanzania.

	Jozani farmed milkfish		**Shakani farmed milkfish**		**Pemba farmed milkfish**		**Mtwara farmed milkfish**		**Mtwara wild milkfish**		**Pemba wild mullets**	
	min	max	min	max	min	max	min	max	min	max	min	max
Lipid%	3.420	15.150	6.302	22.956	2.462	4.381	5.086	11.523	4.603	12.916	3.215	6.701
HCB	0.371	0.479	0.320	0.521	0.067	0.210	<	0.250	0.143	0.277	0.147	0.284
α-HCH	<	0.026	<	<	<	<	<	<	<	<	<	<
β-HCH	<	<	<	<	<	<	<	0.056	<	0.107	<	0.164
γ-HCH	0.011	0.088	0.030	0.123	<	0.115	0.015	0.090	0.024	0.191	<	0.069
∑HCHs	0.011	0.088	0.030	0.123	<	0.115	0.015	0.145	0.024	0.191	0.047	0.201
p,p'-DDE	20.456	39.204	10.235	20.422	3.080	7.922	<	1.247	<	715.273	19.014	119.066
p,p'-DDD	<	3.711	<	1.648	<	<	<	<	<	75.504	5.375	15.432
o,p'-DDT	<	0.920	<	<	<	<	<	<	<	<	<	<
p,p'-DDT	3.078	10.675	1.319	2.553	<	<	<	0.194	<	17.471	0.653	3.488
∑DDTs	23.534	51.156	11.555	22.909	3.080	7.922	<	1.407	<	808.248	25.344	137.509
p,p'-DDE/*p,p'*-DDT	6.65	3.73	7.76	8	-	-	-	6.43	-	40.94	29.12	34.14
Oxychlordane	<	0.040	<	0.059	<	<	<	<	<	<	<	<
trans-chlordane	<	0.014	<	0.057	<	<	<	0.056	<	<	<	<
cis-chlordane	<	0.014	<	0.042	<	<	<	0.034	<	<	<	<
trans-Nonachlor	<	0.103	<	0.178	<	<	<	0.066	<	<	<	<
∑CHLs	<	0.103	<	0.238	<	<	<	0.103	<	<	<	<
Mirex	<	<	<	0.270	<	<	<	<	<	<	<	<
PCB-28	<	<	<	<	<	<	<	<	<	<	<	<
PCB-52	<	<	<	<	<	<	<	<	<	<	<	<
PCB-74	<	<	<	<	<	<	<	<	<	<	<	<
PCB-101	<	0.350	<	0.692	<	<	<	<	<	<	<	<
PCB-99	<	0.428	<	<	<	<	<	<	<	<	<	<
PCB-118	0.141	0.195	0.165	0.452	<	<	<	0.051	<	<	<	0.24
PCB-153	0.299	0.678	0.647	2.914	<	0.465	<	0.522	<	0.555	<	0.733
PCB-138	<	0.276	<	0.974	<	<	<	0.151	<	0.075	<	0.164
PCB-180	0.223	0.458	0.320	2.188	<	0.071	<	0.360	<	<	0.018	0.222
PCB-170	<	0.183	0.085	0.905	<	<	<	0.117	<	<	<	0.109
∑PCBs	0.670	2.406	1.318	8.128	<	0.537	<	1.111	<	0.631	0.024	1.46
BDE-28	<	0.029	<	0.297	<	<	<	<	<	<	<	<
BDE-47	0.547	1.546	0.340	1.111	<	0.468	0.103	0.284	<	1.333	<	0.558
BDE-100	0.902	2.783	<	1.373	<	<	<	<	<	<	<	<
BDE-99	<	<	<	<	<	<	<	<	<	<	<	<
BDE-154	<	1.340	<	0.274	<	<	<	<	<	<	0.392	0.837
BDE-153	<	<	<	<	<	0.512	<	0.182	<	0.375	<	<
BDE-183	<	<	<	0.457	<	<	<	4.329	<	3.448	<	<
BDE-208	<	<	<	<	<	<	<	<	<	<	<	<
BDE-207	<	<	<	<	<	<	<	<	<	<	<	<
BDE-206	<	<	<	<	<	<	<	<	<	<	<	<
BDE-209	0.076	1.363	0.074	0.503	<	1.129	<	0.132	<	1.162	<	0.354
∑PBDEs	1.674	7.033	1.025	2.867	<	1.452	0.103	4.695	0.087	5.300	0.686	1.396
HBCD	<	<	<	<	<	<	<	<	<	<	<	16.927

< Below detected limit

Table S1.2: Maximum acceptable levels (MRLs) in eddible tissue of fish recommended By USFDA and EU and the maximum concentration for DDTs, HCB, Mirex, γHCH, CHLs, PCBs and PBDEs in ng/g ww detected in farmed ana wild nilkfish and mullets the present study.

Contaminant	USFDA	EU	Present study
DDT	5000	100	63.9
HCB	-	100	0.103
Mirex	100	100	0.06
γHCH	-	100	0.013
CHL	300	100	0.04
PCBs	2000	75	1.22
PBDEs	-	-	0.39

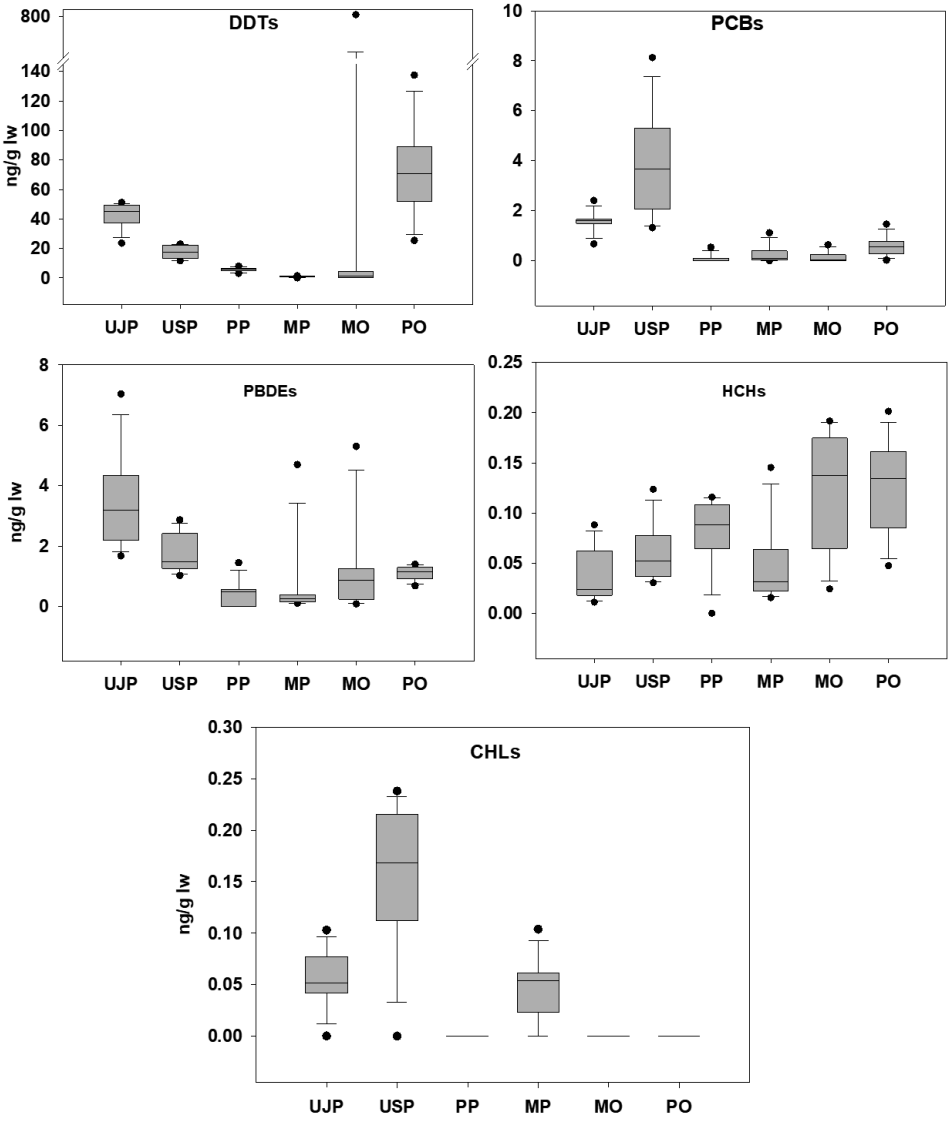

Fig S 2.1: Mean levels of ∑DDTs, ∑PCBs, ∑PBDEs, ∑HCHs and ∑CHLs in farmed milkfish from Jozani (UJP), Shakani (USP), Pemba (PP) and Mtwara (MP) and wild milkfish from Mtwara (MO) and mullet from Pemba (PO) illustrated using a box-plot with median line, the 50% box and inter-quartile range (whiskers).

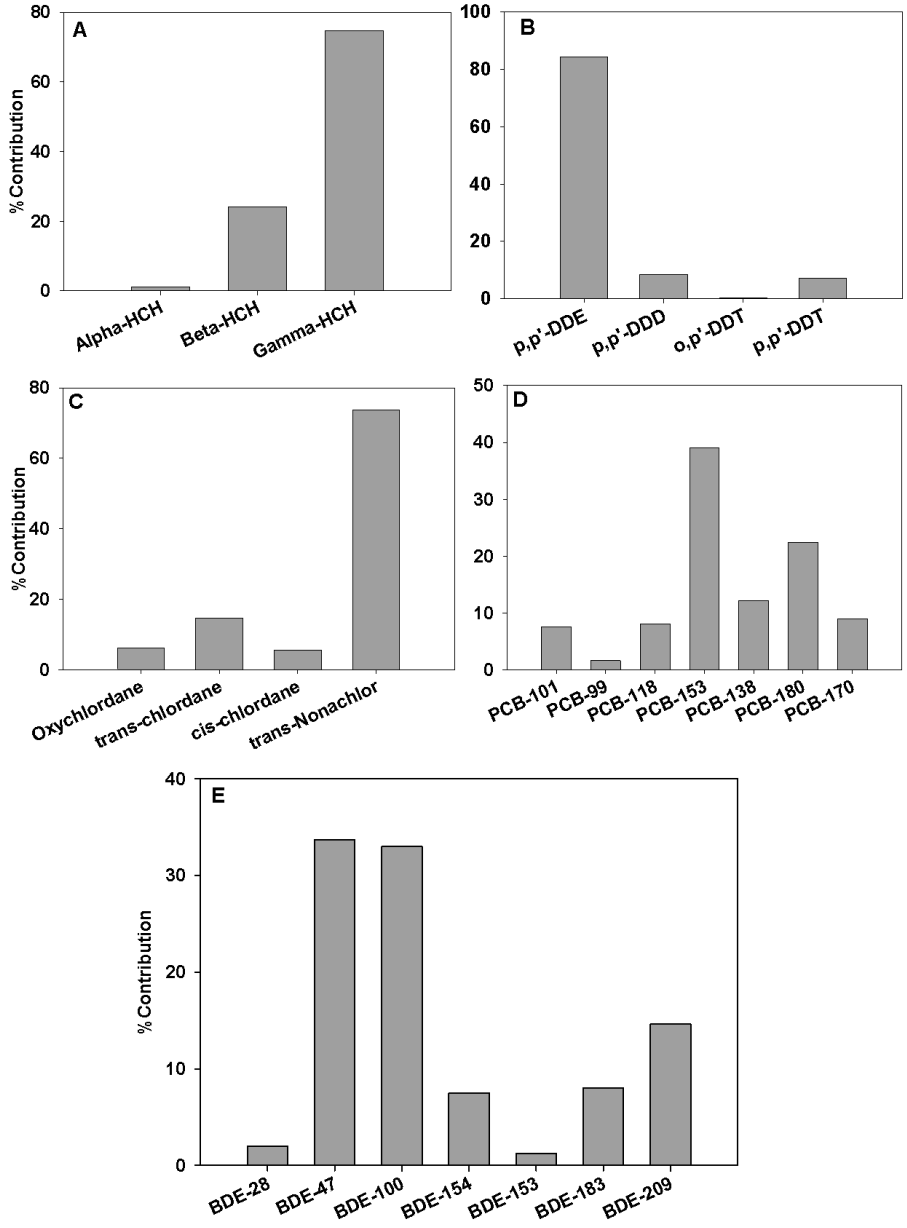

Fig S 2.2: Percent contribution individual detected contaminant to ∑HCHs (A), ∑DDTs (B), ∑CHLs (C), ∑PCBs (D) and ∑PBDEs (E).

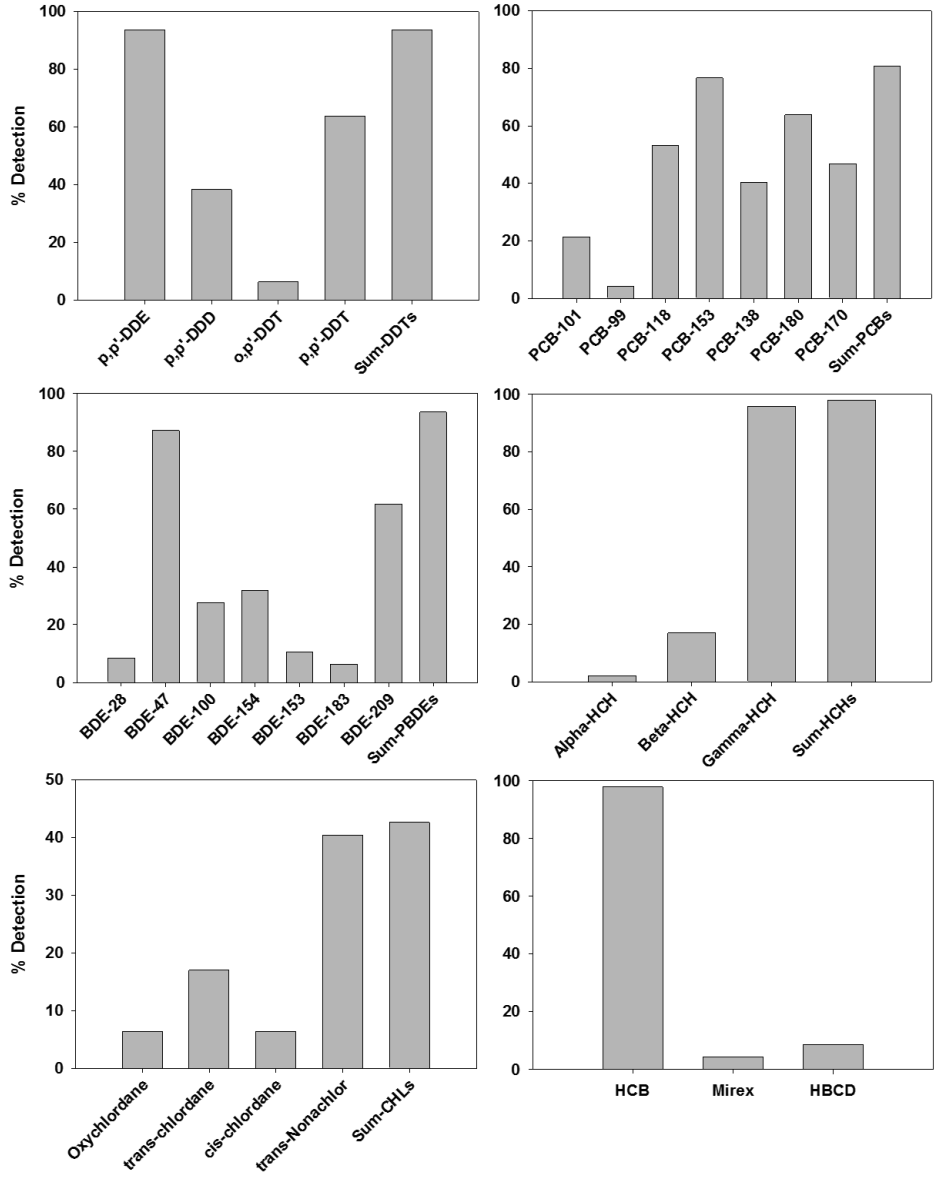

Fig S 2.3: Percent detection of DDTs, PCBs, PBDEs, HCHs, CHLs, HCB, Mirex, and HBCD in analysed samples.

S3. Material and methods

S3.1 Description of study sites

S3.1.1 Unguja Island

Unguja Island is one of the two main Zanzibar islands situated in Tanzanian part of the Western Indian Ocean. Unguja is known for its touristic destination and the production of seaweed and spices. It has a population of 896,455 with most of them being farmers (NBS 2015). Unguja Island is also one of the small scale rice producing regions. The samples were collected from two locations in Unguja; Shakani situated 6°16'30'' south of the Zanzibar stone town and Jozani situated at 6°15'15'' south east of the Zanzibar stone town.

Shakani site is a vacated quarry area which was used for extraction of stones for building purpose. The holes left after quarry digging are filled with seawater seeping from the ocean are now used as ponds. They are stocked with different types of marine organisms including sea turtles, barracudas and milkfish for recreational purposes. The surrounding area has tree shrubs on the landward side and mangrove stand on the seaside. Eleven milkfish samples were collected from one of the ponds.

The Jozani area is located within Jozani Forest reserve famous for its red colobus monkeys. Jozani ponds are owned by the group of villagers for tourist activities. The ponds are concrete bordered by mangrove forest on the south and terrestrial bushes on the north. The main economic activity is tourism but there are some ongoing agricultural activities outside the reserve. The ponds are connected to the ocean by a channel which is used for filling the ponds with seawater during spring tides. A total of 20 milkfish were collected from this site.

S3.1.2 Pemba Island

Pemba Island, also part of Zanzibar islands, is situated north of Unguja and is the second biggest island in Tanzania. The island is well known for clove production and rice farming. It has a population of 406,848 (NBS 2015). In Pemba, thirty milkfish samples were collected from ponds in Pujini situated at 5°19'11.08''S, 39°48'28.85''E in Pemba island about 10 km from Chake Chake town. The ponds are located on salt flats behind the mangroves near to salt pans. The economic activities in the vicinity of the area include salt extraction activities,

small scale agriculture and animal husbandry. A total of 20 mullets, collected from the Indian Ocean, were bought from the fishermen at the landing site in Wete town situated at 5°03'44.25''S, 39°43'6.33''E, 30 km from Chake Chake town. Wete is one of the highly populated and busiest regions in Pemba with a population estimate of 97,246 (NBS & OCGS 2013).

S3.1.3 Mtwara

Mtwara is located on mainland in southern Tanzania bordering Mozambique. It is famous for cashew nuts production, oil/gas industries and a recently built and operating cement industry. It has a population of 1,270,854 with majority being farmers (NBS 2015). Sampling was conducted in Mtwara rural district at Ndumbwe village from the Tujifunze fish farmers group which is among the 27 groups of farmers managed by Mtwara fish farmers' association (UWASA). The ponds are situated on salt flats within the mangrove forest. The ponds are located at 10°13'25.99''S, 39°57'32.66''E about 2 km from the main road and 30 km from Mtwara town. The main economic activity around the ponds is agriculture. Thirty milkfish were collected from the ponds and ten fish (eight milkfish and two mullet fish) were bought from the fishermen who fished from the nearby Ocean.

S3.2 Species description

S3.2.1 Milkfish (*Chanos chanos*, Forsskål 1775)

Milkfish is one of the most important cultured brackish water food fish in Southeast Asia with Philippines and Indonesia producing more than 300 tonnes annually (Garcia 1990). It is the only member of the family Chanidae in the order Gonorychiformes. Milkfish occur near the coast and islands in the Indo-Pacific region and usually their distribution coincides with clear, shallow coral reef and mangrove areas. Milkfish are omnivorous fishes, they feed by filtering, grazing and snap prey. They feed on range of foods from copepods, diatoms, detritus to juvenile clupeids when they are adults (Bagarinao 1991; Garcia 1990; Lee et al. 1986). Milkfish normally attain first sexual maturity between the age of 6-9 years in the ponds and 5-7 years in the wild. They are highly tolerant species, can thrive and grow well in temperature between 8.5 to 42.7^{o}C and salinity between 0 to 70 depending on their

growth stage. In most cases, their tolerance depends on the acclimatization in the environment (Bagarinao 1991; Garcia 1990). Due to the tolerance of milkfish to environmental changes, it has been reared in earthen ponds in commercial fish farms. The market size in most farms is between six months to one year when they attain approximate 1 kg. Milkfish is commercially a very important fish species in Asian countries especially in Indonesia and Philippines, its production has been increasing since 1990s to date with over 1 million tonnes production (FAO 2014; Fishbase 2016; Garcia 1990). In Tanzania Milkfish was caught as a bycatch in salt production sites (Sobo 2014), it became introduced in experimental farms by Institute of Marine Sciences (IMS) from 1996-2003 and introduced to the farmers in 2004 (Mmochi & Mwandya 2003). Since 2007 records have shown that Milkfish production in Tanzania has been increasing (Sobo 2014). It is now a potential marine aquaculture species in the coasts of Tanzania with more than 1300 farmers engaged in its production with estimate of 231 tons (Ministry of Livestock and Fisheries Development 2014).

S3.2.2 Mullet (*Mugil cephalus*, Linnaeus 1758)

Mullet is an important food fish species in the Mugillidae family. It is found in coastal tropical and subtropical waters, it is diurnal coastal specie that enters estuaries and rivers. It usually schools over sand and mud areas and feed on zooplanktons, detritus, diatoms and small vertebrates and invertebrates (Saleh 2008). It is a euryhaline specie, can acclimatize to different levels of salinity and can tolerate temperatures between 8 to 24^{o}C. Mullet grows up to the maximum of 100 cm and attain maturity at the age of 2 years and above. It is similar to milkfish in term of feeding, habitat and they are on the same trophic level in the food chain (Saleh 2008; Schmittou 1973). Due to the similarity with milkfish, mullet can be cultured in polyculture with milkfish to increase profit in the fish farms (Mirera 2011).

II

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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

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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 the maximum levels (ML) set by FAO/WHO (0. 3 mg/kg ww) in 100% of the analysed fish. Other metals were below the respective MLs. Concentrations of Pb were higher in wild fish than in farmed fish, implying different sources. Cd concentrations were generally low, but highest in wild mullet muscle from Pemba (0.02 mg/kg ww). 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 were 40 to 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. However, the detection of Pb in concentrations above ML in all fish suggest that Pb may affect the health of fish and other water living organisms in the area. Future investigations should include regularly monitoring of heavy metals and POPs 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.

Key words

Heavy metals; Pb; Aquaculture; Health risk; Fish health; Tanzania

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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 stock is 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

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 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 1g of liver and 5g 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) followed by heating at the temperature between 210 to 350 °C for about 10 to 20 minutes. Thereafter, 10 ml of concentrated sulphuric acid H_2SO_4 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 and washed using distilled water. The samples were filtered using 15mm 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 dw 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). 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.

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The HI is the sum of all the THQ from individual metal. EWI, THQ and HI were calculated using equations below;

$$EWI = \frac{MC * IRd * 7}{BW}$$

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)

$$THQ = \frac{E_F * E_D * F_{IR} * C}{R_{FD} * W_{AB} * T_A} * 10^{-3}$$

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); R_{FD} 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)

$$HI = \sum_{i=1}^{n} THQ_i$$

THQi 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 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 from 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 the muscle 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. 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% to 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). In farmed milkfish, a significant positive correlation was found between Fe and fish weight, Zn and fish weight and length, while a negative correlation was found between Co and fish weight and length (Table S2A). For wild milkfish, a significant positive correlation was found between Cd and fish weight, and between Zn, Co and Ni and fish weight and length. In addition, a significant positive correlation was found between Cd and Zn, Co and Ni (Table S2B). In wild mullet, a significant positive correlation was found between Cd and Ni (Table S2C). Significant positive correlations were found between Hg and fish weight and length for farmed milkfish and between Hg and fish length in wild mullet (Table S2D).

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 the 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 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 polluted 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). Therefore, the South Equatorial Current (east-west), combined with the East Africa Coastal Current (south-north) could be the main 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 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., 2011), 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., 2011). 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.

4.3 Association among contaminants, fish weight and fish length

Significant positive correlations between Cd, Zn, Co and Ni in wild milkfish muscle, and between Cd, Zn and Ni in wild mullet muscle indicate similar sources for these metals (Table S2 B, C). Fish weight

in farmed milkfish was significantly positively correlated to Fe and Zn. Fish length was only positively correlated to Zn. Fish weight in wild milkfish was significantly positive correlated to Cd, Zn, Co and Ni. Of unknown reasons Co in the farmed milkfish, was negatively correlated to fish weight and length. However, this was a weak correlation ($r<0.5$).

4.4 Possible health risk implications for fish and humans with regard to heavy metals

4.4.1 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 – 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 additionally be exposed to Pb paint on children toys and in playground equipment in African countries.

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.

4.4.2 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.

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 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. This suggest that Pb pollution 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.

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FIGURES AND TABLES

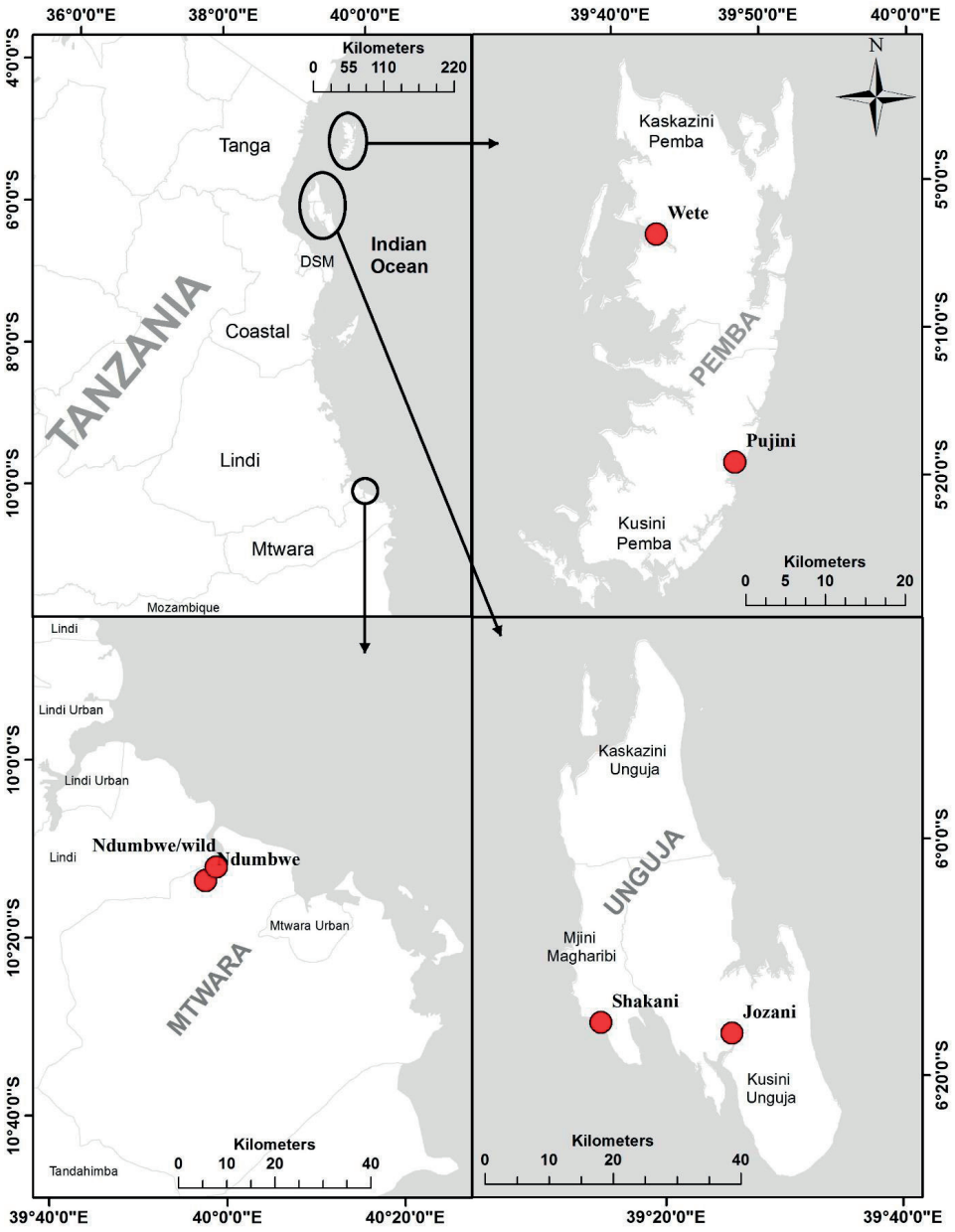
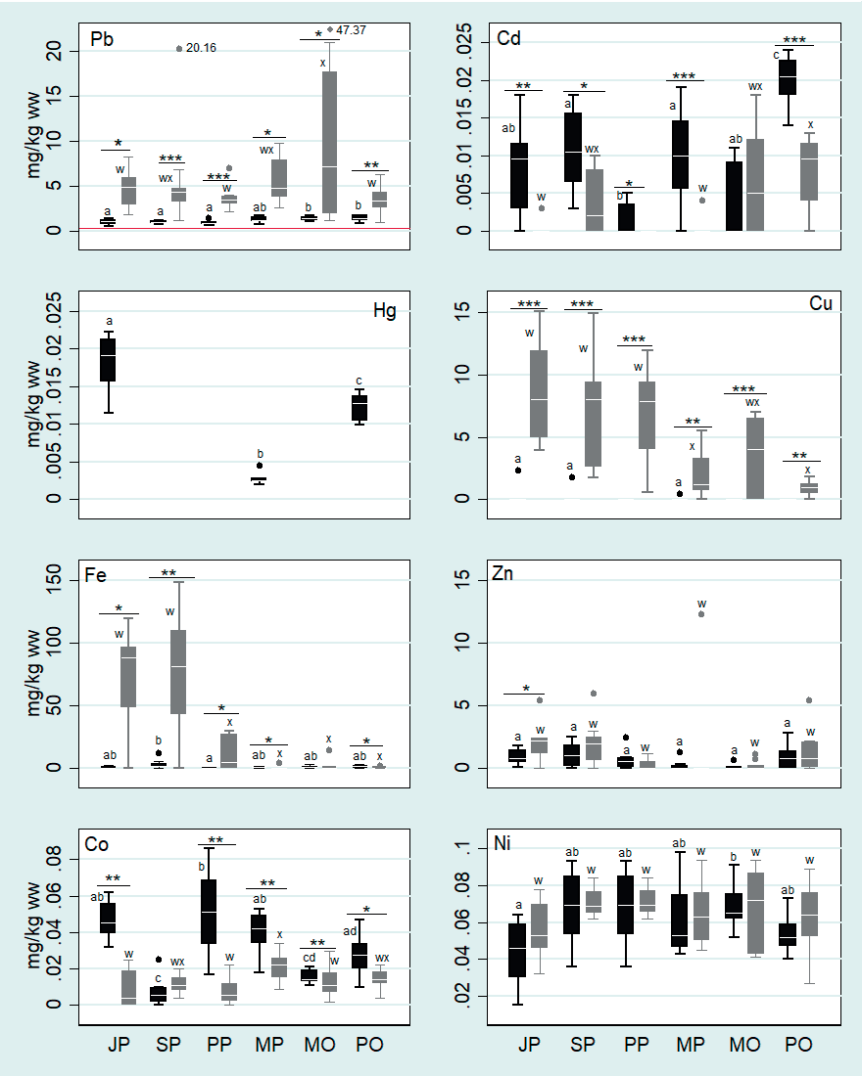


Fig 1. Map of Tanzanian coasts showing location of sampling sites



*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.

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).

(A)

Site	Sampling month/year	Salinity (ppt)	Mean weight (g)	Weight range (g)	Mean length (cm)	No of samples (Muscle)	No of samples (Liver)
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

(B)

Site	Sampling month/year	Salinity (ppt)	Mean weight (g)	Weight range (g)	Mean length (cm)	No of samples (Muscle)
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

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

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	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*

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	0	0	0	30
Ni	0.061	0.073	0.052	
Co	0.041	0.019	0.028	
Cr	0	0	0	

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	$1.96x10^{-5}$	0	$3.15x10^{-5}$	0.004 (EFSA, 2012); JEFCA, 2017
Fe	$1.69x10^{-3}$	$1.54x10^{-3}$	$4.82x10^{-3}$	5.6 (JEFCA, 2017)
Zn	$1.13x10^{-3}$	$4.45x10^{-4}$	$1.90x10^{-3}$	7 (JEFCA, 2017)
Cu	0	0	0	3.5 (JEFCA, 2017)
Ni	$1.5x10^{-4}$	$1.79x10^{-4}$	$1.28x10^{-4}$	0.035 (JEFCA, 2017)
Co	$9.99x10^{-5}$	$4.56x10^{-5}$	$6.78x10^{-5}$	0.21 (Varol et al 2017)
Cr	0	0	0	0.023 (Lin, 2004)
Cd*	$7.4x10^{-5}$	$1.06x10^{-4}$	$2.17x10^{-4}$	0.025 (JEFCA, 2017)

**EMI and PTMI for Cd were calculated based on 30 days*
Farmed milkfish: Jozani, Shakani, Pemba and Mtwara ponds
Wild milkfish: Mtwara, Indian Ocean
Wild Mullet: Pemba, Indian Ocean

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 (USEPA, 2018)
Hg	0.028	0	0.045	0.0001 (USEPA, 2018)
Cd	0.003	0.004	0.007	0.0001 (USEPA, 2018)
Fe	0.0004	0.0003	0.001	0.7 (USEPA, 2018)
Zn	0.0005	0.0002	0.0009	0.3 (USEPA, 2018)
Cu	0	0	0	0.04 (USEPA, 2018)
Ni	0.0011	0.0013	0.0009	0.02 (USEPA, 2018)
Co	0.048	0.022	0.0323	0.0003 (USEPA, 2018)
Cr	0	0	0	0.003 (EFSA, 2014; USEPA, 2018)
Hazard Index	0.177	0.167	0.231	

**RFD, Oral Refence Dose for different heavy metals in fish*

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.

(A)

	Farmed milkfish				Wild milkfish	Wild mullet
	Jozani Pond	Shakani pond	Pemba pond	Mtwara pond	Mtwara	Pemba
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

(B)

	Farmed milkfish				Wild milkfish	Wild Mullets
	Jozani Pond	Shakani pond	Pemba pond	Mtwara pond	Mtwara	Pemba
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*

SUPPLEMENTARY DATA

Table S1: Concentrations of Pb, Cd and Hg in (1) muscles and (2) livers of farmed and wild milkfish and wild mullet from Tanzania with number of samples with concentrations above LOD, percentiles and range (min to max)

1.Muscle	N	Min	25th	50th	75th	95th	Max
Farmed milkfish							
Pb	32	0.535	0.821	0.962	1.130	1.566	1.671
Cd	32	<LOD	0.003	0.007	0.013	0.018	0.019
Hg	12	0.002	0.003	0.008	0.019	0.022	0.022
Wild milkfish							
Pb	8	1.049	1.158	1.387	1.584	1.962	1.962
Cd	8	<LOD	<LOD	0.010	0.013	0.018	0.018
Hg	0	.	.	.	.	.	.
Wild mullet	N						
Pb	8	0.892	1.178	1.426	1.657	1.738	1.738
Cd	8	0.014	0.018	0.021	0.023	0.024	0.024
Hg	6	0.010	0.010	0.013	0.014	0.015	0.015
2.Liver							
Farmed milkfish	N	Min	25th	50th	75th	95th	Max
Pb	32	1.117	3.186	4.132	5.932	9.747	20.160
Cd	32	<LOD	<LOD	<LOD	<LOD	0.009	0.010
Hg	0	.	.	.	.	.	.
Wild milkfish							
Pb	8	4.908	6.402	8.027	19.295	47.370	47.370
Cd	8	<LOD	<LOD	<LOD	0.007	0.010	0.010
Wild mullet							
Pb	8	0.918	2.631	3.338	4.275	6.256	6.256
Cd	8	<LOD	0.004	0.010	0.012	0.013	0.013
Hg	0	.	.	.	.	.	.

Table S2: Correlation between fish weight (FW); fish length (FL)and Pb, Cd, Fe, Zn, Co, Cu and Ni concentrations in the muscles of (A) Farmed milkfish (B) Wild milkfish (C) Wild mullets and (D) Hg. Coefficient of correlation r is given in the table with p-value in italic, significant correlations are highlighted in bold.

(A)

	Pb	Cd	Fe	Zn	Co	Cu	Ni	FW	FL
Cd	-0.14	1							
	0.431								
Fe	0.26	0.34	1						
	0.159	*0.058*							
Zn	-0.17	-0.05	0.26	1					
	0.362	0.796	0.149						
Co	0.18	-0.29	-0.33	-0.08	1				
	0.335	*0.108*	*0.065*	*0.668*					
Cu	-0.05	0.29	0.07	-0.22	-0.20	1			
	0.795	*0.103*	*0.690*	*0.237*	*0.270*				
Ni	-0.16	0.04	0.09	0.31	-0.28	0.05	1		
	0.382	*0.844*	*0.610*	*0.086*	*0.122*	*0.777*			
FW	-0.18	0.3	**0.38**	**0.45**	**-0.43**	0.20	0.01	1	
	0.318	*0.100*	***0.032***	***0.009***	***0.014***	*0.265*	*0.948*		
FL	-0.21	0.17	0.32	**0.45**	-0.35	0.19	-0.06	**0.96**	1
	0.243	*0.358*	*0.075*	***0.011***	*0.050*	*0.298*	*0.718*	***<0.0001***	

(B)

	Pb	Cd	Fe	Zn	Co	Ni	FW	FL
Cd	-0.32	1						
	0.444							
Fe	0.21	-0.24	1					
	0.610	*0.560*						
Zn	0.05	**0.83**	-0.37	1				
	0.909	***0.012***	*0.373*					
Co	-0.57	**0.90**	-0.19	0.66	1			
	0.139	***0.002***	*0.651*	*0.076*				
Ni	-0.55	**0.78**	0.1	0.56	**0.91**	1		
	0.160	***0.022***	*0.823*	*0.148*	***0.002***			
FW	-0.29	**0.98**	-0.26	**0.81**	**0.93**	**0.79**	1	
	0.493	***<0.0001***	*0.531*	***0.016***	***0.001***	***0.021***		
FL	-0.14	**0.93**	-0.21	**0.81**	**0.88**	**0.71**	**0.98**	1
	0.736	***0.001***	*0.610*	***0.016***	***0.004***	***0.047***	***<0.0001***	

(C)

	Pb	Cd	Fe	Zn	Co	Ni	Cr	FW	FL
Cd	0.19	1							
	0.651								
Fe	0.23	-0.22	1						
	0.588	*0.608*							
Zn	0.42	**0.78**	0.07	1					
	0.307	***0.022***	*0.862*						
Co	-0.24	-0.68	0.18	-0.31	1				
	0.568	*0.062*	*0.679*	*0.460*					
Ni	0.06	**0.79**	-0.67	0.54	-0.6	1			
	0.888	***0.020***	*0.070*	*0.167*	*0.119*				
Cr	0.09	0.27	-0.24	0.67	0.345	0.35	1		
	0.826	*0.526*	*0.575*	*0.069*	*0.403*	*0.391*			
FW	-0.42	-0.3	-0.24	-0.55	0.45	-0.31	-0.22	1	
	0.301	*0.471*	*0.565*	*0.156*	*0.268*	*0.450*	*0.601*		
FL	-0.29	-0.08	-0.49	-0.32	0.34	-0.03	0.03	**0.93**	1
	0.490	*0.844*	*0.220*	*0.441*	*0.405*	*0.944*	*0.941*	***0.001***	

(D)

		Hg	FW	FL
Fmi	FW	**0.79**	1	
		0.002		
	FL	**0.8**	**0.99**	1
		0.002	***<0.0001***	
Wmu	FW	0.32	1	
		0.538		
	FL	0.09	**0.93**	1
		0.872	***0.008***	

Table S3: Mean concentration in mg/kg ww of heavy metals (HM) in the muscles and livers of wild and farmed milkfish and wild mullets

	Farmed Milkfish		Wild Milkfish		Wild Mullet	
HM	Muscle	Liver	Muscle	Liver	Muscle	Liver
Pb	1.018	5.006	1.408	14.964	1.394	3.458
Cd	0.008	0.001	0.008	0.003	0.02	0.0079
Hg	0.011	-	-	-	0.012	-
Fe	1.315	39.071	0.852	3.566	1.786	0.26
Zn	0.714	1.4996	0.357	0	0.876	1.423
Cu	0.142	6.100	0	4.65	0	0.898
Ni	0.060	0.066	0.073	0.062	0.054	0.063
Co	0.036	0.012	0.0189	0.009	0.027	0.014
Cr	0	0	0	0	0.011	0

Table S4: The ratios of heavy metals concentration between muscles and livers for (A) For each group and (B) For each site

(A)

	Pb	Cd	Fe	Zn	Co	Cu	Cr	Ni
Farmed milkfish								
Mean	0.203	6.075	0.034	0.476	2.949	0.023	-	0.908
Media n	0.233	-	0.045	0.773	3.857	0.000	-	0.924
Wild milkfish								
Mean	0.094	2.667	0.239	-	2.041	0.000	-	1.185
Media n	0.173	-	-	-	2.176	0.000	-	1.381
Wild mullet								
Mean	0.403	2.540	6.871	0.615	1.904	0.000	-	0.855
Media n	0.427	2.158	-	0.955	1.964	0.000	-	0.813

(B)

	Pb	Cd	Fe	Zn	Ni	Co	Cr	Cu
Jozani pond								
Mean	0.199	19.250	0.013	0.422	0.765	5.561	-	0.034
Median	0.211	-	0.004	0.373	0.868	11.375	-	0.000
Shakani pond								
Mean	0.165	2.932	0.047	0.530	0.964	0.632	-	0.031
Median	0.212	5.250	0.024	0.517	1.007	0.500	-	0.000
Pemba pond								
Mean	0.243	-	0.014	2.323	0.954	6.613	-	0.000
Median	0.258	-	0.000	-	1.000	9.364	-	0.000
Mtwara pond								
Mean	0.223	19.750	1.642	0.129	0.944	1.899	-	0.027
Median	0.257	-	-	-	0.841	1.909	-	0.000
Mtwara wild								
Mean	0.094	2.667	0.239	-	1.185	2.041	-	0.000
Median	0.173	-	-	-	1.381	2.176	-	0.000
Pemba wild								
Mean	0.403	2.540	6.871	0.615	0.855	1.904	-	0.000
Median	0.427	2.158	-	0.955	0.813	1.964	-	0.000

Table S5: 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.
(A)

	Farmed milkfish				**Wild milkfish**	**Wild mullet**
	Jozani Pond	**Shakani pond**	**Pemba pond**	**Mtwara pond**	**Mtwara**	**Pemba**
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

(B)

	Farmed milkfish				Wild milkfish	Wild Mullets
	Jozani Pond	Shakani pond	Pemba pond	Mtwara pond	Mtwara	Pemba
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

III

Manuscript

Concentrations and patterns of persistent organic pollutants (POPs) and heavy metals residues in tilapia (*Oreochromis niloticus)* imported to Tanzania. Assessment of human health risks

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Keywords

Farmed Fish, DDTs, Arsenic, Dioxin-like PCBs, Global transport

Abstract

Fish is an important part of the diet for people in Tanzania. The high price of Tanzanian tilapia and insufficient production has led to increased import from China. In 2017, imported tilapia from China were collected and analysed for POPs and heavy metals. Generally, the concentrations of POPs and heavy metals were low. However, the levels and patterns of compounds in individual fish varied, suggesting that tilapia from the same package were from different locations in China. This was further confirmed by the differences in percent contribution of OCPs, PCBs and PBDEs to the sum of POPs between individual fish. The dominating POP was *p,p'*-DDE and its highest concentration was 5.99 ng/g wet weight. The ratio of *p,p'*-DDE to *p,p'*-DDT indicated both historic and recent use of DDT in the Chinese environment. HCB, HCHs and mirex were detected in more than 85% of the samples. The PCB pattern was dominated by PCB-153>PCB-138>PCB-209. Finding of PCB-209 may suggest that imported goods can introduce PCB-209 to the Tanzanian food web. HBCDD was the dominating BFR. For lifetime exposure, no potential health risks were observed for any POPs or heavy metals. However, the 95th percentile of estimated weekly intake (EWI) for dioxin-like (DL)PCBs; PCB-118 and -105 exceeded the recently adjusted TWI for dioxins and DL-PCBs (2 pgTEQ/kg bw/week), suggesting potential health risks to high fish consumers among Tanzanian adults. In addition, this study found a high contribution of As to the hazard index, which is of concern. The As cancer risk (CR) assessment indicated a potential of carcinogenic risk to Tanzanians from consuming imported tilapia. Nevertheless, it is important to recognise positive effects of a varied diet including fish. For traceability, it is recommended that future monitoring studies include individual samples.

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1. Introduction

Fish is an important part of the diet worldwide, due to its high-quality protein and long-chain omega-3 fatty acids, essential minerals and vitamins (Bosch et al., 2016; FAO, 2018). Due to rapid population growth and stagnation of capture fisheries (FAO, 2014), fish farming has become of increased importance around the world (FAO, 2018). After carp, tilapia is among the most cultured and traded fish species (FAO, 2018). It belongs to the cichlid family, it is omnivorous, feeding on plankton and aquatic plants. Tilapia grow very rapid, which is essential for the aquaculture industry. Nile tilapia, originally from African fresh waters, was exported to other countries in the 1940s and 1950s for fish farming purposes (FAO, 2005; Modadugu and Acosta, 2004). Today, China is the leading country in the production and export of farmed fish worldwide. The Chinese tilapia production in 2017 was 1.93 million metric tons of which 47% accounted for export to other countries including Africa (FAO, 2018; USDA, 2017). Fish import in Tanzania has increased from 3,000 tons in 2010 to 14,000 tons in 2016 with tilapia contributing about 8%. The high price of Tanzanian tilapia and insufficient production has led to the increased import from China (MALF, 2016).

Rapid human population growth, urbanization, industrialization and agricultural development, have increased the number of potentially harmful chemicals in the environment, such as persistent organic pollutants (POPs) and heavy metals (Fernández and Grimalt, 2003; Gu et al., 2015; Lohmann et al., 2007). POPs are persistent, they bioaccumulate in biota and biomagnify through food chain (Lohmann et al., 2007; Walker et al., 2012). POPs and heavy metals can be transported long distance from their origin through long range atmospheric transport (LRAT) (Breivik et al., 2016; Wania and Mackay, 1993) (Kelly and Gobas, 2001). Most of the POPs and heavy metals end up in aquatic environment through river flow, rain runoff and waste discharges. In addition, trade of consumer products can distribute POPs and heavy metals globally (Rotterdam Convention, 2017). POPs have been manufactured since 1940s and are utilized in agriculture, industries, construction and in consumer products (Stockholm Convention, 2018). POPs may cause adverse health effects to animals and humans (Walker et al., 2012; WHO, 2010), and due to their toxic potency and persistence in the environment, more than 20 POPs have been internationally prohibited under the Stockholm Convention, a global treaty for protecting human health and the environment against POPs contamination (Stockholm Convention, 2018). Heavy metals are naturally occurring elements in the earth's crust; they have been used in industries, constructions and in consumer products (Odukudu et al., 2014). However due to various anthropogenic activities such as improper waste management, mining and emission, they may reach levels above recommendation in the environment and biota including fish (Bosch et al., 2016). Due to the potential health effects to humans and biota, mercury (Hg), lead (Pb) and cadmium (Cd) have been included in UNECE (United Nations Economic Commission for Europe) and convention on Long-range Transboundary Air Pollution (LRTAP) aiming at protecting human and the environment from air pollution (UNECE, 1979).

Fish can bioaccumulate POPs and heavy metals in various tissues directly from the surrounding water or indirect through feeding on other organisms. In China, several studies documented levels of POPs and heavy metals in fish for consumption (Gu et al., 2016; Huo et al., 2017; Kong et al., 2005; Shi et al., 2013; Sun et al., 2018a, 2018b). In Tanzania, levels of POPs and heavy metals in wild and farmed fish were documented earlier (Mwakalapa et al., 2018; Mziray and Kimirei, 2016; Polder et al., 2014; Saria, 2016). Despite the fact that fish import is growing in Tanzania, little is known about occurrence and levels of POPs and heavy metals and potential human health risks of consuming imported fish. The Tanzanian Food and Drug authority and Fisheries Division inspect imported fish among others on chemical pollutants. However, governmental inspections like this are in general performed on pooled samples. Therefore, the objectives of the present study were to determine the concentration of POPs and heavy metals in muscles of individual imported tilapia, and to assess health risks to humans.

2. Materials and Methods

2.1. Origin of the samples, sampling and sample treatment

The imported tilapia from China were purchased from a fish trading company in Dar es Salaam, Tanzania. The producer (named on the box) was located in Fujian, East China. The fish (n=23) was degutted before sending and arrived frozen in a paper box.

Sampling was done in Dar es Salaam in March 2017. Four of the fish were discarded based on weight/length and 19 fish remained for analyses. The frozen fish were thawed before subsampling. The skin was removed before collecting a piece of the dorsal muscle. The muscle samples were wrapped in a labelled aluminium foil and frozen at -20°C until transportation to Norway for analysis. During transportation, the samples were kept frozen and stored at -20°C until analysis.

2.2. Sample analysis

The analysis of Persistent Organic Pollutants (POPs) was conducted at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences (NMBU), Campus 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 analysis of heavy metals was done at the Laboratory for Soil and Water analysis, Faculty of Environmental Sciences and Natural Resource Management (MINA), NMBU, Campus Ås, Norway.

The fish muscle samples were analysed for organochlorine pesticides (OCPs): hexachlorobenzene (HCB), α-, β- and γ-hexachlorocyclohexanes (∑HCHs), bis-2,2-(4-chlorophenyl)-1,1,1- trichloroethane (*p,p′*-DDT) and its metabolites *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD and *p,p′*-DDT (∑DDTs), chlordanes:, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor (∑CHLs) and mirex and polychlorinated biphenyls (PCBs): PCB-66, -74, -87, -99, -101, -105, -110, -114, -118, -128, -137, -138, -141, -149, -151, -153, -156, -157, -170, -180, -183, -189, -194, -206 and -209 ($\sum_{25}$PCBs). It was not possible to determine PCB-28, -31, -47, -52 and -187 due to instrumental difficulties on the MS. Because of the analytical problems with PCB-28 and -52, the sum of non-dioxin-like (NDL) indicator (ID) PCBs include only PCB-101, -138, -153 and -180 (∑NDL-ID-$_4$PCBs). Because of low detection frequency of PCB-114 and -189 (<26%), the sum of dioxin-like (DL) PCBs: include only PCB -105, -118, -156, -157 (∑DL-$_4$PCBs). Furthermore, *o,p'*-DDT, oxychlordane, PCB-56, -136, -196 and -199 were analysed but not detected in levels >LOD in any of the 19 fish samples and were not included in sums or discussed further.

The analysed brominated flame retardants (BFRs) were polybrominated diphenyl ethers (PBDEs): BDE-28, -47, -99, -100, -153, -154, -183, -206, -207, -208 and -209 ($\sum_{11}$PBDEs). When nona- and deca- BDEs were excluded for comparison reasons, the $\sum_7$BDEs included BDE-28, -47, -99, -100, -153, -154, -183. Other analysed BFRs were hexabromocyclododecane (HBCDD), pentabromotoluene (PBT), pentabromoethylbenzene (PBEB), 2,3-Dibromopropyl-2,4,6-tribromophenyl ether (DPTE) and hexabromobiphenyl (HBB).

In addition, the fish muscles were analysed for the non-essential heavy metals: Lead (Pb), cadmium (Cd), mercury (Hg), aluminum (Al), arsenic (As), and essential heavy metals: Cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), nickel (Ni), selenium (Se), vanadium (V) and zinc (Zn).

2.3 Analysis of POPs

The analytical method was based on Brevik, (1978) with slight modification as described in Mwakalapa et al (2018) and Polder et al (2008). In brief, approximately 0.5-1 g of homogenized fish muscle was weighed into the pre-cleaned centrifuge glass tubes and internal standards PCB -29, -112 and -207 (Ultra-Scientific, RI, USA); BDE -77, -119, -181, and 13C12-209, 13C12-TBBP-A (Cambridge Isotope Laboratories, Inc., MA, USA) were added. Lipid extraction was done twice following homogenization using Ultra Turax (IKA Ultra-Turrax T25, IKA Laboratory Technology, Staufen, Germany) and ultrasonic (Cole Parmer CPX 750, Vernon Hills IL, USA) homogenizers and centrifuging using Allegra X-12R Centrifuge (Beckman Coulter, Fullerton, CA, USA). The lipid content was measured gravimetrically using 1 ml aliquot of lipid extract and lipid removal/cleanup was done using 96% H_2SO_4 (Fluka Analytika, Sigma-Aldrich, St. Louis, USA). The final extracts were transferred into the 2 ml amber vials for GC analysis after evaporation on a sand bath at 40°C with the blow of N_2.

The separation and detection of OCPs and PCBs were performed by high resolution GC (Agilent 6890 Series gas chromatography system; Agilent Technologies, Avondale, PA, USA) equipped with an injector and auto sampler (Agilent 7683), a dual column system with specifications SPB-5 and SPB-1701, both 60 m, 0.25 mm i.d. and 0.25 μm film thickness (Supelco, Bellefonte, PA, USA) coupled to two 63Ni micro (μ) electron capture detectors (Agilent 6890 μ – ECD). The separation detection of BFRs were performed on HRGC-LRMS configured with a split/splitless injector (Agilent Technologies, Santa Clara, Us). The BFRs were monitored using ECNI on SIM mode at m/z 79/81 for PBDEs, HBCDD, PBT, PBEB, at m/z 79/551 for HBB and at m/z 160/79 for DPTE. The details of the analytical method, instrumental specifications and temperature program have been described earlier in (A Polder et al., 2014; A. Polder et al., 2008b; Polder et al., 2016).

2.3.1 QA/QC for POPs

For every analytical series included three procedural blanks (solvents), one blind (non-spiked cod (*Gadus morhua*)), two spiked samples of cod for recoveries and the laboratory's own reference materials (LRMs) of blubber of harp seal (*Pagophilus groenlandicus*) and minke whale (*Balaenoptera acutorostrata*). The analytical quality was successfully approved by routinely analysing different Certified Reference Materials (CRMs), In addition, the laboratory successfully participated in Arctic Monitoring and Assessment Program (AMAP) ring test for PCBs, OCPs and PBDEs in human serum 2016, and Quasimeme 2016, round 1: QOR126BT, QOR127BT, QBC046BT, QBC047BT for OCs in fish muscle, fish liver and shellfish tissue inter-laboratory studies. The lowest levels of detection (LODs) for individual compounds were defined as three times the noise level. The LODs (ng/g wet weight (ww)) ranged from 0.0007 to 0.013 for OCPs, 0.0006 to 0.0903 for PCBs and 0.001 to 0.005 for BFR. Positive procedural blanks were found for some OCPs and some BDEs. The relative recoveries for OCPs were between 55 and 142%, for PCBs between 87 and 118% and for BFRs between 99 and 112%. The results above and below the limit (80 to 120%) were corrected for recoveries.

2.4 Analysis of Heavy metals

The method used for heavy metals analysis has been previously described in Mwakalapa et al (2018). In brief, approximately 500 mg of muscle sample were weighed in a nitric acid (HNO_3) and Milli-Q water rinsed teflon tubes, then digested using 5 mL HNO_3. Ultrapure concentrated HCl was added to prevent loss of Hg. Internal standard consisting of Sc, Ge, Rh, In and Bi was added. The samples were then decomposed

at 260°C in an UltraClave (Milestone, Italy) then diluted to 50.0 mL using distilled water in centrifuge tubes from Sarstedt. The samples were then analysed on Agilent 8800 QQQ ICP-MS against standards for each element.

2.4.1 QA/QC for heavy metals

For each series, at least one certified reference material (CRM) and 3 blanks were analysed. For this series, DORM-3 (Fish Protein Certified Reference Material for Trace Metals) from National Research Council Canada was analysed. The values assigned for the following metals: Cr, Fe, Ni, Cu, Zn, As, Cd, Hg, and Pb are all within the acceptable range of uncertainty. The limit of detection (LOD, mg/kg ww) were: Li (0.0003), Al (0.02), V (0.0004), Cr (0.001), F (0.04), Co (0.0001), Ni (0.003), Cu (0.0006), Zn (0.006), As (0.001), Se (0.001), Cd (0.0001), Hg (0.0002), and Pb (0.0001).

2.5. Ethical Clearance and permissions to conduct research

Permission to conduct this research in the selected fish was given by the management of Institute of Marine Sciences, University of Dar es Salaam and the Ministry of Livestock and Fisheries Development. The permission to transport samples from Tanzania to Norway was granted by The Ministry of Livestock and Fisheries and The Norwegian Food Safety Authority.

2.6 Estimation of potential human health risks

The potential health risk for humans related to exposure to POPs and heavy metals were assessed using the median and 95th percentiles of measured concentrations, and the average and highest per capita fish consumption (Gerber et al., 2016; Pheiffer et al., 2018; Yohannes et al., 2014).

The assessment of human health risk was done by comparing the concentration of POPs and heavy metals to the Maximum Residue Limits (MRLs) and Maximum Limits (MLs) set by EU food safety and FAO/WHO (EU Food Safety, 2018; FAO/WHO, 1995) , respectively. Estimated Weekly Intake (EWI), Target Hazard Quotient (THQ) and Hazard Index (HI) were calculated to estimate long-term health risks associated with consumption of tilapia, details for the estimation are described in supplementary information S3.1. Due to carcinogenic effect of the As, (USEPA, 1986) has established the highest ($1x10^{-4}$) and lowest ($1x10^{-6}$) acceptable safe standards level range for cancer risk over a lifetime exposure. Carcinogenic risk (CR) for As was calculated using the cancer slope factor (CFS) of 1.5 mg/kg day provided by (USEPA, 2005) and compared with the safe standards.

For assessment of Tanzanian exposure to DL-PCBs, we used the WHO toxic equivalent factor (TEF_{2006}) (Berg et al., 2006) to estimate the toxic equivalents (TEQs) for the DL-PCBs. TEQs were used to calculate the EWIs for DL-PCBs. TEQs values were calculated using the following equation;

$$TEQ_{total} = \sum (C_i * TEF_i)$$

Where TEQ_{total} is the summation of individual dioxin-like PCB, C_i and TEF_i are the concentration and toxic equivalent factor, respectively for the dioxin-like PCBs.

2.7. Statistical data analysis

Data were organized in MS Excel, 2016. JMP 11 statistical software was used for further analysis. Compounds detected in less than 60% of the samples were only reported with range and not included in statistical analysis. The compounds which were detected in more than 60% of the samples were reported with mean, median and range and were included in further statistical analysis. To reduce overestimation the compounds that were detected below LOD were treated as zero during analysis as the concentrations of POPs in general were close to LOD. Shapiro-Wilk Test W was used to test for the distribution of the data. Spearman rank correlation was used to assess the correlation between variables.

3. Results

The fish weight, length, lipid percentages and the concentrations of POPs with their detection frequency are presented in Table. 1. The corresponding results expressed as ng/g lipid weight are presented in Table S1. The fish weight and length ranged between 260 – 396 g and 23.3 – 26.8 cm respectively. The median lipid % was 0.81 ranging from 0.24% to 2.24%. In 84% of the fish the ∑OCPs (∑DDTs, ∑HCHs, ∑CHLs) contributed more than 50% to the ∑POPs (Fig 1A).

3.1 Chlorinated pesticides (OCPs)

DDTs, HCB, HCHs and mirex were detected in more than 85% of the fish in concentrations >LOD. DDTs were the dominating OCPs (Table 1). The median concentration of ∑DDTs was 0.38 ng/g ww (range 0.05 - 6.36 ng/g ww). *p,p'*-DDE was the dominant DDT metabolite contributing 78.3% to the ∑DDTs followed by *p,p'*-DDD (18%) and *p,p'*-DDT (3.7%). The median concentration of *p,p'*-DDE was 0.37 ng/g ww (range 0.04-5.99 ng/g ww). The ratios of *p,p'*-DDE/*p,p'*-DDT ranged from 2 to 1930. The median concentrations of HCB, ∑HCHs, ∑CHLs and Mirex were 0.03, 0.02, 0.01 and 0.02 ng/g ww, respectively. The dominant HCH was β-HCH with a median of 0.01 ng/g ww. The α-HCH, β-HCH and γ-HCH contributed on average 36.51%, 49.51% and 13.98% to the ∑HCHs, respectively. *Trans*-nonachlor was the dominant chlordane contributing on average 44.6% to ∑CHLs, followed by *cis*-chlordane (33.1%) and *cis*-nonachlor (22.3%), respectively.

3.2 PCBs

In general, the penta- and hexa-chlorinated PCBs dominated the PCB pattern in the fish (Fig 1B). In three fish, PCB-209 contributed from 25 to 35% to ∑PCBs. The individual PCB congener pattern (median values) were dominated by PCB-153, followed by PCB-138>PCB-209> PCB-180>PCB-118> PCB-149≈PCB-170>PCB-105>PCB-206, contributing 17.9%, 13.3%, 10.9%, 10.9%, 10.3%, 6.7%, 6.7%, 4.2% and 3.7% to $\sum_{25}$PCBs, respectively. The highest concentrations of PCB-153, -138, -209 and -118 were 0.06, 0.05, 0.05 and 0.04 ng/g ww (Table 1). The ∑NDL-ID-$_4$PCBs contributed 42.1% to $\sum_{25}$PCBs. The ∑DL-$_4$PCBs contributed 18.7% to $\sum_{25}$PCBs. PCB-118 was the dominating DL-PCB, contributing 54.9% to ∑DL-$_4$PCBs.

3.4 BFRs

HBCDD was the dominant BFR (Table 1) and detected in 100% of the fish samples with a median concentration of 0.33 ng/g ww ranging from 0.02 to 1.39 ng/g ww. The median HBCDD concentration contributed 76% to ∑BFRs. The median concentration of $\sum_{11}$PBDEs was 0.03 ng/g ww ranging from 0.01

to 0.25 ng/g ww (Table 1). The individual PBDE congener pattern (median values) was dominated by BDE-209, followed by BDE-47>BDE-154> BDE-206>BDE-100>BDE-153, contributing 45.9%, 18.1%, 14.2%, 9.5%, 8.5%, 3.9% to the $\sum_{11}$PBDEs. The highest concentration of BDE-209 was 0.12 ng/g ww. When excluding the nona- and deca BDEs (BDE-209, BDE-208, BDE-207 and BDE-206), BDE-47 was the dominating BDE congener contributing 40.5% to the $\sum_{7}$PBDE followed by BDE-154 (31.8%) and BDE-100 (19%). HBB was detected in only 37% of the samples. PBT, PBEB and DPTE were not detected in any of the samples. Contributions of individual PBDEs for the individual fish are shown in Fig 1C.

3.5 Heavy metals

Except for Pb and Cd all other non-essential metals were detected in 100% of the samples (Table 2, Figure S1). Pb and Cd were detected in 94.7% and 84.2% of the samples respectively. The dominant non-essential metal was Al with highest median concentration of 0.58 mg/kg ww (range 0.25-1.5 mg/kg ww) followed by As>Hg>Pb>Cd. The essential metals were detected in 100% of the samples, except for Ni, which was detected in 84% of the samples. The dominant essential metal was Zn which had a median concentration of 3.3 mg/kg ww (range 2.6-7.5 mg/kg ww) followed by Fe>Se>Cu>Co≈Ni>Cr>V≈Li (Table 2).

3.6 Associations between POPs and metals

Spearman rank correlation for lipid percentage and dominant POPs are presented in Table S2A and between heavy metals in Table S2B. A significant strong correlation ($r>0.7$) was observed between HCB and *p,p'*-DDT; between trans-nonaklor and BDE-47, PCB-138 and PCB-153; between mirex and BDE-47; between PCB-153 and PCB-138, BDE-47; between PCB-138 and BDE-47 and BDE-47 and HBCDD. For the heavy metals, a significant correlation was observed between Pb and Zn, V; between Cd and Se; between As and Cu; between Al and Zn, V and between Zn and V.

3.7 Human health risk

3.7.1 Maximum Residue Limits (MRLs) and Maximum Limits (MLs)

The MRLs and MLs for POPs and heavy metals are presented in Table S3. The concentrations of POPs and heavy metals in the present study were all below recommended values set by EU and WHO/FAO (EU Food Safety, 2018; FAO/WHO, 1995).

3.7.2 Estimated weekly intake (EWI) for POPs and heavy metals

The median and 95th percentile concentrations were used for estimating EWI for POPs ($\sum$DDTs, HCB, y-HCH, mirex, DL-$_4$PCBs, HBB) and heavy metals (Pb, Hg, Cd, As, Al, Se, Fe, Co, Ni, Cu, Zn, V, Cr, Li) from consumption of the imported tilapia in Tanzania (Tables S4 A, B). The EWI for $\sum$DDTs, HCB, y-HCH, mirex and HBB were below the TWI based on USEPA (2007) and WHO/JECFA (2018). The EWI for DL-PCBs were compared with the previous TWI set by (EU Council, 2001) and the most recent from EFSA (2018) (Table S4 A). The 95th percentile of EWI for DL-PCB-118 for the average fish consumer and the 95th percentile of EWI for DL-PCB-105 and -118 for the high fish consumer exceeded the current TWI (2 pgTEQ/kg bw/week) set by EFSA (EFSA, 2018). When compared to the previous TWI (14 pgTEQ/kg bw/week) all DL-PCBs were far below the recommended value (Fig 2). The 95th percentile of EWI for

∑DL-PCBs for both the average and the high fish consumers exceeded the current TWI but was also below the previous TWI (Table S4 A). For the average and high consumer, the median and 95th TEQ for PCB-118 contributed 55% and 59% to ∑TEQ-DL-$_4$PCBs followed by PCB-105 contributing with corresponding percentages of 23% and 27%, respectively.

For the heavy metals, none of the EWI and EMI exceeded the PTWI and PTMI (Table 4B).

3.7.3 Target Hazard Quotient (THQ) and (HI)

The THQ and HI for the analysed POPs and heavy metals are presented in Tables 3 A and B. All the THQs for POPs were below 1. The highest THQ was observed for *p,p'*-DDE followed by *p,p'*-DDD>PCB-118>BDE-47>PCB-105 contributing 92%, 38.8%, 8.7%, 4.3% and 3.9% to the 95th percentile of HI for high consumers. The HI for both the average and high consumers were less than 1.

For heavy metals all the THQ were less than 1, however the highest THQ was observed for As followed by Hg>Se>Co>Pb contributing 53%, 23%, 7.8%, 7% and 2.9% to the 95th percentile of HI for high consumers. The HI for both the average and high consumers were less than 1, however the 95th percentile of HI for the high consumers was close to 1 (HI=0.76). The 95th percentile carcinogenic risk (CR) value for the As due to exposure from tilapia consumption were 2×10^{-4} and $1.2x10^{-4}$ for both the average and high consumer, which were above the acceptable lifetime cancer risk levels range ($1x10^{-6}$ to $1x10^{-4}$).

4. Discussion

4.1 POPs

In general, the concentrations of POPs were relatively low. However, the levels and patterns of the individual compounds differed between the fish (Fig S2), which suggest that the fish are from different locations. In addition, we found that the percentages of contributions of the sum OCPs, PCBs and PBDEs to the sum of POPs were different between the individual fish (Fig 1A), which support the hypothesis that the fish, packed in one box, were not from the same location in China. This was not expected. For food safety reasons, it is preferable that traded goods have high traceability and standards (FAO/WTO, 2017; Moretti et al., 2003). For this reason, studies as this, analysing individual fish, rather than pooling, can give important information to Food Safety, Health and Trade authorities.

DDTs were the dominating POPs. In two of the fish samples, the ratio of *p,p'*-DDE/*p,p'*-DDT were lower than five (<5) suggesting recent use of DDT while others had higher ratios reflecting more historic use of DDT (Table 1). The pattern of HCHs, dominated by β-HCH, suggested the use of technical mixture of HCHs in the past. Although in low and varying concentrations, the present study showed that mirex still is present in the Chinese environment. Mirex was used as insecticide to eliminate ants (Alley, 1973) and was banned in 1978 by the Stockholm Convention (USEPA, 2009).

Just like the OCPs, PCB levels and patterns were in general low and varying between the individual fish (Fig 1B). Surprisingly, PCB-209 contributed 9.6% to $\sum_{25}$PCBs in the present study (Fig 1B). PCB-209 was present in less than 1% of all commercial PCBs in the past. However, it is produced as a by-product of several chemical industries and wastewater treatment in USA, China and in other countries (Howell et al., 2008; Huo et al., 2017; Knutzen et al., 2001). In Eastern China, Huo et al., (2017) found PCB-209 in sediment of a freshwater lake and suggested a pigment industry as possible source. To our knowledge,

PCB-209 has never been analysed and/or detected in any study in Tanzania. Therefore, the import of fish contaminated with PCB-209 might introduce this compound to the Tanzanian food web.

HBCDD was in general the dominating BFR. Studies in China confirm ongoing production and distribution of HBCDD (Li et al., 2016; Sun et al., 2018a). Sun et al., (2018) suggested e-waste recycling activities, harbor construction and shipment to be the sources of HBCDD in the coastal environment of China. Li et al., (2016) points out that emission of HBCDD might come from the production and disposal of HBCDD containing products such as insulation boards. The Stockholm Convention banned HBCDD in 2013 (Stockholm Convention, 2018). However, the estimation of future production of HBCDD in China may be as much as 238 Kilo tones (Kt) by 2020 (Li et al., 2016). HBCDD was found present in the Tanzanian environment earlier, but only in few samples of tilapia, wild mullet, chicken eggs and humans (Müller et al., 2016; Mwakalapa et al., 2018; Polder et al., 2016, 2014). These findings were related to the disposal of imported consumer products. The present study found that BDE-209 dominated the PBDE pattern in 68% of the analysed fish. This was not unexpected, as BDE-209 is still produced and used in China in large quantities (Zhang et al., 2017) although it is listed for elimination by the Stockholm convention. The rest of the PBDE pattern showed ongoing exposure to penta- and hexa-BDEs in Chinese aquatic environment.

4.2 Metals

In general, metals were in low concentrations, with wide ranges between fish. The weak correlations between fish (Table S2B) suggest that tilapia were exposed to different sources of heavy metals. This supports again the hypothesis that the studied tilapia might be originating from different locations. Industrial activities such as mining discharges from chemical industries, atmospheric deposition were suggested as major sources of As pollution in China (Chen et al., 2015; He and Charlet, 2013; Huang et al., 2018). The As is highly toxic and it is found naturally in high concentrations in ground water in various places worldwide (National Research Council, 1999; WHO, 2018). Consumption of the imported tilapia from China is a potential additional exposure route to As for humans apart from drinking water in Tanzania (Kassenga and Mato, 2008).

4.3 Assessment of human health risk

Various organizations have set international guidelines for maximum levels for the consumption and trade of fish contaminated with POPs and heavy metals. The POPs and metals concentration in imported tilapia from China were below MRLs and MLs, suggesting these fish are safe for trading and consumption.

Humans are exposed to POPs and heavy metals through consumption of fish. There is a need for assessing human health risks from longterm exposure of POPs and heavy metals. The EWI for ∑DDTs, HCB, y-HCH, mirex and HBB were all below the TWI set by WHO/JECFA (2018) and USEPA (2007), indicating no potential health risk from consumption of tilapia from China (Table 3A). Recently (November 2018) EFSA published a new TWI (2 pg TEQ/kg bw/week) for DL-PCBs (EFSA, 2018) which is 7 times lower than the previous TWI (14 pg TEQ/kg bw/week) (EU Council, 2001). Based on the current TWI, the consumption of tilapia from China will pose health risk from the DL-PCBs PCB-118 and -105 for the average and high consumer (Table 3A). However, when using the previous TWI, there will be no potential health risk from consumption of imported tilapia.

Moreover, the THQs for the POPs and heavy metals that are considered as non-carcinogenic indicated no potential human health risk over a lifetime from single or mixture of POPs and heavy metals by consuming imported tilapia in this study. However, the major contributions of DDTs (85%) and As (53%) to the HI

for POPs and heavy metals, respectively, is of concern. The HI (0.8) for sum THQs for heavy metals for high consumers, which is close to 1, is also of concern to the general public. Moreover, the carcinogenic risk for the As exceeded acceptable cancer risk safe level range ($1x10^{-6}$ to $1x10^{-4}$). This indicate possible carcinogenic risk due to consumption of tilapia imported from China.

5. Conclusion

This study revealed that tilapia imported from China is a source of exposure of POPs and heavy metals to the Tanzanian population. Furthermore, the study revealed that the concentrations and patterns for the POPs and heavy metals varied between the individual fish. Based on the varying concentration and different patterns, the study suggests that the tilapia, which were in one package, might originate from different locations in China. The dominating POP was *p,p'*-DDE and its ratio to the *p,p'*-DDT suggest both previous and recent use of DDT in the Chinese environment. To our knowledge, PCB-209 has never been analyzed and/or been detected in any study in Tanzania. The finding of PCB-209 in the imported Chinese tilapia suggests that PCB-209 can be introduced to the Tanzanian food web and environment by imported goods.

This study found relatively low levels of As in the imported tilapia. However, because of the high toxicity of As and its high contribution to the hazard index, As in imported fish are of concern as fish consumption may thus be an additional source of exposure to natural ground water sources in Tanzania.

In general, POPs and heavy metals did not pose any potential human health risks from consumption of the imported fish. However, the EWI for DL-PCB-105 and -118, for both average and high fish consumers, exceeded the TWI, which means that PBC-105 and -118 may pose health risk for humans when consuming tilapia from China. Moreover, the 95th percentile cancer risk levels for As, for both average and high fish consumers, exceeded the acceptable cancer risk safe level range, indicating potential carcinogenic effect to humans when consuming tilapia from China. The present study only assessed health risks related to POPs and heavy metals from consumption of fish. Thus, other food items and exposure sources may increase the risk. On the other hand, according to the Norwegian Scientific Committee for Food Safety (VKM, 2006) the positive effects of a varied diet that includes fish may counterbalance the potential of negative effects.

For future studies, it is suggested that the Food Safety authorities in Tanzania may routinely include individual sampling for monitoring purposes in order to elucidate the homogeneity of the traded fish, and to enable a more thorough risk assessment.

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FIGURES AND TABLES

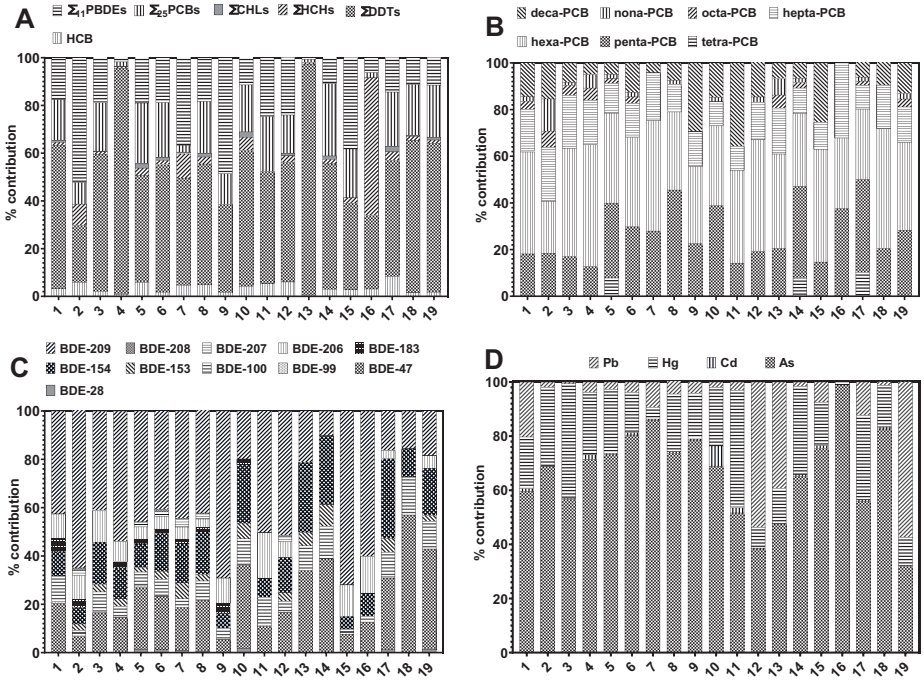

Fig 1: Pattern of POPs in individual tilapia muscle tissue for A) POP groups as percent contribution to the sum POPs B) PCB homologues percent contribution to the sum PCBs C) PBDE percent contribution to the sum of PBDEs D) Toxic heavy metals percent contribution to the sum of toxic heavy metals

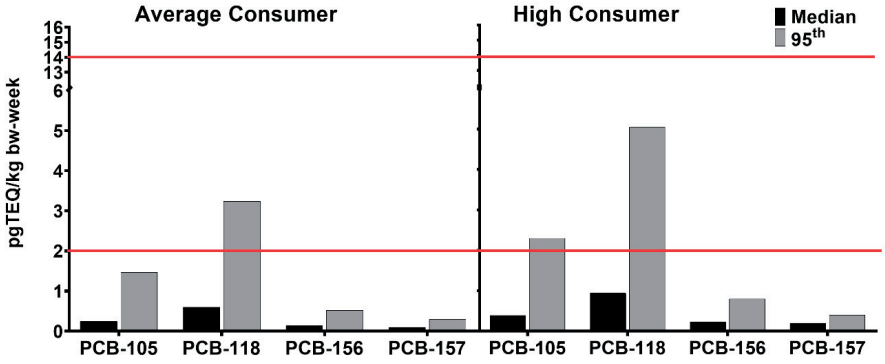

Figure 2: Median and 95th Estimated weekly intake (EWI) of dioxin like PCBs, DL-PCB-105, 118, 156, 157 in pgTEQ/kg bw-week for the average and high fish consumer. The red lines represent the current and previous maximum tolerable weekly intake (TWI) for DL-PCBs.

Table 1: Concentrations (ng/g wet weight) of organochlorine pesticides (OCP), polychlorinated biphenyls (PCBs), brominated flame retardants (BRFs) in muscle tissue of imported tilapia in Tanzania, number of samples, percent detection > LOD, percentiles and range (min to max)

	N>LOD	%>LOD	Min	25th	50th	75th	95th	Max
Weight			260	310	336	360	396	396
Length			23.3	24.7	25.4	26	26.8	26.8
Lipid%			0.24	0.34	0.81	1.25	2.24	2.24
HCB	19/19	100	0.004	0.01	0.03	0.04	0.18	0.18
α-HCH	17/19	89.5	<LOD	0.003	0.01	0.01	0.04	0.04
β-HCH	13/19	68.4	<LOD	<LOD	0.01	0.02	0.18	0.18
γ-HCH	12/19	63.2	<LOD	<LOD	0.003	0.006	0.028	0.028
∑HCHs	17/19	89.5	<LOD	0.01	0.02	0.04	0.24	0.24
p,p'-DDE	19/19	100	0.04	0.12	0.37	0.66	5.99	5.99
p,p'-DDT	14/19	73.7	0.0001	0.0001	0.02	0.05	0.09	0.09
∑DDTs			0.05	0.13	0.38	0.81	6.36	6.36
p,p'-DDE/*p,p'*-DDT			2.85	13.4	23.5	492.6	1930	1930
trans-nonachlor	12/19	63.2	<LOD	<LOD	0.002	0.01	0.02	0.02
cis-nonachlor	13/19	68.4	<LOD	<LOD	0.001	0.005	0.01	0.01
∑CHLs			<LOD	<LOD	0.01	0.02	0.05	0.05
Mirex	18/19	94.7	<LOD	0.01	0.02	0.04	0.07	0.07
PCB-101	7/19	36.8	<LOD					0.031
PCB-105	19/19	100	0.001	0.001	0.003	0.01	0.02	0.02
PCB-114	5/19	26.3	<LOD					0.002
PCB-118	19/19	100	0.002	0.004	0.01	0.03	0.04	0.04
PCB-138	19/19	100	<LOD	0.003	0.01	0.04	0.05	0.05
PCB-153	17/19	89.5	0.003	0.006	0.01	0.04	0.05	0.06
PCB-156	12/19	63.2	<LOD	<LOD	0.002	0.005	0.01	0.01
PCB-157	10/19	52.6	<LOD	<LOD	0.001	0.002	0.003	0.003
PCB-180	19/19	100	0.002	0.002	0.01	0.02	0.03	0.03
PCB-189	5/19	26.3	<LOD					0.001
PCB-209	18/19	94.7	<LOD	0.00	0.01	0.02	0.05	0.05
∑NDL-ID$_4$PCBs			0.005	0.01	0.04	0.12	0.16	0.16
∑DL-$_4$PCBs			0.003	0.01	0.01	0.05	0.07	0.07
∑$_{25}$PCBs			0.01	0.03	0.08	0.33	0.50	0.50
BDE-47	19/19	100	0.003	0.01	0.02	0.05	0.09	0.09
BDE-99	5/19	26.3	<LOD					0.002
BDE-100	19/19	100	0.001	0.004	0.01	0.02	0.03	0.03
BDE-153	13/19	68.4	<LOD	<LOD	0.003	0.01	0.02	0.02
BDE-154	19/19	100	0.002	0.01	0.01	0.04	0.10	0.10
BDE-183	9/19	47.4	<LOD					0.005
BDE-209	19/19	100	0.004	0.02	0.04	0.09	0.12	0.12
∑$_7$PBDEs			0.03	0.05	0.10	0.23	0.31	0.31
∑$_{11}$PBDEs			0.01	0.02	0.03	0.12	0.25	0.25
HBCDD	19/19	100	0.02	0.05	0.33	0.69	1.39	1.39

∑DDTs: *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDT

∑HCHs: α-HCH, β-HCH, γ-HCH

∑CHLs: *trans*-chlordan, *cis*-chlordan, *trans*-nonachlor, *cis*-nonachlor

∑$_{25}$PCBs: PCB-66, -74, -87, -99, -101, -105, -110, -114, -118, -128, -137, -138, -141, -149, -151, -153, -156, -157, -170, -183, -180, -189, -194, -206, -209

∑DL-$_4$PCBs: PCB-118, -105, -156, -157

∑NDL-ID$_4$PCBs: PCB-101, -153, -138, -180

$\sum_{11}$PBDEs: BDE-28, -47, -99, -100, -153, -154, -183, -206, -207, -208 and -209
$\sum_{7}$PBDEs: BDE-28, E-47, -100, -99, -154, E-153, -183

Table 2: Concentrations of heavy metals (mg/kg wet weight) in muscle tissue of imported tilapia in Tanzania with number of samples, percentiles and range (min to max)

N=19	mean	min	25th	50th	75th	95th	max
Weight	100	330	260	310	336	390	396
Pb	0.02	<LOD	0.001	0.003	0.012	0.14	0.14
Hg	0.02	0.002	0.016	0.017	0.021	0.051	0.051
Cd	0.001	<LOD	0.0004	0.0005	0.0008	0.0038	0.0038
As	0.07	0.021	0.042	0.061	0.077	0.22	0.22
Al	0.63	0.25	0.37	0.58	0.81	1.5	1.5
Se	0.41	0.28	0.36	0.41	0.47	0.54	0.54
Fe	2.18	0.75	1.6	2	2.3	4.8	4.8
Co	0.01	0.0028	0.009	0.01	0.016	0.029	0.029
Ni	0.01	<LOD	0.01	0.01	0.01	0.012	0.012
Cu	0.13	0.076	0.11	0.13	0.15	0.24	0.24
Zn	3.72	2.6	2.9	3.3	3.8	7.5	7.5
V	0.01	0.001	0.0016	0.003	0.0082	0.093	0.093
Cr	0.02	0.003	0.004	0.0081	0.012	0.067	0.067
Li	0.00	0.001	0.002	0.003	0.0034	0.0079	0.0079

Table 3: Total hazard quotient (THQ) for (A) POPs and (B) heavy metals, Hazard Index and the % contribution of individual metal to Hazard index

A)

	Average consumer		High consumer		
	Median	95th	Median	95th	%Contribution*
p,p'-DDE	4.29E-04	7.03E-03	6.67E-04	1.09E-02	***59.11***
p,p'-DDD	9.83E-04	2.97E-03	1.53E-03	4.61E-03	***24.93***
p,p'-DDT	2.05E-05	1.10E-04	3.18E-05	1.71E-04	0.92
HCB	1.30E-05	8.08E-05	2.02E-05	1.26E-04	0.68
γ-HCH	3.96E-06	3.34E-05	6.16E-06	5.20E-05	0.28
α-HCH	3.88E-07	1.97E-06	6.03E-07	3.07E-06	0.02
Mirex	4.26E-05	1.25E-04	6.63E-05	1.94E-04	1.05
PCB-105	1.85E-04	3.01E-04	2.89E-04	4.69E-04	2.53
PCB-114	1.43E-05	2.89E-05	2.23E-05	4.49E-05	0.24
PCB-118	4.23E-04	6.63E-04	6.58E-04	1.03E-03	5.58
PCB-156	6.88E-05	1.05E-04	1.07E-04	1.64E-04	0.89
PCB-157	3.44E-05	5.27E-05	5.35E-05	8.21E-05	0.44
PCB-189	1.40E-05	1.51E-05	2.18E-05	2.35E-05	0.13
BDE-47	5.49E-05	3.30E-04	8.55E-05	5.13E-04	2.77
BDE-99	1.76E-06	7.61E-06	2.74E-06	1.18E-05	0.06
BDE-153	5.92E-06	3.25E-05	9.22E-06	5.05E-05	0.27
BDE-209	1.99E-06	5.90E-06	3.10E-06	9.18E-06	0.05
HBCDD	5.80E-07	2.44E-06	9.03E-07	3.80E-06	0.02
HBB	8.80E-08	2.19E-06	1.37E-07	3.42E-06	0.02
Hazard index	2.30E-03	1.19E-02	3.58E-03	1.85E-02	

**% percent contribution to Hazard Index calculated for 95th percentile of the high consumers; significant percent contribution to the hazard index are presented in bold and italic*

B)

	Avg consumer		High consumer		
HM	median	95th	median	95th	%Contribution*
Pb	0.0003	0.0141	0.0004	0.0219	2.90
Hg	0.0374	0.1122	0.0582	0.1747	***23.15***
Cd	0.0002	0.0013	0.0003	0.0021	0.28
As	0.0716	0.2582	0.1114	0.4019	***53.25***
Al	0.0002	0.0005	0.0003	0.0008	0.11
Se	0.0289	0.0380	0.0449	0.0592	7.84
Fe	0.0010	0.0024	0.0016	0.0038	0.50
Co	0.0117	0.0340	0.0183	0.0530	7.02
Ni	0.0002	0.0002	0.0003	0.0003	0.04
Cu	0.0011	0.0021	0.0018	0.0033	0.44
Zn	0.0039	0.0088	0.0060	0.0137	1.82
V	0.0001	0.0036	0.0002	0.0057	0.75
Cr	0.0010	0.0079	0.0015	0.0122	1.62
Li	0.0005	0.0014	0.0008	0.0022	0.29
Hazard Index	0.1581	0.4849	0.2460	0.7547	

**% percent contribution to Hazard Index calculated for 95th percentile of the high consumers; significant percent contribution to the hazard index are presented in bold and italic*

SUPPLEMENTARY DATA

Table S1: Lipid percentage, weight and length of the fish and concentrations in ng/g lw of organochlorine pesticides (OCP), polychlorinated biphenyl (PCBs) and brominated flame retardants (BRFs) in the muscles of imported tilapia presented in median and range (min-max)

	Min	Median	Max
Weight	260	336	396
Length	23.3	25.4	26.8
Lipid%	0.24	0.81	2.24
HCB	1.05	2.61	8.20
α-HCH	<LOD	1.14	5.38
β-HCH	<LOD	1.48	19.9
γ-HCH	<LOD	0.29	6.81
∑HCHs	<LOD	2.78	25.7
p,p'-DDE	3.50	41.8	269
p,p'-DDT	<LOD	2.08	7.87
∑DDTs	4.73	52.5	285
p,p'-DDE/p,*p'*-DDT	2.85	23.5	1930
trans-nonachlor	<LOD	0.43	1.48
cis-nonachlor	<LOD	0.31	0.89
∑CHLs	<LOD	0.86	2.85
Mirex	<LOD	2.50	14.9
PCB-101	<LOD	<LOD	4.16
PCB-105	0.08	0.86	1.97
PCB-114	<LOD	<LOD	0.24
PCB-118	0.25	1.96	4.82
PCB-138	<LOD	2.14	6.09
PCB-153	0.28	2.38	6.67
PCB-156	<LOD	0.16	0.77
PCB-157	<LOD	0.06	0.41
PCB-180	0.21	1.18	4.14
PCB-189	<LOD	<LOD	0.12
PCB-209	<LOD	1.98	6.25
∑NDL-ID$_4$PCBs	0.51	6.24	20.05
∑DL-$_4$PCBs	0.33	3.21	7.93
∑$_{25}$PCBs	0.99	15.53	58.2
BDE-47	0.35	3.84	10.5
BDE-99	<LOD	<LOD	0.17
BDE-100	0.07	1.33	2.92
BDE-153	<LOD	0.38	3.63
BDE-154	0.26	1.86	8.90
BDE-183	<LOD	<LOD	1.14
BDE-209	0.41	6.76	24.4
∑$_7$PBDEs	0.69	9.18	25.8
∑$_{11}$PBDEs	1.91	19.0	54.5
HBCDD	2.01	32.8	223

∑DDTs: *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDT

∑HCHs: α-HCH, β-HCH, γ-HCH

∑CHLs: *trans*-chlordan, *cis*-chlordan, *trans*-nonachlor, *cis*-nonachlor

∑$_{25}$PCBs: PCB-66, -74, -87, -99, -101, -105, -110, -114, -118, -128, -137, -138, -141, -149, -151, -153, -156, -157, -170, -183, -180, -189, -194, -206, -209

∑DL-$_4$PCBs: PCB-118, -105, -156, -157
∑NDL-ID$_4$PCBs: PCB-101, -153, -138, -180
∑$_{11}$PBDEs: BDE-28, -47, -99, -100, -153, -154, -183, -206, -207, -208 and -209
∑$_7$PBDEs: BDE-28, -47, -100, -99, -154, -153, -183

Table S2: Correlation between dominant A) POPs and B) Heavy metals

A

	Lipid	HCB	β-HCH	γ-HCH	*p,p'*-DDE	*p,p'*-DDT	*trans*-nonachlor	Mirex	PCB-153	PCB-138	BDE-47	BDE-209
HCB	0.23	1										
β-HCH	**0.55***	**0.54***	1									
γ-HCH	-0.07	0.28	0.37	1								
p,p'-DDE	0.12	0.33	-0.07	-0.11	1							
p,p'-DDT	**0.51***	**0.68***	**0.53***	0.41	**0.51***	1						
trans-nonachlor	0.06	**0.82*****	0.4	0.34	0.36	**0.62***	1					
Mirex	-0.25	**0.60***	0.17	0.34	**0.46***	0.43	**0.68***	1				
PCB-153	-0.41	0.38	-0.15	0.15	0.45	0.24	**0.72****	**0.61***	1			
PCB-138	-0.26	0.41	-0.12	0.09	**0.49***	0.29	**0.74****	**0.64***	**0.97*****	1		
BDE-47	-0.17	**0.68***	0.3	0.38	0.36	**0.52***	**0.85*****	**0.90*****	**0.70****	**0.70****	1	
BDE-209	-0.43	0.18	-0.15	-0.11	-0.16	-0.09	0.15	0.37	0.16	0.09	0.28	1
HBCDD	-0.06	**0.67***	0.22	**0.52***	0.2	**0.53***	**0.71****	**0.68***	**0.49***	**0.51***	**0.76****	0.21

<0.05, *<0.001, *<0.00001*

B

	Pb	Hg	Cd	As	Al	Se	Zn	Cu	Ni	Co	Fe	Cr	V
Hg	0.07	1											
Cd	0.20	0.06	1										
As	0.08	0.004	-0.23	1									
Al	**0.68***	-0.16	0.20	-0.15	1								
Se	0.01	0.45	**0.52***	-0.21	0.02	1							
Zn	**0.73****	-0.17	-0.06	0.27	**0.59***	-0.25	1						
Cu	0.25	-0.23	-0.05	**0.46***	0.05	-0.26	0.27	1					
Ni	0.18	0.26	0.09	-0.39	-0.08	0.38	-0.18	-0.11	1				
Co	0.25	-0.23	0.22	**-0.54***	0.40	-0.01	0.11	-0.15	0.06	1			
Fe	0.01	-0.04	0.18	-0.07	0.19	-0.20	0.22	-0.01	-0.04	0.28	1		
Cr	0.43	0.21	-0.001	-0.19	0.13	0.21	0.43	0.12	0.41	0.17	0.02	1	
V	**0.65***	0.23	0.26	-0.22	**0.49***	-0.01	**0.51***	0.01	0.10	0.16	0.33	0.45	1
Li	0.06	-0.10	-0.05	-0.22	0.07	-0.14	0.02	-0.25	-0.13	0.45	0.20	0.04	0.19

**<0.05, *<0.001*

Table S3: Maximum acceptable levels (MRLs) and Maximum Limits (MLs) in mg/kg ww in eddible tissue of fish recommended By EU for POPs and WHO/FAO for heavy metals

Contaminant	EU	WHO/FAO
DDT	100	-
HCB	100	-
Mirex	100	-
γ-HCH	100	-
CHL	100	-
PCBs	75	-
Pb	-	0.3
Cd	-	0.1
Hg	-	0.5
Fe	-	43
Zn	-	30
Cu	-	30

Table S4: (A) Estimated weekly intake (EWI) for POPs in 1) ng/kg body weight (bw)/week; 2) pgTEQ/kg bw/week and (B) EWI for heavy metals (mg/kg bw/week) and estimated monthly intake for Cadmium ((EMI) mg/kg bw/month)

A)

	Average consumer		High consumer			
	Median	95th	Median	95th		
1. ng/kg bw-week					USEPA (2018)	FAO/WHO (2005)
DDTs	0.04	0.23	0.07	0.36	3500	70000
HCB	0.07	0.45	0.11	0.70	5600	
γHCH	0.01	0.07	0.01	0.11	2100	
Mirex	0.06	0.17	0.09	0.27	1400	
HBB	0	0.03	0	0.05	14000	
2. pgTEQ/kg bw-week					EC (2001)	EFSA (2018)
PCB-105	0.25	1.47	0.39	***2.30***	14	2
PCB-118	0.60	***3.24***	0.94	***5.05***	14	2
PCB-156	0.14	0.52	0.22	0.80	14	2
PCB-157	0.1	0.3	0.2	0.4	14	2
sum-TEQs	1.1	***5.5***	1.7	***8.5***	14	2

**TWI set by EC in 2001, **TWI set by EFSA in 2018, EWIs above safe levels are presented in bold and italic.*

B)

	Avg consumer		High consumer			
	median	95th	median	95th	TWI/TMI**	
Pb	6.90E-06	3.45E-04	1.07E-05	5.37E-04	0.025	JECFA, 2018
Hg	4.19E-05	1.26E-04	6.52E-05	1.96E-04	0.004	JECFA, 2011
Cd*	5.28E-06	4.01E-05	8.22E-06	6.25E-05	0.025	JECFA, 2013
As	1.50E-04	5.42E-04	2.34E-04	8.44E-04	0.015	JECFA, 1988
Al	1.43E-03	3.70E-03	2.22E-03	5.75E-03	2	JECFA, 2011
Se	1.01E-03	1.33E-03	1.57E-03	2.07E-03	0.035	USEPA, 2007
Fe	4.93E-03	1.18E-02	7.67E-03	1.84E-02	5.6	JECFA, 1983
Co	2.47E-05	7.15E-05	3.84E-05	1.11E-04	0.21	Finley et al 2012
Ni	2.47E-05	2.96E-05	3.84E-05	4.60E-05	0.035	USEPA, 2007
Cu	3.20E-04	5.92E-04	4.99E-04	9.21E-04	3.5	JECFA, 1982
Zn	8.13E-03	1.85E-02	1.27E-02	2.88E-02	2.1	JECFA, 1982
V	7.40E-06	2.29E-04	1.15E-05	3.57E-04	0.007	USEPA, 2007
Cr	2.00E-05	1.65E-04	3.11E-05	2.57E-04	2.1	EFSA,2014
Li	7.40E-06	1.95E-05	1.15E-05	3.03E-05	14	USEPA, 2007

**calculated for Estimated Monthly Intake (EMI) (30 days), **Tolerable Monthly Intake (TMI) for Cd*

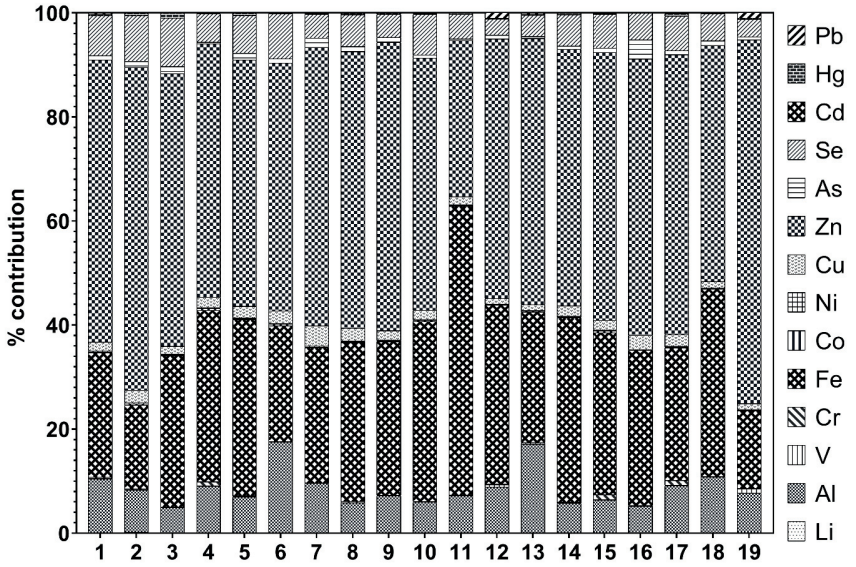

Figure S1: Heavy metals profile in individual tilapia muscle tissue showing percent contribution to total metal burden, based on metal concentrations in mg/kg wet weight

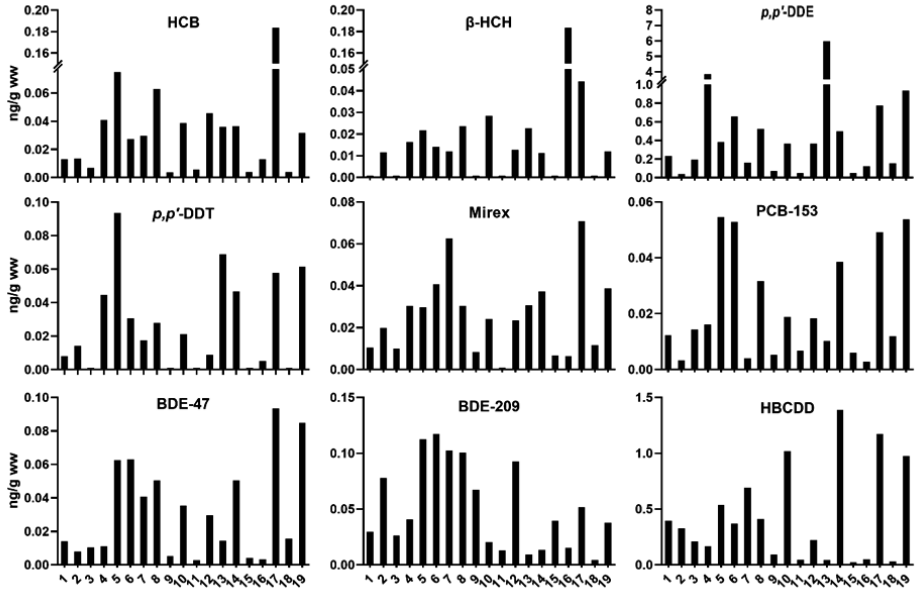

Figure S2: Concentrations of dominant POPs in ng/g wet weight in individual tilapia muscle tissue.

S3. Material and methods

S3.1 Risk assessment of health

Risk assessment of health from consumption of POPs and heavy metals was assessed by comparing the detected concentration of contaminants in sampled imported tilapia with the maximum residue limits (MRLs) and maximum limits (MLs) set by the European Union (EU), joint Food and Agricultural Organization and World Health Organization (FAO/WHO and European Commission (EC). Furthermore, anticipated heavy metals contamination burden and long-term effect was estimated by using Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI) as adopted from (USEPA, 1989). These were calculated for all the analysed metals in the present study. EDI entails the amount of heavy metals concentration ingested by an adult person due to consumption of contaminated fish muscles per day. THQ as a ratio of amount of chemical ingested over a period of time to the reference dose (RFD) of each metal entails the non-carcinogenic risk to human being from individual mental through consumption of fish. THQ<1 assumes the level of exposure of individual metal below which it is unlikely to experience adverse. The HI is the sum of all the THQ from individual metal. EDI, THQ and HI were calculated using equations below;

$$EDI = \frac{MC * IRd}{BW}$$

Where, MC is the metal concentration in fish muscles (mg/kg ww), IRd is the daily average fish consumption by an adult person (24.66 g/day) (MALF., 2016) and BW is an average body weight for an adult individual (70 kg). FAO, 2018, assumes 38.36 g/day for the high consumer in East Africa.

$$THQ = \frac{EF * ED * FIR * C}{RFD * WAB * TA} * 10^{-3}$$

Where, EF is the is the exposure frequency to heavy metals (365 days/yr.); ED is the exposure duration (61.8 years) equivalent to life expectancy; FIR is the fish ingestion rate (x g/day); C is the metal concentration in fish muscle (mg/kg ww); RFD is the oral reference dose (mg/kg/day); WAB is the average body weight of an adult person (70 kg) and TA is the average exposure time with non-carcinogenic effect (EF*ED).

$$HI = \sum_{i=1}^{n} THQ_i$$

Where, HI is the hazard index and THQi is the THQ of the individual metal and n is the number of metals analysed in the study.

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