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Persistent organic pollutants (POPs) and per- and polyfluoroalkyl substances (PFASs) in liver from wild and farmed tilapia (*Oreochromis niloticus*) from Lake Kariba, Zambia: Levels and geographic trends and considerations in relation to environmental quality standards (EQSs)

Chalumba Kachusi Simukoko [a,b], Eliezer Brown Mwakalapa [c], Kaampwe Muzandu [b], Stephen Mutoloki [d], Øystein Evensen [d], Erik Magnus Ræder [a], Mette Bjørge Müller [e], Anuschka Polder [a,*], Jan Ludvig Lyche [a]

[a] Department of Paraclinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P. O. Box 5003 NMBU, 1432 Ås, Norway
[b] Department of Biomedical Sciences, School of Veterinary Medicine, University of Zambia, P. O. Box 32379 Lusaka, Zambia
[c] Department of Natural Sciences, Mbeya University of Science and Technology, P. O. Box 131, Mbeya, Tanzania
[d] Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P. O. Box 5003 NMBU, 1432 Ås, Norway
[e] Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P. O. Box 5003 NMBU, 1432 Ås, Norway

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ABSTRACT

The current study was carried out to investigate a wide variety of persistent organic pollutants (POPs) in wild and farmed tilapia *(Oreochromis niloticus)* in Lake Kariba, Zambia, and assess levels of POPs in relation to Environmental Quality Standards (EQSs). Concentrations of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyls (PBDEs), and perfluoroalkyl substances (PFASs) were determined in liver samples of tilapia. PFASs compounds PFOS, PFDA and PFNA were only detected in wild fish, with the highest median PFOS levels in site 1 (0.66 ng/g ww). Concentrations of POPs were in general highest in wild tilapia. The highest median $\sum$DDTs (93 and 81 ng/g lw) were found in wild tilapia from sites 1 and 2, respectively 165 km and 100 km west of the fish farms. Lower DDE/DDT ratios in sites 1 and 3 may indicate relatively recent exposure to DDT. The highest median of $\sum_{17}$PCBs (3.2 ng/g lw) and $\sum_{10}$PBDEs (8.1 ng/g lw) were found in wild tilapia from sites 1 and 2, respectively. The dominating PCB congeners were PCB-118, -138, −153 and −180 and for PBDEs, BDE-47, -154, and −209. In 78% of wild fish and 8% of farmed fish $\sum_{6}$PBDE concentrations were above EQS_{biota} limits set by the EU. This warrants further studies.

1. Introduction

Fish serves as a major source of proteins to most people in the world and is essential for food security and sustainability (FAO, 2020). The growing human population has led to increased demand for fish, which in turn has led to overexploitation of wild fisheries, and reduction of some fish stocks (FAO, 2005). The aquaculture industry is rapidly growing across Africa to meet the increased demand and fish consumption on the continent (FAO, 2018). Nile tilapia (*Oreochromis niloticus*) is a fast-growing species in tropical and subtropical climate and therefore commonly used for commercial purposes. According to Genschick et al. (2017), Zambia is the major producer of tilapia in the Southern African Development Community (SADC) and the sixth-largest producer of farmed fish in Africa. Based on recent reports, 27 per cent of Zambia's fish production, approximately 30,000 tons, comes from fish farms (WorldFish, 2022).

During commercial fish farming, organic substances, chemicals, and antibiotics can be released in the water bodies and may disturb biodiversity and threaten freshwater ecosystems. (Berg et al., 1992; Kishimba et al., 2004; Li et al., 2011; Mwakalapa et al., 2018; Nonga et al., 2011; Polder et al., 2014; Simukoko et al., 2021; Ssebugere et al., 2014). Pollution of aquatic environments by persistent organic pollutants

* Corresponding author. Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P. O. Box 5003 NMBU, 1432 Ås, Norway.
E-mail address: anuschka.polder@nmbu.no (A. Polder).

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(POPs) may also affect fish and human health (Barni et al., 2016; Burreau et al., 2004; Rodriguez-Hernandez et al., 2017; VKM, 2014). POPs include compounds like organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) and Perfluoroalkyl substances (PFASs) (Stockholm Convention, 2021). Except for PFASs, which are protein bound (EPA, 2019), POPs are lipophilic and can therefore accumulate in fatty tissues of organisms including fish, and bioaccumulate in the food chain (Burreau et al., 2004; Deribe et al., 2011; Letcher et al., 2010; Sharma et al., 2009; Squadrone et al., 2013; Ssebugere et al., 2009). Their semi-volatile nature coupled with long environmental half-lives results in long-range transport and global distribution (Wania and Mackay, 1993). Anthropogenic activities such as industries, mining, agriculture, and waste from human settlements are known sources of POPs (Covaci et al., 2008; Glüge et al., 2020; Henry and Kishimba, 2006; Liu et al., 2022; Lyche et al., 2015). The Stockholm Convention of 2001 aims to protect human health and the environment from POPs, but despite global measures taken, POPs are still present in most parts of the world (Ashraf, 2017). Zambia ratified the Stockholm Convention in 2006.

The growing aquaculture industry in Africa may be threatened by the presence of POPs and other contaminants in the water and fish. To ensure sustainable aquaculture development, it is of key importance to gain knowledge on toxicological risk factors and the potential adverse effects of pollutants and other environmental factors on fish health. The current study was carried out to establish knowledge on the concentrations of a wide variety of POPs in wild and farmed tilapia from Lake Kariba, Zambia to elucidate possible geographic differences, and assess levels of POPs in relation to Environmental Quality Standards (EQSs) under the EU's Water Framework Directive.

2. Materials and methods

2.1. Description of sampling area

Lake Kariba is an artificial lake and reservoir. The lake is 220 km long and is on the border between Zambia and Zimbabwe (16° 28′ to 18° 04′S; 26° 40′ to 29° 03′ E). The runoff of the lake is eastwards, and several rivers from both the Zambian and Zimbabwean sides flow into the lake on their way east (Fig. 1). On the Zambian side it covers three districts (Sinazongwe, Gwembe and Siavonga). The district of Sinazongwe is known for coal mining, while Gwembe and Siavonga districts are dominated by agricultural activities. Five different locations in the lake (sites 1, 2 and 3, and farms 1 and 2) (Fig. 1) were selected for collecting of tilapia samples. The sites in the lake were chosen for investigating the general POP exposure to wild and farmed tilapia as well as the influence on POPs in the lake from anthropogenic activities on land. The fish farms (farm 1 and 2) in site 3 are supplied with a local fish feed processing plant. Both farms use high quality feed, based on locally grown soya beans and supplements (https://www.lakeharvest.com/, https://www.aller-aqua.com/zm). Sites 1 and 2 are situated 165 km and 100 km west of the farms, respectively. Further details and characteristics of the lake and sites were described previously (Simukoko et al., 2021).

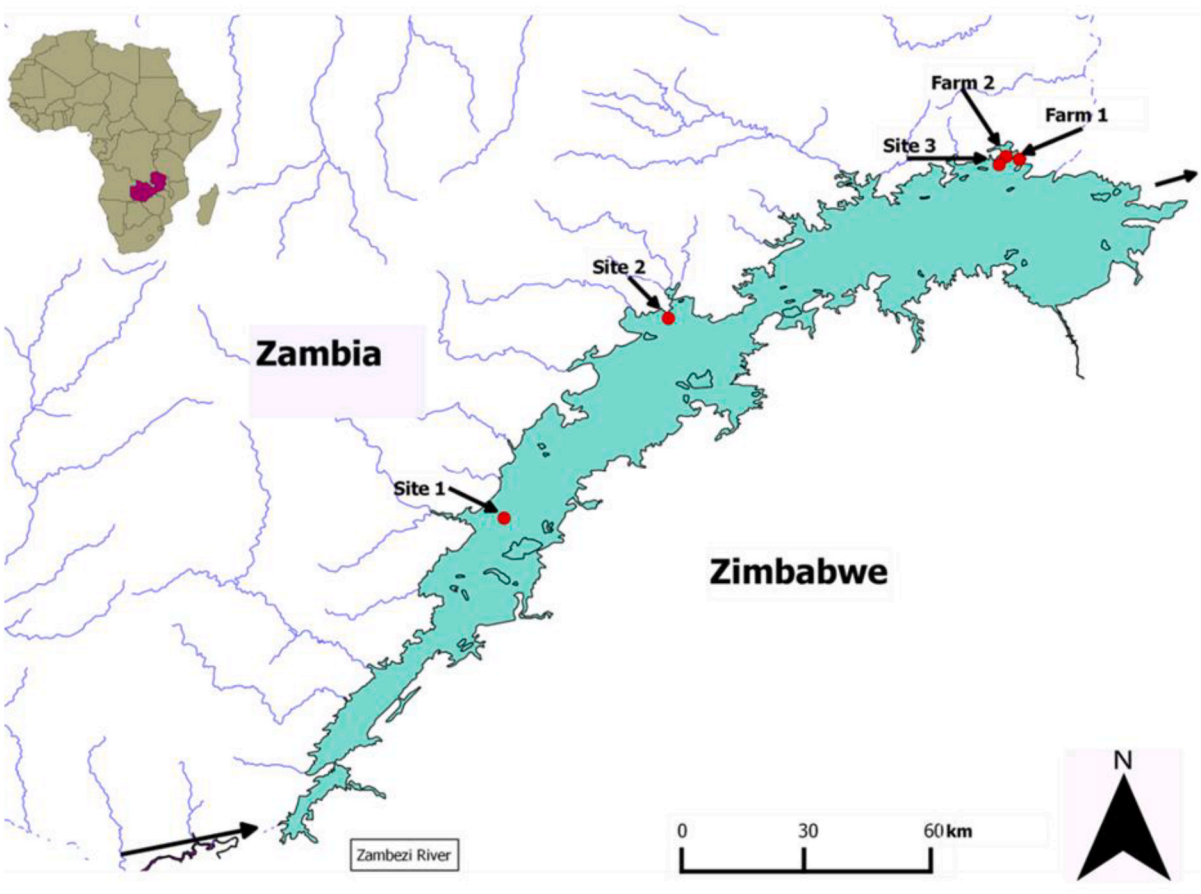

Fig. 1. Map of lake Kariba, showing the 5 sampling locations (sites1-3 and farms 1 and 2).
Courtesy of Eliezer Mwakalapa.

2.2. Ethical consideration and permission for the study

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

2.3. Sample collection

A total of 142 wild and farmed tilapia samples were collected from June to July 2017. Physicochemical parameters (pH, temperature, conductivity, and total dissolved solutes) were measured in all sites (not shown). Live wild tilapia were bought from local fishermen as they pulled in their catch from the water. The fish were placed on ice in a container and transported to the shore for dissection. Farmed tilapias were sampled by dip netting and placed on ice in containers. The length and sex of the fish were recorded (Tables 1A and 1B). The farmed fish were approximately 6 months of age. The age of wild fish was not defined but supposed to be older due to slower growth rate than farmed fish. Only length was used in statistical analyses because fish weight measured in the field was considered as not precise. The fish were dissected by veterinarians and the work was done as fast as possible, to avoid external contamination. Tables placed on the shore were covered with aluminium foil. All equipment, including aluminium foil, dissection tools, and tubes were pre-cleaned with ethanol. Liver tissue was removed and placed in the pre-cleaned 15 ml Eppendorf tubes. The samples were transported to the University of Zambia, Veterinary Medicine School on ice in a cooler box, where they were stored at −20 °C. The samples were later transported on ice by courier to the Laboratory of Environment Toxicology at the Norwegian University of Life Science (NMBU) at Ås, Norway, and stored at −20 °C until analyses. More details were described earlier (Simukoko et al., 2021).

2.4. Sample analysis of OCPs, PCBs and BFRs

Based on fish size, out of the 142 fish collected, 82 liver samples from male fish were selected and pooled for POPs analyses (Table 1A). Each homogenate contained two or three liver samples to get the preferable amount of 3 g of matrixfor the analysis.

Before analyses the samples were thawed at room temperature and protected from light. The samples were analysed for organochlorinated pesticides (OCPs): hexachlorobenzene (HCB), α, β- and γ-hexachlorocyclohexanes (HCHs), heptachlor, 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, polychlorinated biphenyls PCBs: PCB-101, -105, −110, −118, −128, −138, −141, −149, −151, −153, −156, −170, −180, −183, −194, −206 and −209 ($\sum_{17}$PCBs), and brominated flame retardants (BFRs): polybrominated diphenyl ethers PBDEs: BDE -47, −99, −100, −153, −154, −183, −196, −202, −206 ($\sum_{9}$PBDEs) and BDE-209 ($\sum_{10}$PBDEs is $\sum_{9}$PBDEs + BDE-209), and hexabromocyclododecane (HBCDD). Mirex, PCB -28, −52, −56, −66, −74, −87, −99, −114, −136, −137, −157, and −187; BDE-28, -207, and −208 and HBCDD were analysed but not detected above LOD. These compounds were not included in further data analysis.

2.5. Sample analysis of PFASs

PFAS compounds were analysed in individual liver samples (N = 26) in other fish than used for POP analyses, but from the same catch. The perfluoroalkyl substances analysed were: perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonamide (PFOSA) and perfluorooctane sulfonate (PFOS)* and 9 PFCAs: perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA)*, perfluorodecanoic acid (PFDA)*, perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), and perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA). Because of low sample amounts available, some female fish were included for PFAS analyses (Table 1B). Compounds marked with * were included in $\sum_{3}$PFAS. Other PFAS components were not detected in levels > LOD and were not used in further data analysis.

2.6. Chemical analyses of OCPs, PCB, BFRs

The analytical method of the analysis of OCs was first described by Brevik (1978) and modified by Polder et al. (2014), in which full details are described. In short, the method is based on repeated fat extraction of the homogenized liver with acetone, cyclohexane, and water, using an ultrasonic homogenizer and separation of the fat using centrifugation. Lipid determination was done gravimetrically using 1 ml aliquot of the fat extract. The rest of the extract was treated with 96% H_2SO_4 for cleaning of fat and the final extract was concentrated before GC analyses. Before extraction, 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 to all samples and controls. During the analyses, the samples were protected from light and amber GC-vials were used.

2.7. Chemical analyses of PFASs

The analytical method for PFAS was described by Gronnestad et al. (2017). 40 μl of a standard mixture with internal standards $^{13}C_5$ PFHxA, $^{13}C_4$ PFHxS, $^{13}C_4$ PFHpA, $^{13}C_4$ PFOA, $^{13}C_4$ PFOS, $^{13}C_5$ PFNA, $^{13}C_2$ PFDA, $^{13}C_2$ PFUnDA, $^{13}C_8$ FOSA, $^{13}C_2$ PFDoDA (500 ng/ml, dissolved in methanol) (Wellington laboratories, ON N1G 3M5, CA) were added to 0.5 g homogenized liver samples. Extraction was performed twice with 5 ml methanol and an ultrasonic probe sonicator followed by centrifugation. The supernatants were combined and cleaned with approximately 0.2 g graphitized carbon (EnviCarb). Finally, the samples were evaporated to near dryness and dissolved in 500 μl Methanol/water 1:1. Analysis of the samples on HPLC-MS resulted in substantial matrix effects, suggesting that further cleanup was necessary. An additional 0.2 g EnviCarb was added to the samples followed by filtration with Spin-X centrifuge filters (Corning). The EnviCarb and filters were washed

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

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

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

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

n.d. = not documented.

with 500 μl methanol, and the filtrates were combined and concentrated to dryness and reconstituted in 200 μl MeOH.

2.8. Instrumental analysis

2.8.1. Separation and detection of the POPs

OCPs, PCBs and BFRs were separated and detected using GC-MS methods as previously described (Mwakalapa et al., 2018; Polder et al., 2014), on a HRGC (Agilent 6890 Series) coupled to a MS detector (Agilent 5975C Agilent Technologies). The OCPs and PCBs (injection volume of 1 μl) were separated on a DB-5 MS column (J&W Scientific, Agilent Technologies) (60 m, 0.25 mm i. d., 0.25 mm film thickness). BFRs (injection volume of 2 μl) were separated on a DB-5 MS column (J&W Scientific, Agilent Technologies) (30 m, 0.25 mm i. d., 0.25 mm film thickness). The separation and identification of BDE-209 (injection volume of 10 μl) were performed on a GC-5-MS (Agilent 6890 Series/5973 Network) configured with a programmable temperature vaporization (PTV) injector (Agilent Technologies) equipped with a DB-5-MS column (10 m, 0.25 mm i. d., 0.10 mm film thickness) (J&W Scientific, Agilent Technologies). For all components, five-to eight-point linear calibration curves were used and calculations were done within the linear range for the component. OCPs, PCBs and BFRs were monitored using negative chemical ionization (NCI) in selected ion monitoring (SIM). Target and qualifier ions and internal standards for OCPs, PCBs and BFRs are listed in Table S1.1.

2.8.2. Separation and detection of the PFASs

Samples were analysed on an Agilent 1200 HPLC-system coupled to an Agilent 6460 Triple Quad Mass Spectrometer (Agilent Technologies). A Phenomenex C18 Luna Omega 3 μm 100 × 4,6 mm (Phenomenex) was used as the analytical column and a 50 mm version of the same column was installed between the pump and the injector to act as a delay-column to reduce blank contamination. The injected amount was 20 μl. The LCMS was used with electrospray ionization (ESI). Target and qualifier ions and internal standards for PFASs are listed in Table S1.2.

2.8.3. Quality assurance (QA)/quality control (QC)

Chemical analyses of the liver samples were conducted at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences in Oslo, Norway. The laboratory is accredited, and annually approved for testing chemicals in biological samples by the Norwegian accreditation (NA) according to the requirement of the NS-EN ISO/IEC 17025 (TEST 137).

2.8.4. 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 (LRM) of the blubber of a harp seal (*Pagophilus groenlandicus*). Results were corrected for mean of the three blank values. Blank values of BDE-209 were very low and consistent in the series and therefore corrected similarly to the other analytes. The results of the LRM were within acceptable ranges. Covering the period of analyses the analytical quality was approved by routinely analysing Certified Reference Materials of different fish tissues with different concentrations levels (CRMs: 2525, 350, 1946, 598). All K-values were within ± 1 for the year the samples were analysed, and number of components within two standard deviations and 20% of the 'true' value were within the accepted range for the accreditation. Within the same period, the laboratory successfully participated in round 1 and 2 of Quasimeme inter-laboratory studies for POPs in fish muscle, fish liver and shellfish tissue in 2016 (Z-scores within ± 2 for OCs for 89% and 88% and for BFRs 100% and 96% for rounds 1 and 2, respectively) and 2017 (Z-scores within ± 2 for OCs for 81% and 90% and for BFRs 92% and 100%, respectively). The limits of detections (LOD) for individual analytes were defined as 3 times the noise level of each analyte. The LODs (ng/g wet weight, ww) ranged from 0.003 to 0.166 for OCPs, 0.003 to 0.101 for PCBs and 0.003 to 0.036 for BFRs (Table S2.1). The relative recoveries were 82–137% for OCPs, 92–127% for PCBs, and 68–120% for BFRs. Based on assessment of recovery and test results of standard drifts and LRM, results were not corrected for recoveries.

For PFASs, every analytical series included three blanks of solvent, two samples of spiked Atlantic cod (*Gadus morhua*) for recoveries and one blind sample of non-spiked Atlantic cod. LOD was calculated as 3 times the noise in the chromatogram. The LOD for PFAS ranged between 0.093 ng/g ww to 0.706 ng/g ww (Table S2.3). Matrix-matched calibration curves ranged from 0 to 50 ng/ml and were linear with $R^2 >$ 0,99, except for PFTrDA. The analytical quality of the method was assessed by including an inter-laboratory test (AMAP) in the analysis of samples. The relative recoveries were 67–115%. None of the reported PFAS compounds (PFNA, PFDA, and PFOS) were detected in the procedural blanks.

2.9. Statistical data analysis

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

3. Results

3.1. Fish characteristics

Fish weight and length correlated strongly for fish below 1 kg for both wild fish ($r = 0.93$) and farmed fish ($r = 0.92$). Since fish weight above 1 kg was not specified, fish length was used as indicator of fish size. Fish from farm 1 were significantly longer (mean 36 cm), ($p < 0.05$)

than the other locations (Table 1A). The median liver lipid contents (%) of farmed fish from farms 1 (7.5%) and 2 (8,8%) were significantly higher ($p < 0.05$) than for wild fish from site 2 (4.9%) and were also significantly higher ($p < 0.05$) in farm 1 than in site 1 (4.1%). In addition, there was a significant difference ($p < 0.05$) in liver lipid content between the wild fish at site 2 (4.9%) and 3 (7.2%) (Fig. 2). Length of the individual fish for PFAS analyses were all in the same range (Table 1B).

3.2. Levels of OCPs, PCBs, BFRs

Concentrations expressed as ng/wet weight (ww) and lipid weight are presented in Table 2A and 2B, respectively. HCB and *p,p'*-DDE were detected in 100% of the liver samples. Median concentrations of HCB (ng/g lipid weight (lw)) were significantly higher ($p < 0.05$) in wild fish from all sites compared to farmed fish (both farms) (Table 2B, Fig. 2). Of the HCHs, γ-HCH (lindane) and α-HCH were detected in 77% and 50% of the samples, respectively. The highest median concentration of ∑HCHs was 0.05 ng/g lw in site 3. The γ-HCH was the dominant HCH, contributing 56%, 69% and 65% to ∑HCHs in wild fish from sites 1, 2 and 3, and 79% and 58% in fish from farms 1 and 2 (Fig. 3). α-HCH contributed between 21 and 34% to the ∑HCHs and β-HCH between 1 and 14%. The median ∑HCHs was significantly higher ($p < 0.05$) in sites 2 and 3 compared to farm 1 (Fig. 2). DDTs were the most abundant OCPs in all locations with the highest median concentrations of ∑DDTs in wild fish from site 1 and 2 (93 and 81 ng/g lw) (Table 2B). *P,p'*-DDD and *p,p'*-DDT were detected in 93% and 73% of the samples, while *o,p'*-DDD and *o,p'*-DDT were detected in 17% and 3% of the samples, respectively. *P,p'*-DDE and ∑DDTs were significantly higher ($p < 0.05$) in sites 1 and 2 compared to farms 1 and 2, while site 3 was only significantly higher ($p < 0.05$) than farm 1 (Fig. 2). The contribution of *p,p'*-DDE to the ∑DDTs was highest in wild fish from site 2 (61%), lower in wild fish from sites 1 and 3 (48% and 46%) but lowest in farmed fish from farms 1 and 2 (30% and 31%), respectively (Fig. 3). In farms 1 and 2, the contribution of *p,p'*-DDD to the ∑DDTs was higher than that of *p,p'*-DDE with 68% and 63%, respectively (Fig. 3). The ratio of *p,p'*-DDE/*p,p'*-DDT was highest in wild fish from site 2 and farmed fish from farm 1 and lowest in wild fish from site 3. *Trans*-nonachlor was detected in 73%, and *cis*-nonachlor, *cis*-chlordane and *trans*-chlordane in only 17%, 13% and 7% of the samples, respectively. *Trans*-nonachlor contributed 66–87% to ∑CHLs. Median ∑CHLs were significantly higher ($p < 0.05$) in wild fish for all sites compared to farmed fish (both farms). Mirex and heptachlor were not detected in any of the samples.

PCBs were detected in all locations in low concentrations. PCBs-118, -138, −153 and −180 were the most abundant PCBs and found in 53%, 60%, 90% and 77% of the samples, contributing average 8%, 14%, 25% and 16% to $\sum_{17}$PCBs respectively (Fig. 3). The highest median concentration of $\sum_{17}$PCBs was found in site 1 at 3.2 ng/g lw < site 3<site 2<farm 2<farm 1 (Table 2B, Fig. S1). Median $\sum_{17}$PCBs was significantly higher ($p < 0.05$) in site 1 compared to site 2, farm 1 and farm 2. Site 3 was significantly higher ($p < 0.05$) compared to farm 1 and farm 2 (Fig. 2).

PBDEs were detected in all samples, except one. BDE-47, -99, 154 and −209 were the most abundant BDEs detected in 89%, 50%, 50% and 94% in wild fish, and in 33%, 0 (zero), 67%, and 100% in farmed fish, respectively. BDE-209 dominated the PBDE pattern and contributed average 84% to $\sum_{10}$PBDEs (Fig. 3, Fig S1). The highest median concentration of $\sum_{10}$PBDEs (including BDE-209) was 8.1 ng/g lw in site 2. Median concentrations of BDE-209 were 1.43, 7.78, 1.05 ng/g lw, in sites 1, 2, and 3, respectively, and 1.01 and 0.41 ng/g lw in farms 1 and 2. Median $\sum_{9}$PBDEs (excluding BDE-209) were significantly higher ($p < 0.05$) in site 1 and site 2 compared to farm 1 and farm 2, while the median $\sum_{9}$PBDEs was significantly ($p < 0.05$) higher in all sites compared to farm 1. Site 2 was also significantly higher ($p < 0.05$) than farm 2 (Fig. 2).

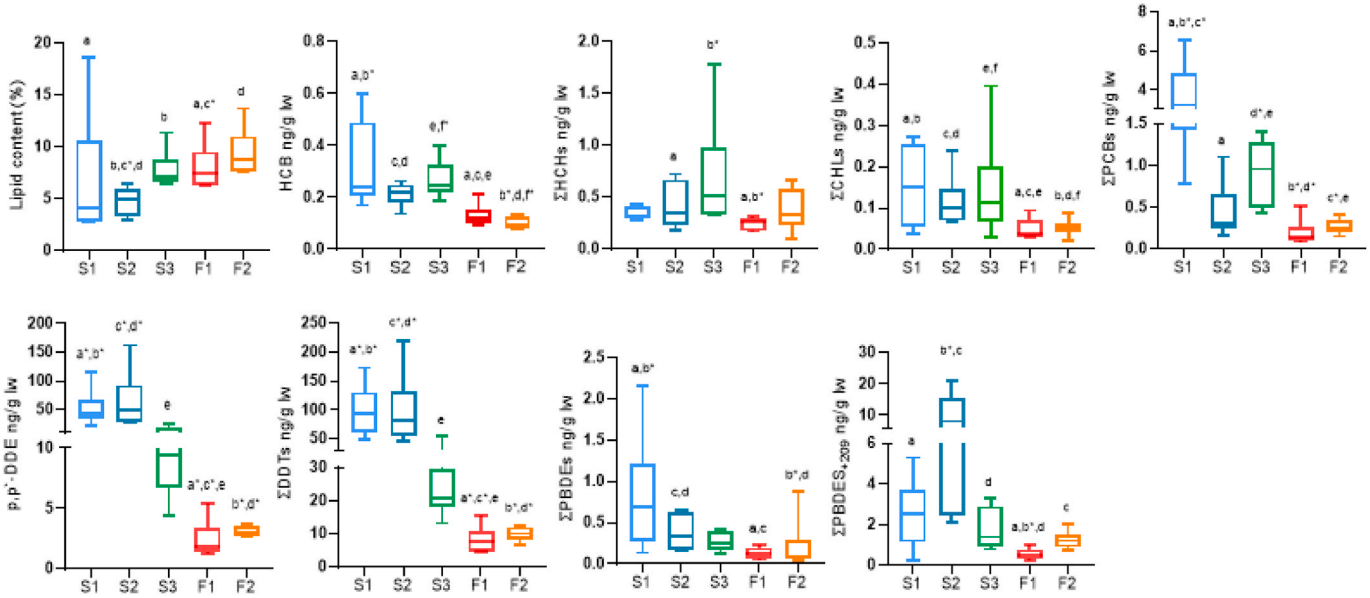

Fig. 2. Liver lipid content and contaminant concentrations in livers from wild fish (site S1-3) and farmed fish (farm F1-2) in Lake Kariba, Zambia.
Liver lipid content is given in % and liver concentrations are presented as ng/g lipid weight (lw). Box plots show median (line), IQR (box) and minimum to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

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

Location		Ww						
		Lipid %	HCB	∑HCH	∑DDTS	∑CHLs	$\sum_{17}$PCBs	$\sum_{10}$PBDEs
Site 1	Mean	**6.77**	**0.02**	**0.03**	**6.90**	**0.01**	**0.18**	**0.14**
	Median	4.17	0.01	0.01	4.20	0.01	0.15	0.16
	Min	2.77	0.01	0.01	1.38	<LOD	0.04	0.01
	Max	18.66	0.05	0.08	15.7	0.03	0.34	0.27
	N = 6							
Site 2	Mean	**4.73**	**0.01**	**0.02**	**4.67**	**0.01**	**0.02**	**0.36**
	Median	4.92	0.01	0.02	3.68	<LOD	0.02	0.31
	Min	2.91	0.01	0.01	1.36	<LOD	0.01	0.13
	Max	6.45	0.01	0.03	10.11	0.02	0.03	0.63
	N = 6							
Site 3	Mean	**7.82**	**0.02**	**0.05**	**2.16**	**0.01**	**0.07**	**0.14**
	Median	7.15	0.02	0.04	1.51	0.01	0.07	0.09
	Min	6.53	0.01	0.02	0.88	<LOD	0.03	0.06
	Max	11.37	0.03	0.12	6.10	0.05	0.16	0.32
	N = 6							
Farm 1	Mean	**8.09**	**0.01**	**0.02**	**0.63**	**<LOD**	**0.02**	**0.04**
	Median	7.51	0.01	0.02	0.56	<LOD	0.01	0.04
	Min	6.26	0.01	0.02	0.31	<LOD	0.01	0.03
	Max	12.31	0.02	0.02	1.07	0.01	0.04	0.07
	N = 6							
Farm 2	Mean	**9.44**	**0.01**	**0.03**	**0.94**	**<LOD**	**0.02**	**0.11**
	Median	8.77	0.01	0.03	0.91	<LOD	0.02	0.11
	Min	7.62	0.01	0.01	0.51	<LOD	0.02	0.07
	Max	13.72	0.02	0.06	1.35	0.01	0.03	0.17
	N = 6							

Σ-HCHs: Sum of α-, β- and γ-HCHs
Σ-CHLs: Sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor
Σ-DDTs: Sum of *p.p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT and *p,p′*-DDT
Σ-$_{17}$PCBs: Sum of CB-101, -105, -110, -118, -128, -138, -141, -149, -151, -153, -156, -170, -180, -183, -194, -206 and -209
Σ-$_{10}$PBDEs: Sum of BDE-47, -99, -100, -153, -154, -183, -196, -202, -206 and -209.

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

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

Σ-HCHs: Sum of α-, β- and γ-HCHs
Σ-CHLs: Sum of oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor
Σ-DDTs: Sum of *p.p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT and *p,p′*-DDT
Σ-$_{17}$PCBs: Sum of CB-101, -105, -110, -118, -128, -138, -141, -149, -151, -153, -156, -170, -180, -183, -194, -206 and -209
Σ-$_{10}$PBDEs: Sum of BDE-47, -99, -100, -153, -154, -183, -196, -202, -206 and -209.

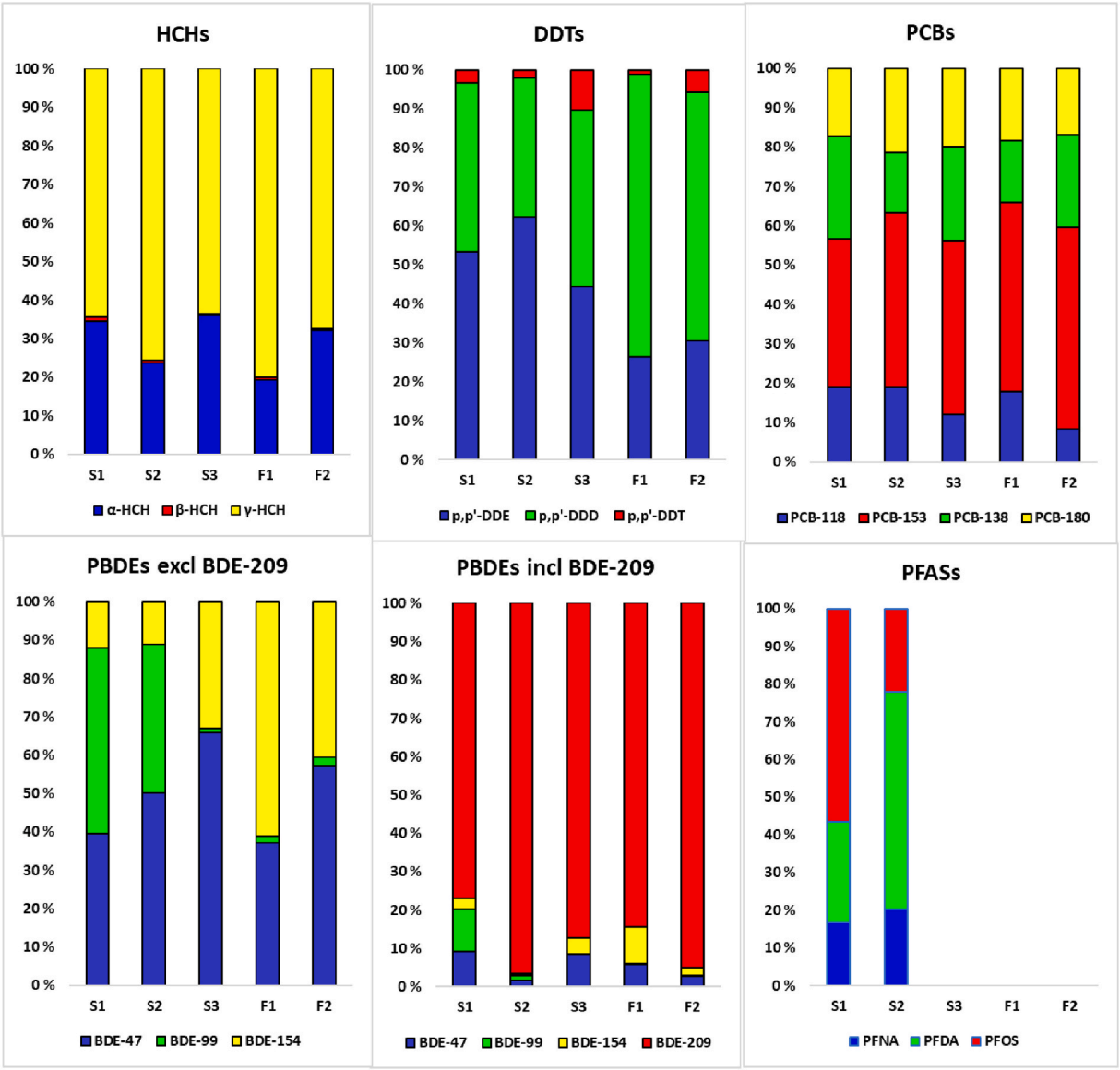

Fig. 3. Relative contribution (in percentages) of individual compounds to ∑HCHs, ∑DDTs, ∑CHLs, ∑PCBs, ∑PBDEs and PFASs.

3.3. Occurrence and levels of PFASs

PFOS, PFDA and PFNA were the only PFASs detected in levels > LOD in individual wild fish (Table 3). PFOS was detected in 100% and 40% in wild fish from site 1 and 2, respectively, while PFNA was detected in 20% in sites 1 and 2. The highest median level of PFOS (0.66 ng/g ww) was found in wild fish from site 1 while highest median level of PFDA (0.37 ng/g ww) was found in site 2 (Table 3; Fig. S1). No PFASs were detected in site 3, farm 1 or farm 2.

3.4. Correlations

Spearman correlations coefficients for the main compounds HCB, ∑DDTs, $\sum_{17}$PCBs, $\sum_{9}$BDEs and $\sum_{10}$BDE are presented in Table S4. In site 1, strong correlations were found between HCB and $\sum_{10}$BDE (r = −0.83) and between $\sum_{17}$PCBs and $\sum_{9}$BDEs. In site 2, strong correlations were found between HCB and $\sum_{17}$PCBs (r = 0.77) and between $\sum_{9}$BDEs and $\sum_{10}$BDE (r = 0.89). In site 3, $\sum_{17}$PCBs and $\sum_{9}$BDEs strongly correlated (r = 0.77). In farm 1, $\sum_{17}$PCBs and ∑DDTs showed strong correlations (r = 0.83), while in farm 2, no strong correlations were found. No correlations were found between BDE-47, -154 and −209 (Table S5) (detection rate >50%).

3.5. Compliance with reference levels

Compared to recommended Environmental quality standards (EQS) from European commission most of the POPs were below the limits for fish except for $\sum_{6}$PBDEs (sum of BDE-28, -47, −99, −100, −153 and −154). In 78% of the wild fish, and 8% of the farmed fish the EQS_{biota} for PBDEs was higher than the EQS limits of 0.0085 ng/g set by the EU (European Commission, 2013).

4. Discussion

The main goal in fish farming industry is to obtain high quality fish for sale in short time. The fish must fulfil criteria set by health authorities, regarding nutritional value as well as to the presence of environmental contaminants/pollutants (VKM, 2014). The compounds in fish feed are therefore specially put together for the purposes of fast growth, with a high content of nutrients and low content of pollutants. Both fish farms use high quality fish feed, based on 80% locally grown soya products, plus supplements (https://www.lakeharvest.com/,

Table 3
Mean concentrations (ng/g ww), median (in brackets) and range of PFAS compounds detected in individual wild and farmed tilapia from Lake Kariba, Zambia. Mean and median were only given in cases where PFAS compounds were detected in more than 50% of the samples.

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

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

https://www.aller-aqua.com/zm). Unfortunately, it was not possible to achieve samples of fish feed in the present study for analyses of POPs. Analysing of fish feed is recommended in future studies. The higher lipid contents in fish from farms 1 and 2, and from wild fish from site 3 (Table 2B, Fig. 2), indicate good conditions for the farmed fish, and for the wild fish foraging around the fish farms. Other studies confirm that wild fish living close to fish cages feed on organic waste and spillage of feed from the cages (Ballester-Moltó et al., 2017; Bustnes et al., 2010; Varol, 2019).

4.1. Levels and congener patterns of OCPs, PCBs and BFRs

DDTs were the dominant OCPs in both wild and farmed fish liver tissues from Lake Kariba, but wild fish had significantly ($p < 0.05$) higher levels of median ∑DDTs compared to the farmed fish (Table 2B, Fig. 2). The dominance of DDTs in the OCP pattern was similar to findings in other studies (Berg et al., 1992; Mwakalapa et al., 2018) (Table 4). The countries around Lake Kariba (Zambia and Zimbabwe) have a history of DDT use for vector control in combatting malaria and tsetse control operations in addition to agriculture (Berg et al., 1992). Lake Kariba was filled with water in 1958–1963. Because the flooded areas were earlier treated with DDT, the sediment of the lake is still a reservoir of DDT residues. Due to the long half-life of DDT and different metabolism under anaerobic conditions, DDT and its metabolites originating from the time before the lake was filled may still contribute to exposure of living organisms in Lake Kariba today (Berg et al., 1992; Brevik et al., 1996). In addition, DDT may have entered Lake Kariba via run-off and atmospheric deposition (Banda and Mundia, 2009; Berg et al., 1992; Ssebugere et al., 2009). Use of DDT was banned globally in the 1970s but is still allowed for use in indoor residual spraying (IRS) and for production of insecticide-treated mosquito nets (ITN) (WHO, 2011). Due to these campaigns, the levels of DDT are expected to decrease in the environment of the Southern African region. Biodegradation of DDT results mainly in the more persistent metabolites *p,p'*-DDE and *p,p'*-DDD. In the present study, the contribution of *p,p'*-DDE to ∑DDTs was highest in wild fish from sites 1 and 2 but decreased eastwards in wild fish from site 3 (Fig. 3). In the farmed fish, *p,p'*-DDD was contributing most to ∑DDTs (Table 2B, Fig. 3). Higher ratio of *p,p'*-DDD/p,p'-DDE is related to anaerobic degradation in soil and sediments and uptake in plant roots (Buah-Kwofie and Humphries, 2017; Chen et al., 2007). Periods of drought or increased water flow in the farming area may contribute to an increased bioavailability of chemical pollutants stored in sediments below the fish cages. However, this needs to be studied further. The ratios of *p,p'*-DDE/*p,p'*-DDT were lower in several pooled samples of wild fish from site 1 and site 3, indicating relatively recent use of DDT in the area (Table 4). In addition, one of the pooled samples from site 1 contained *o,p'*-DDT, strengthening this observation. Levels of mean ∑DDTs in the wild tilapia were lower than those reported in Lake Victoria (Henry and Kishimba, 2006; Polder et al., 2014), but higher than from other areas (Deribe et al., 2011; Gbeddy et al., 2015; Mdegela et al., 2009) (Table 4). Farmed tilapia liver tissue in the present study had comparable lipid adjusted levels of mean ∑DDTs than to those reported three decades ago (Berg et al., 1992) in farmed tilapia muscle from Lake Kariba, Zimbabwean side (Berg et al., 1992) (Table 4). One must be cautious with this comparison because the present study is based on liver, a lipid rich organ with higher levels of POPs. Still, the results indicate that farmed fish in the eastern part of Lake Kariba is exposed to DDT. It is not known if this is caused by DDT contents in the fish feed or by redistribution from sediments. This warrants further research.

The second dominant OCP, HCB, was detected in low levels which were below the EQS set by the EU (Table 6). Median levels of HCB were significantly higher ($p < 0.05$) in wild fish from all sites compared to farmed fish (Table 2B, Fig. 2). This is in accordance with previous studies from Lake Kariba (Berg et al., 1992). HCB was used as a fungicide, in rubber synthesis and wood preservation among other uses, but was banned by international bodies in the 1970s–80s (Stockholm Convention, 2021). The low levels observed in the present study may reflect a general background level related to long range atmospheric transport from emission of industries at far distance (Polder et al., 2014). Levels of HCB in the current study were in the same range as levels reported in tilapia liver from Tanzania (Mwakalapa et al., 2018; Mdegela et al., 2009), but lower than in tilapia muscle from Tanzania (Polder et al., 2014) (Table 4).

Although levels of HCHs generally were low in all study sites, the median ∑HCHs was significantly higher in wild fish from sites 2 and 3 compared to farm 1 ($p < 0.05$) (Fig. 2). Lindane (γ-HCH) was used as an insecticide on fruit and vegetable crops, for seed treatment and treatment of lice and scabies, mainly in Western -European and Asian countries (Vijgen et al., 2019). Pure lindane is contaminated with α-HCH and β-HCH of which β-HCH is the most persistent isomer. Due to regulations, levels of lindane are decreasing. The patterns of HCHs found in this study may thus reflect historic use of the technical mixture combined with relatively recent, but limited use of γ-HCH. The general HCH lipid adjusted levels in tilapia liver from Lake Kariba were in the same range as those reported earlier in liver from several fish species in Tanzania (Mwakalapa et al., 2018), in tilapia muscle from Lake Kariba, Zimbabwe (Berg et al., 1992), and in tilapia muscle from Tanzania (Polder et al., 2014), higher than in muscle from *Distichodus fasciolatus* in DR Congo (Verhaert et al., 2013), but much lower than in tilapia muscle from South Africa (Verhaert et al., 2017), *Hydrocynus vittatus* (Wepener et al., 2012) and in tilapia muscle from Ghana (Gbeddy et al., 2015) (Table 4). The difference in levels of γ-HCH between countries may be a result of whether regulations or ban for γ-HCH (lindane) from 2009 have

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

Country	Location	Sample	Lipid%	HCB	∑HCH	p.p'-DDE	p.p'-DDT	∑DDTs	DDE/DDT	∑CHLs	∑PCBs	∑PBDEs	∑HBCDD	References
Zambia	Wild S1*Oreochromis niloticus*	Liver	6.77	0.32	0.35	52.3	6.88	97.9	17	0.15	3.29	2.55	<LOD	Current study
	Wild S2		4.73	0.21	0.42	64.4	2.0	98.1	60	0.12	0.44	9.29	<LOD	
	Wild S3		7.82	0.27	0.70	12.2	2.37	24.9	5.8	0.14	0.92	1.77	<LOD	
Zambia	Farm F1*Oreochromis niloticus*	Liver	8.09	0.13	0.24	2.41	0.07	8.20	32	0.05	0.19	0.55	<LOD	Current study
	Farm F2		9.44	0.10	0.38	3.07	0.57	9.96	20	0.05	0.26	1.24	<LOD	
Zimbabwe	Caged Oreochromis spp	Muscle		<LOD	0.3	4.2	1.5	6.9						Berg et al. (1992)
Zimbabwe	Wild Oreochromis spp	Muscle		0.5	0.4	26	2.4	31.9						Berg et al. (1992)
Zimbabwe	Pond *Tilapia rendalli*	Muscle		0.1	0.4	4.1	1.7	6.7						Berg et al. (1992)
Zimbabwe	Wild *Tilapia rendalli*	Muscle		0.2	0.6	14.7	4.3	21.1						Berg et al. (1992)
Tanzania	Pond *Chanos*	Liver	7.98	0.22	0.05	13.5	3.2	16.60			1.47	1.64	<LOD	Mwakalapa et al. (2018)
Tanzania	Indian ocean *Chanos*	Liver	7.81	0.20	0.10	103	2.50	177	41		0.20	1.30	<LOD	Mwakalapa et al. (2018)
Tanzania	Indian ocean *Mugil cephalus*	Liver	4.85	0.20	0.04	60.7	2.2	73.3	35		0.6	1.10	5.20	Mwakalapa et al. (2018)
Tanzania (LT)	*Oreochromis niloticus*	Muscle	3.30	1.20	1.10	116	41.8	273	2.8	0.90	17.2	4.10		Polder et al. (2014)
Tanzania (LV)	*Oreochromis niloticus*	Muscle	0.4	2.5	<LOD	27.1	1.4	34.7	9.4	0.5	12	34.4	<LOD	Polder et al. (2014)
Tanzania	*Oreochromic niloticus*	Muscle	1.50					500						Henry and Kishimba (2006)
Tanzania	*Oreochromis urolepis*	Muscle	8.00	2.50		25.0	21.3	46.3		2.50				Mdegela et al. (2009)
DR Congo	*Distichodus fasciolatus*	Muscle	1.2	0.02	0.04			0.14			0.89	0.2		Verhaert et al. (2013)
South Africa	*Oreochromis mossambicus*	Muscle	0.98		34.7			520		10.2	<LOD	<LOD		Verhaert et al. (2017)
South Africa	*Hydrocynus vittatus*	Muscle	3.83	117.5	12.9	4162	937	5537		2.9	532	5.8		Wepener et al. (2012)
Ethiopia	*Oreochromis niloticus*	Muscle						6.90			0.05			Deribe et al. (2011)
Uganda	*Oreochromis niloticus*											5.6		Ssebugere et al. (2014)
Ghana	*Oreochromis niloticus*	Muscle	2.8		41.6	0.15	0.53	10.7		4.02				Gbeddy. et al. (2015)
Ghana (LVA)	*Oreochromis niloticus*	Muscle	1.9								54	7.1	0.75	Asante et al. (2013)
Romania	*Cyprinus carpio*	Muscle	0.76	57	209	1271	201	2847			1248	14.3		Covaci et al. (2006)
Norway (LF)	*Salmo trutta*	Muscle	3	18							108	27	15	Lyche et al. (2018)

<LOD: lower than limit of detection
LT-Lake Tanganyika, LV- Lake Victoria, LVA- Lake Volta (Akosombo), LF -Lake Femund.

Table 5
Comparison of mean concentrations and ranges (ng/g ww) of PFASs in tilapia and other fish species from various countries.

Country	Location (industry)	Remote	Industry	fish species	matrix	PFOS	∑PFAS	
Zambia	Sinazongwe	x		Tilapia	liver	0.64	0.94	present study
Norway	Femund	x		Brown trout	liver	3.96	73.4	Lyche et al., 2018
Italy	Lake Varese	x		European perch	muscle	9.6		Squadrone et al. (2015)
South Africa	Vaal River	x	x	Multiple sp.	liver	13–460		Groffen et al. (2018)
South Africa	Olifant River basin		x	Multiple sp.	muscle	0.15–2.7		Verhaert et al. (2017)
Norway*	Tyrifjord		x	Brown trout	liver	<180	90.5–288	Langberg et al. (2022)
Canada**	St. Lawrence River		x	Diff species	whole	12–140	21–92	Munoz et al. (2022)
China***	Chaohu Lake		x	Eurasian carp	whole	±80	±170	Chen et al. (2022)

*Paper industry
**Different industries
***Estimated values, based on graphs.

been implemented (Stockholm Convention, 2021). The present low γ-HCH levels in Lake Kariba may indicate that regulations for use of lindane have been successful.

The ∑CHL levels were very low, but nevertheless significantly higher ($p < 0.05$) in wild than in farmed fish (Fig. 2). Chlordanes are banned compounds and are no longer used as insecticides (Stockholm Convention, 2021) and their levels are expected to decrease. Mean levels of ∑CHL were less than those reported by Polder et al. (2014) and Mdegela et al. (2009) in Tanzania and Gbeddy et al. (2015) in Ghana. Mirex and Heptachlor are also banned substances and were below detection limit in all samples (Table S2.1).

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

Table 6
Environmental quality standards (EQS) for contaminants in fish and median levels (expressed as ng/g wet weight (ww)), (dioxin-like PCBs, DL-PCBs as pg/g TEQs ww) in wild and farmed (grouped and individual) tilapia from Lake Kariba, Zambia.

Contaminant	EU EQS	Present study				
		Wild S1	Wild S2	Wild S3	Farm F1	Farm F2
DDT		4.2	3.68	1.51	0.56	0.91
HCB	10	0.01	0.01	0.02	0.01	0.01
Y–HCH	–					
CHL	–	0.01	<LOD	0.01	<LOD	<LOD
PCBs	0.6	0.15	0.02	0.07	0.01	0.02
DL-PCB: PCB-118	6.5*	0.014	0.002	0.005	0.001	0.001
∑6PBDE**	0.0085	0.0268	0.0134	0.0178	0.0064	0.0062
PFOS	9.1	0.66	0.14	<LOD	<LOD	<LOD

*: pg TEQ/g ww
**: Sum of BDE-28, -47, 99, -100, -153, -154.

PBDEs were used as flame retardants in thermoplastics (computer and TV housing), textiles, foams, furniture, electronics, building materials, and interiors of cars, busses, and airplanes (Covaci et al., 2008; Lyche et al., 2015). In general, low PBDE levels were detected in the present study. However, BDE-209, showed a large geographic difference with median concentrations up to 8 times higher in fish liver from site 2 than in sites 1 and 3, and farms 1 and 2 (Fig. 2, Fig.S1). Deca-BDE, mainly consisting of BDE-209, was used as a replacement for the tetra and penta- BDE mixtures after the latter were banned (Stockholm Convention, 2021). The half-life of BDE-209 may vary in different species and Luo et al. (2019) found that the dose dependent half-life of BDE-209 in the muscle of rice fish was from 17 to 19.4 days. Deca-BDE debrominates to lower brominated and more persistent BDE congeners, such as BDE-47 (Stapleton et al., 2004). Tetra-BDE (BDE- 47), contributing about 40% to the commercial penta BDE mixture, is one of the most abundant, toxic, and bioaccumulative PBDE congeners (Ssebugere et al., 2014; Asante et al., 2013; Mwakalapa et al., 2018). Due to differences in metabolism, lower brominated congeners such as BDE 47 accumulate more in aquatic organisms, while higher brominated congeners like BDE 154 and 209 accumulate more in terrestrial organisms (Luo et al., 2019). In this study, no correlation was found between BDE-47, -154 and −209. This may indicate that the high BDE-209 concentration in site 2 (7.8 ng/g lw) comes from more recent exposure while BDE-47 and -154 reflect more historic use of the commercial, now banned penta-BDE mixture (Stockholm Convention, 2021). Similar observations were made in a study on tilapia from Lake Victoria (Polder et al., 2014). Industries along the rivers and limited wastewater control may cause runoff of pollutants such as PBDEs to the lake, where they can enter the lake's food web. Textile industries are reported to contribute to Deca-BDE pollution in the environment (Derden and Huybrechts, 2013). Cotton production and textile industry is increasing in Zambia, and there is a cotton ginnery in Gwembe (site 2) (RATES, 2003). Further studies should be done to elucidate possible BDE-209 sources. In addition to local sources, a certain contribution from precipitation of long-range atmospheric transported BDE-209 may be suggested (Li et al., 2011) as BDE-209 was detected in almost 100% of the studied fish samples from Lake Kariba. Interestingly, BDE-99, contributing 45–49% to the penta-BDE mixture, was not detected in any of the farmed fish. Higher sample amounts and analyses of individual samples in future studies may give a better picture of the sources of PBDE and biotransformation of BDE-209 in the lake. $\sum_{10}$PBDE levels in wild tilapia from sites 1 and 2

were from four to thirteen-fold lower than in tilapia muscle from Lake Victoria, comparable to findings in the other lakes in Tanzania (Polder et al., 2014), and levels in Ghana, South Africa, and Uganda (Asante et al., 2013; Wepener et al., 2012; Ssebugere et al., 2014), but higher than in Milkfish (*Chanos*) from Tanzania (Mwakalapa et al., 2018) (Table 4). A global deca-BDE ban was adopted under the UN Stockholm Convention in 2017 and thus levels of BDE-209 and its debromination products are expected to decrease in the environment. The study shows that levels of POPs were higher in wild tilapia than in farmed tilapia. Farmed fish fed on a high nutrient rich diet grows much faster than wild fish. At collection time, the farmed fish were about 6 months at age and had a shorter exposure time in the lake than the smaller (but older) wild fish, who feed on available plants and small insects. From a human health point of view, it seems that farmed fish is preferable compared to wild fish for human consumption. However, in such a comparison between farmed and wild fish, it is important to include specific, for humans' beneficial nutrients, residues of antibiotics and other contaminants (VKM, 2014).

4.2. Levels and pattern of PFAS

This is the first time PFASs are detected in fish from Lake Kariba, Zambia. PFASs is a large group of perfluorinated substances produced since the 1950's and used in various consumer products such as impregnated outdoor textiles, shoes, food containers, kitchen ware and firefighting foam due to their water repellant properties. In addition, they are useful in special processes within mining industries (Glüge et al., 2020; Liu et al., 2022). They are very persistent to degradation and may cause adverse health effects in living species (Fenton et al., 2021). In contrast to the lipophilic POPs, PFAS bind to proteins and are more soluble in water (Groffen et al., 2018; Gronnestad et al., 2017; Lam et al., 2014; Mudumbi et al., 2014). In the present study PFAS were only detected in wild tilapia from sites 1 and 2 (Table 3). PFDA and PFOS were detected in levels > LOD in 100% of the tilapia from site 1. In site 2, PFDA was found in 80%, and PFOS was found in 40% of the tilapia (Table 3). PFNA was only found in one sample from site 1 (20%) and site 2 (20%), respectively. The PFAS patterns differed between site 1 and 2, with a higher contribution of PFOS to $\sum$PFASs in site 1 compared to site 2 (Fig. 3). The occurrence of PFASs in the wild fish from Sinazongwe (site 1)) may be influenced by runoff from the mining industry as well as other anthropogenic activities in this area (Barfoot et al., 2022; Glüge et al., 2020; Liu et al., 2022). As Gwembe district is an agricultural area, with a smaller population than in Sinazongwe (Simukoko et al., 2021), the source of PFAS in fish from site 2 may be more influenced by anthropogenic activities on land than the upstream pollution from mining activity in Sinazongwe. Interestingly, no PFAS were found in fish from site 3 and farms 1 and 2, situated near the outlet of the lake, thus reducing a hypothesis of atmospheric transported PFAS to the region. However, since the PFAS results in this study are based on only a few individual samples from each site, future studies are needed on PFAS occurrence and its sources along the whole Lake Kariba.

There are to our knowledge only two studies on PFAS in South African fish. Verhaert et al. (2017) found comparable concentrations of PFOS and PFNA in muscle tissue ranging from 0.15 to 2.7, and <LOQ to 0.14 ng/g ww, respectively, in muscle of fish from the Olifants River basin, while Groffen et al. (2018) found PFOS in fish liver from Vaal River in ranges of 13–460 ng/g ww which were similar or higher than in USA, Europe and Asia. The high PFAS levels in fish from Vaal River were explained by exposure from industrial and mining activities (Groffen et al., 2018). In both studies from South Africa (Groffen et al., 2018; Verhaert et al., 2017), PFOS was the most dominant PFAS measured in fish, similar to findings in site 1 in the present study. In comparison with literature from other parts of the world, there is a clear difference between remote and industrialized areas (Table 5). Langberg et al. (2022), described differences (fingerprints) between PFAS patterns and levels in fish exposed to nearby airports, paper industries and atmospheric exposure. The PFAS found in the present study were long chained (>6C), and of these, PFOS is regulated under the Stockholm Convention since 2009. Although PFAS components are very stable, PFOS levels are expected to decline in the future, due to this regulation.

4.3. Levels of POPs in relation to environmental quality standards (EQSs) and human health

In the present study, only levels of $\sum_6$PBDEs exceeded the EQS_{biota} in 78% of wild fish and 8% of farmed fish (Table 6). However, since the EQS threshold is expressed in ng/g ww, use of fish muscle would have resulted in lower PBDE levels, and fewer samples were expected to exceed the EQS. In addition, the low level of EQS_{biota} for PBDEs in fish has recently been discussed in the EU because this threshold may have been based on a limited number of congeners and species (Teunen et al., 2022). It should be mentioned that EQS is a standard for protection of fish and human health, but that other parameters such as infectious agents, poor nutrition, water temperature, oxygen contents and others may also influence fish health. Follow up studies are warranted, to ensure that international regulations result in decreased levels of these contaminants that may threaten the aquatic environment.

Dioxin-like (DL) PCB-118 TEQ levels (pg/g ww) were below EQS for DL-PCBs (Table 6). Liver tissues were used in this study because lipophilic POPs would be detected at the highest levels in the lipid-rich liver. Results from this study can therefore not directly be used in a risk assessment for humans since humans consume fish muscle. However, the percentage of lipid in tilapia liver (range 3–19%) (present study) is higher than in its muscle, (0.4–4%) (Polder et al., 2014). Therefore, one can assume that POP levels in wild and farmed tilapia liver from the present study are much higher than in the muscle tissue. In 2011, the EU set an MRL for Σ non-dioxin like (NDL) PCBs (PCB-28, -52, −101, −138, −153, and −180) in fish filet to 75 ng/g ww. The highest sum of ΣNDL-PCBs in the present study (sum of PCB 118, 138, 153, 180) was 0.2 ng/g ww in liver, and thus far below this EU MRL. POP levels occasionally varied greatly between the pooled samples from the same area. This may have consequences when calculating risk in future studies. Therefore, analyses of individual samples are recommended to get a better view on the variation in the specified areas.

5. Conclusion

The present study shows generally low levels of OCPs, PCBs, BFRs and PFAS in fish from Lake Kariba, and that POP levels were lower in farmed fish compared to wild fish. Levels of $\sum_6$PBDEs exceeded the EQS_{biota} in 78% of wild fish but only in 8% of farmed fish. The study revealed a geographical trend with higher levels of DDTs, PCBs, PBDEs and PFAS from west to east of Lake Kariba. However, levels of HCB and HCHs were also higher in fish that had foraged near the farms. This needs further investigation to elucidate possible sources. The contribution of *p,p'*-DDD to ΣDDTs increased eastwards, possibly due to higher environmental impact of anaerobic processes and historic use. PCB levels were low, and PCB profiles were dominated by the most persistent PCB congeners PCB-118, -138, −153, and −180. This indicated exposure to historical used PCB, but to a different PCB mixture than what has been found in some other African countries. The finding of the now banned PFOS and BDE-209 in wild tilapia from the western part of the lake warrants further research for finding possible sources. Future studies could cover the whole lake from west to east, increased number of individual samples, and more species for investigation of POPs levels in relation to the food chain. Even though the study was based on liver samples, a theoretical and thus limited assessment indicated that farmed fish from Lake Kariba is safe for human consumption.

Author contribution statement

Chalumba K Simukoko: Collection of samples, chemical analyses,

treated data, original draft, statistical analyses for original draft. Contribution to revisions.; Eliezer B Mwakalapa: Collecting of samples, analyzing data, design of map and original draft.; Kaampwe Muzandu: Supervision of whole study: Design, collection of samples, evaluation of data, and manuscript writing.; Stephen Mutoloki: Study design, collection of samples, reviewing the writing.; Øystein Evensen: Study design, collection of samples, reviewing the writing.; Erik M Ræder: Analyses of PFAS and evaluation of PFAS results. Writing of PFAS chapter.; Mette B Müller: Evaluation of chemical data. Review and revision of the manuscript, revision of statistical analyses, revision of tables and figures.; Anuschka Polder: Supervision, contributing with analyzing data and writing of original draft. Editing of the manuscript. Corresponding author.; Jan L Lyche: Supervision of all stages of study steps: Design, collection of samples, evaluation of data, and manuscript writing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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