Occurrence and levels of persistent organic pollutants (POPs) in wild and farmed tilapia (Oreochromis niloticus) from Lake Kariba, Zambia: possible impact on fish health

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Title: Occurrence and levels of persistent organic pollutants (POPs) in wild and farmed tilapia (*Oreochromis niloticus*) from Lake Kariba, Zambia: possible impact on fish health.

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Abstract

The current study was carried out to determine occurrence and levels of persistent organic pollutants (POPs) in wild and farmed tilapia *(Oreochromis niloticus)* in Lake Kariba, Zambia, and possible implications for fish health. Concentrations of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyls (PBDEs), hexabromocyclododecane (HBCDD) and perfluoroalkyl substances (PFASs) were determined

in liver samples of tilapia. Concentrations of POPs in wild tilapia were in general higher than in farmed tilapia, however, concentrations of DDTs and PCBs in wild tilapia foraging near the fish farms were more in the range of the farmed fish. The highest median ∑DDTs (93 and 81 ng/g lw) were found in wild tilapia from sites 1 and 2, respectively 165 km and 100 km west of the farms. DDE/DDT ratios seem to indicate recent exposure to DDT. The highest median of $\sum_{17}$PCBs (3.2 ng/g lw) and $\sum_{10}$PBDEs (8.1 ng/g lw) were found in wild tilapia from site 1 and 2, respectively. The dominating PCB congeners were PCB-118, -138, -153 and -180 and for PBDEs, BDE-47, -154, and -209. Of PFASs, only PFOS, PFDA and PFNA were detected in wild fish with highest median PFOS levels in site 1 (0.66 ng/g ww). The PCB and BDE concentrations in wild and farmed fish were above EQS_{biota} limits set by the EU. This may suggest a risk to the fish species and threaten biodiversity.

1. Introduction

Fish serves as a major source of proteins to most people in the world and is essential for food security and sustainability (FAO 2020). The growing human population has led to increased demand for fish, leading to overexploitation of wild fisheries and depletion of some fish stocks. Human fish consumption was estimated at 151.2 million tonnes in 2016, while production from capture and aquaculture fisheries were 91 and 80 million tonnes, respectively (FAO 2018; Golden et al. 2017). Apart from being a source of protein, aquaculture is also a source of income for many people estimated at USD 231.6 billion in 2018 (FAO 2018). Since the 1980s aquaculture has seen a constant growth. Currently, African aquaculture industry is undergoing a rapid growth (FAO 2018). Zambia is the sixth-largest producer of farmed fish in Africa, and biggest producer of tilapia in the Southern African Development Community (SADC) (Genschick et al., 2017). Zambia produced 85,000 metric tonnes of fish according to the Department of Fisheries, of which 30,000 tons was produced in fish farms (WorldFish 2022). Despite all benefits, increasing of fish farming may have negative impact on the environment due to release of organic effluents, chemicals and antibiotics in the waterbodies and by acting as a source of diseases or genetic contamination of wild species (Berg et al. 1992; Jarić et al. 2011; Kishimba et al. 2004; Li et al. 2011; Mwakalapa. et al. 2018; Nonga et al. 2011; Polder et al. 2014; Ssebugere et al. 2014; Subasinghe 2005).

Pollution of aquatic environments by persistent organic pollutants (POPs) can affect fish and human health (Barni et al. 2016; Burreau et al. 2004; Jarić et al. 2011; Rodriguez-Hernandez et al. 2017). 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) (Deribe et al. 2011; Rodriguez-Hernandez et al. 2017). Anthropogenic activities such as industries, mining, agriculture and waste from human settlements are sources of POPs (Covaci et al. 2008; Henry and Kishimba 2006; Lyche et al. 2015; Nieuwoudt et al. 2009; Santonen et al. 2017). Their semi volatile nature coupled with long environmental half-lives results in long-range transport and global distribution (De Boer et al. 1994; Nie et al. 2006; Panseri et al. 2019; Squadrone. et al. 2013). Except for PFASs, which are protein bound, 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). Despite the Stockholm Convention of 2001, protecting human health and the environment from POPs (Stockholm Convention 2021), they are still present in most parts of the world (Ashraf 2017).

The growing aquaculture industry in Africa may be threatened by the presence of POPs and other contaminants in the water and fish. Therefore, to enable 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. Contaminant residues in fish may also represent a food safety risk. Environmental stressors including harmful chemical contaminants and biotoxins and other water quality parameters such as pH, oxygenation and eutrophication, have impact on health in wild stocks and farmed fish. Lack of knowledge about levels, sources, environmental behaviour and toxicity, hampers evidence-based decision-making regarding implementation of protective measures. The current study was carried out to establish the occurrence and concentrations of POPs in wild and farmed tilapia from Lake Kariba, Zambia, with emphasis on fish health.

2. Materials and methods

Description of sampling area and species

Nile tilapia (*Oreochromis niloticus*) were collected from Lake Kariba, located on the southern border of Zambia with Zimbabwe (16° 28′ to 18° 04′S; 26° 40′ to 29° 03′ E). Lake Kariba is a

man-made lake in the Zambezi River basin spanning across three districts (Sinazongwe, Gwembe and Siavonga) on the Zambia side. The lake is 320 Km long and covers an estimated area of 5400 Km², with an average depth of 29 meters. The water flow is from west to east where a hydroelectric power plant is located at the Kariba Gorge (Fig. 1). Five locations (Sites 1, 2 and 3, and farms 1 and 2) were selected for this study. Site 1 is in Sinazongwe district with human activities that include coal mining, commercial fishing, crop, livestock, and wildlife farming, with a population of 98,246 (Zambia census 2010). Site 2 is in Gwembe district with agricultural activity (crop and livestock), commercial fishing and a population of 50,136 (Zambia census 2010). Sites 1 and 2 are situated respectively 165 km and 100 km west of the farms. Site 3, which is in Siavonga district, has agricultural activities with crop, livestock, crocodile and commercial fish farms and a feed processing plant. It has a population of 85,811 (Zambia census 2010). Fish farms 1 and 2 are also located in Siavonga district. The farms use cages for rearing tilapia on the lake. Nile tilapia is a fish native to river Nile, which has been spread across the world (Eknath AE and Hulata G 2009). It is found as both wild and farmed fish. It is an omnivorous species that feeds on plankton and higher plants, like algae. Tilapia thrives in tropical and subtropical climates with environmental temperatures of 9 – 42^{o}C, living in shallow waters. It is a fast grower and is fairly resistant to harsh conditions making it favourable for aquaculture.

The climate around Lake Kariba is sub-tropical with a cool and hot dry season from May to October and the wet season between November and April. Annual rainfall ranges from 400 to 700 mm whilst temperature ranges between 13°C and 40°C. Rainfall and intra-seasonal distribution of rain vary greatly from year to year. Due to these climatic conditions, the southern part of Zambia is a drought-prone area and rain-fed agriculture is highly unpredictable.

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 conducting of the study in their areas. Managers at the fish farms gave their permission for samples to be collected from their farms. 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.

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 fishermen as they pulled in their catch from the water. The fish were then placed in a container containing ice water and transported to the shore for dissection. Farmed tilapia were sampled by dip netting and placed in containers containing water. The length and sex of the fish were recorded (Table 1A, 1B). The size of the fish was sometimes considerably different within the study sites. The scale used in the field could only weigh fish up to 1kg. Therefore, only length was used in statistical analyses. Using forceps and scalpel blades, the fish was dissected on a board and liver tissue removed and placed in clean 15 ml Eppendorf tubes then placed on ice in a cooler box. The samples were then transported to the University of Zambia, Veterinary Medicine School, and stored at -20^{o}C. The frozen samples were later transported on ice to the Laboratory of Environment Toxicology at the Norwegian University of Life Science in Oslo, Norway and stored at -20^{o}C until analyses.

Sample analysis of OCPs, PCBs and BFRs,

A total of 82 liver samples were selected and pooled based on size (Table 1A). Each homogenate contained two or three liver samples. Only male fish was included for POP analyses. Before analyses the samples were thawed at room temperature and protected from light during the analyses. 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 were analysed but were not detected above LOD. Those compounds were not included in any data analyses.

Sample analysis of PFASs,

Individual liver samples (N=26) from the same sampling areas and same catch were analysed for perfluoroalkyl substances (PFAS): perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonamide (FOSA) 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 any data analyses further.

Chemical analyses of OCPs, PCB, BFRs

The analytical method for analysing 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 homogenised 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 in all the samples. During the analyses the samples were protected from light and amber GC-vials were used.

Chemical analyses of PFASs

The analytical method was described by Grønnestad et al (2017). In short, internal standards (Wellington laboratories) were added to 0,5 g homogenized liver samples. Extraction was performed twice with 5ml 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 with 500µl methanol and the filtrates were combined and concentrated to dryness and finally reconstituted in 200µl MeOH.

Instrumental analysis

Separation and detection of the POPs

OCPs, PCBs and BFRs were separated and detected using GC-MS methods as described by Mwakalapa et al. (2018) and Polder et al. (2014), 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). 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/5973Network) 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).

Separation and detection of the PFASs

Samples were analyzed on an Agilent 1200 HPLC-system coupled to an Agilent 6460 Triple Quad Mass Spectrometer (Agilent Technologies). A Phenomenex C18 Luna Omega 3µm 100x4,6 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.

Quality Assurance (QA)/ Quality control (QC)

The chemical analysis of the liver 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 the requirement of the NS-EN ISO/IEC 17025 (TEST 137).

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). Within the same period, 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 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. The relative recoveries were 82-137% for OCPs, 92-127% for PCBs, and 68-120% for BFRs.

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. Matrix-matched calibration curves ranged from 0 – 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.

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 and further included in the statistical analyses. Levels below LOD were replaced with 1/2 LOD. Compounds with a detection rate lower than 50% were reported with range and were not included in further statistical analyses. For this latter group, 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. If data from one of the locations failed the Shapiro-Wilk test, the data of all locations were log-transformed. The nonparametric Kruskal-Wallis test were 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

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, Fig. 2). The median liver lipid contents (%) of farmed fish from farm 1 (8.8 %) and 2 (7.5 %) were significantly higher than for wild fish from site 2 (4.9 %), and farm 1 was significantly higher than site 1 (4.1 %). There was also a significant difference in liver lipid content between the wild fish at site 2 and 3 (7.2 %). Length of the individual fish for PFAS analyses were all in the same range (Table 1B).

Occurrence and levels of OCPs, PCBs, BFRs

HCB and *p,p'*-DDE were the OCPs detected in 100% of the liver samples. Median concentrations of HCB were significantly higher in wild fish from site 3 (Table 2B) compared to site 2 and in farm 1 and 2 (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 site 1, 2 and 3, and 79 % and 58 % in fish from farm 1 and 2 (Fig. 3). α-HCH contributed between 21-34 % to the ∑HCHs and β-HCH between 1-14%. The median ∑HCHs was significantly higher in site 3 compared to site 1,2 and farm 2 (Fig. 2). DDTs were the most abundant OCPs in all the 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 %, 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 in site 1 and 2 compared to farm 1 and 2, while site 3 was only significantly higher than farm 2 (Fig.2). The contribution of *p,p'*-DDE to the ∑DDTs was highest in wild fish from site 1 and 2 (48 % and 61 %), but lower in wild fish from site 3, and farmed fish from farm 1 and 2 (46 %, 30 % and 31 %) (Fig. 3) . In farm 1 and 2, the contribution of *p,p'*-DDD to the ∑DDTs was higher than that of *p,p'*-DDE with 63 % and 68 %, 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 2 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. ∑CHLs were not significantly different between any of the locations. 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 3.3 ng/g lw <site 3<site 2<farm 1<farm 2 (Table 2B, Fig. S1). Median $\sum_{17}$PCBs was significantly higher in site 1 compared to 2 and farm 1 and 2. Site 3 was significantly higher compared to site 2 and farm 2 (Fig. S1).

PBDEs were detected in all samples, except one. BDE-47, -99, -154, and 209 were the most abundant BDEs detected in 70 %, 30 %, 57 % and 97 % of the samples. BDE-209 dominated the PBDE pattern and contributed average 84 % to $\sum_{10}$PBDEs (Fig. 3). The highest median concentration of $\sum_{10}$PBDEs (including BDE-209) was 8.1 ng/g lw in site 2. Median $\sum_{9}$PBDEs (excluding BDE-209) were not significantly different between the locations, however the median $\sum_{9}$PBDEs was significantly lower in farm 2 compared to site 1,2,3 and farm 1 (Fig. 2).

HBCDD was only detected in one sample from farm 1, with a concentration of 0.27 ng/g lw.

Occurrence and levels of PFASs

PFOS, PFDA and PFNA were the only PFASs detected in levels >LOD in individual wild fish (Table 2B). No PFASs were detected in site 3, farm 1 or farm 2. PFOS and PFNA were detected in 100 % and 40 % and in 100 % and 40 % in wild fish from site 1 and 2, respectively and PFNA was detected in 20 % in site 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).

Correlations

Spearman correlations coefficients for the main compounds HCB, ∑DDTs, ∑PCBs, ∑BDEs and ∑BDE including BDE-209 are presented in Table S2. Strong correlations were found between HCB, ∑DDTs and ∑PCBs (r>0.86), and between ∑DDTs and ∑BDE including BDE-209 (r=0.86) in site 1. In site 2, only ∑BDEs and ∑BDE including BDE-209 showed strong correlations (r=0.84). In site 3, ∑DDTs, ∑PCBs, ∑BDEs and ∑BDE including BDE-209

297 strongly correlated (r>0.8), and ∑PCBs and ∑BDEs with r=0.93. In site 4, HCB and ∑BDEs
298 showed strong correlations (r=0.86), and in site 5, HCB and ∑BDEs (r=0.86) and ∑DDTs and
299 ∑PCBs (r=0.88).

300 *Compliance with reference levels*

301 Compared to recommend Environmental quality standards (EQS) from European commission
302 most of the POPs were below the limits for fish except for $\sum_{10}$BDE (mean 0.04 - 0.36 ng/g
303 ww) which was higher than the EQS limits of 0.0085 ng/g set the EU (European Commission
304 2013; Jürgens et al. 2015).

305 **4. Discussion**

306 Food security is a global issue and farming of fish is essential as supplement to wild fish
307 industry for the growing global population (FAO 2020). The main goal in fish farming industry
308 is to obtain high quality fish for sale in short time. The fish must fulfil criteria set by health
309 authorities, as regards to nutritional value as well as to the presence of environmental
310 contaminants/pollutants (Saavedra et al 2017; Skåre et al. 2014). Fish feed is therefore specially
311 composed for the purposes of fast growth, high content of nutrients and low content of
312 pollutants. As expected, the farmed fish had significantly higher liver lipid content compared
313 to the wild fish (Table 2, Fig 2), which indicates good conditions for the farmed fish. Spillage
314 of feed and organic waste from the cages into the environment around the farms makes more
315 nutrients available to wild fish (Ballester-Moltó et al 2017; Bustnes et al. 2010; Varol. 2019).
316 Fish from site 3 forages around the fish farms and shows significantly higher fat content than
317 in wild fish from site 2, confirming that wild fish in site 3 makes use of nutrient spill from the
318 fish farms (Table 2). There is also a possibility that farmed tilapia escape from the cages and
319 thus being caught as wild fish, thus influence the fat content of fish in site 3 (Azevedo-Santos
320 et al. 2011).

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

322 DDTs were the dominant OCPs in both wild and farmed fish liver tissues from Lake Kariba.
323 Wild fish had significantly ($p<0.05$) higher levels of median ∑DDTs compared to the farmed
324 fish (Table 2, Fig. 2). This was similar to findings in other studies (Berg et al. 1992; Mwakalapa
325 et al. 2018) (Table 5). The countries around Lake Kariba (Zambia and Zimbabwe), have
326 historically been influenced by use of DDT for vector control in combatting malaria and tsetse

control operations in addition to agriculture (Berg et al. 1995). Lake Kariba was filled with water in 1958-1963. Because the flooded areas were earlier treated with DDT, the sediments of the Lake will still be a reservoir of DDT residues. Due to 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. 1995; Brevik 1996). In addition, DDT may have entered the Lake Kariba by 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 South 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 site 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 2, 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 et al. 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 wild fish from site 1 and site 3, indicating relatively recent use of DDT in the area (Table 5). In addition, one of the pooled samples from site 1 contained *o,p'*-DDT, strengthening this observation. The *p,p'*-DDE/*p,p'*-DDT ratios in fish from farm 2 were higher than in farm 1 and indicate stronger relationship to historic use of DDT (Ssebugere et al. 2009). Levels of mean ∑DDTs in the wild tilapia were lower than those reported in Lake Victoria (Henry and Kashimbi 2006; Polder et al. 2014), but higher than from other areas (Deribe et al. 2011; Gbeddy et al. 2015; Mdegela et al. 2009) (Table 5). Farmed tilapia in the present study had similar levels of mean ∑DDTs to those reported from Lake Kariba, Zimbabwean side, by Berg et al. (1992) (Table 4).

The second dominant OCP, HCB, was detected in low levels below the EQS set by the EU (Table 4). Median levels of HCB were significantly higher in wild fish from site 3 compared to site 2 and farm 1 and 2 (Table 2B, Fig. 2). Previously, Berg et al (1992) found larger differences between wild and farmed fish in Lake Kariba. HCB was used as a fungicide, in rubber synthesis and wood preservation among other uses, but is banned under the Stockholm Convention (Stockholm Convention 2021). The low levels observed may reflect a general

360 background level related to long range atmospheric transport from emission of industries at far
361 distance (Polder et al. 2014). Levels of HCB in the current study were in the same range as
362 levels reported in tilapia liver from Tanzania by Mwakalapa et al. (2018) and Mdegela et al
363 (2009), but lower than those reported by Polder et al. (2014) in tilapia muscle, and much lower
364 than in brown trout in Norway reported by Lyche et al. (2018) (Table 5).

365 Although levels of HCHs generally were low in all study sites, the median ∑HCHs was
366 significantly higher in wild fish from site 3 than in site 1 and 2 and in farm 2 ($p<0.05$) (Fig. 2).
367 Median ∑HCHs in farmed fish from farm 1 was not significantly different from site 3,
368 suggesting that HCH in site 3 and farm 1 have a common source. Lindane (γ-HCH) was used
369 as an insecticide on fruit and vegetable crops, for seed treatment and treatment of lice and
370 scabies, mainly in West-European and Asian countries (Vijgen et al. 2006). Non-scientific
371 confirmed information suggests use of locally produced lindane as a fishing technique in
372 Ghana. Pure lindane is contaminated with α-HCH and β-HCH of which β-HCH is the most
373 persistent isomer. Due to regulations, levels of lindane are decreasing. The patterns of HCHs
374 found in this study may thus reflect historic use of the technical mixture combined with
375 relatively recent use of γ-HCH. The general HCH levels in tilapia from Lake Kariba were in
376 the same range as those reported by Berg et al. (1992), Mwakalapa et al. (2018) and Polder et
377 al. (2014) in Tanzania, but much lower than findings in South Africa by Verhaert et al. (2017)
378 and in Ghana by Gbeddy et al. (2015) (Table 5).

379 *Trans*-Nonaklor was the dominant chlordane found in low levels in the present study.
380 Chlordanes are banned compounds and are no longer used as insecticides (Stockholm
381 Convention 2021). Mean levels of ∑CHL were less than those reported by Polder et al. (2014)
382 and Mdegela et al. (2009) in Tanzania and Gbeddy et al. (2015) in Ghana. Mirex and
383 Heptachlor are also banned substances and were below detection limit in all samples (Table S
384 1.1).

385 PCBs were detected in very low levels in wild and farmed tilapia and those levels were in the
386 same range as in other East African countries (Deribe et al. 2011; Kidd et al. 2004; Mwakalapa
387 et al. 2018), but lower than in tilapia from Tanzania and Ghana, and other fish from South
388 Africa and Norway (Polder et al. 2014; Wepener et al. 2012; Asantae et al. 2013; Lyche et al.
389 2018). Occurrence and levels of PCB (17.2 ng/g lw) in wild tilapia from Lake Tanganyika was
390 suggested to be related to human activities and small local industries (Polder et al. 2014). Wild
391 tilapia from site 1 showed the highest mean $\sum_{17}$PCBs (3.2 ng/g lw) (Table 2B). Emission from

392 a coal mine in the area may be a possible source. Other possible sources of PCBs in countries
393 with limited historic use of PCBs are waste burning, transportation, household heating,
394 discharges from cities, sewage processing, e-waste burning, hospital waste incineration, and
395 transformer oil (Pius et al. 2019). In the present study only PCB-118, -138, -153 and -180 were
396 detected in more than 50% of the samples. These congeners are the most persistent PCBs and
397 contributed more than 60 % to $\sum_{17}$PCBs. Dominance of PCB-153, PCB-180 and PCB-138 was
398 similar to findings in other studies (Asante et al. 2013; Hayward et al. 2007; Mwakalapa et al.
399 2018; Polder et al. 2014). In the present study, PCB-118 was detected in 72 % of the wild
400 tilapia, but only in 25 % of the farmed tilapia (data not shown). PCB-118 is a mono-*ortho*
401 substituted PCB and has a toxic equivalent factor of 0.00003 (Van den Berg et al. 2006). In the
402 study by Polder et al. (2014) PCB-118 was only detected in one tilapia sample from Lake
403 Victoria and one from Lake Babati in Tanzania. It seems thus, that the environment in Lake
404 Kariba is exposed to a different historic PCB mixture than in other studies in the region. The
405 finding of significantly higher $\sum_{17}$PCBs in fish from site 1 and 3 (0.92 ng/g lw) than in the
406 nearby farms 1 (0.27 ng/g lw) and 2 (0.19 ng/g lw), may be related to higher age of the wild
407 fish, rather than higher exposure to unknown sources.

408 PBDEs were used as flame retardants in thermoplastics (computer and TV housing), textiles,
409 foams, furniture, electronics, building materials and interiors of cars, busses, and airplanes
410 (Covaci et al. 2008; Lyche et al. 2015). In general, low PBDE levels were detected in the
411 present study. BDE-47, -99 and -154 were detected in more than 50 %, whereas BDE-209 was
412 detected in 97 % of the samples in all areas. Tetra-BDE (BDE- 47) and penta-BDE (BDE-99)
413 are the most abundant, toxic, and bioaccumulative PBDE congeners (Ssebugere et al. 2014;
414 Asante et al. 2013; Mwakalapa et al. 2018). Due to differences in metabolism, lower
415 brominated congeners such as BDE 47 and 100 accumulate more in aquatic organisms, while
416 higher brominated congeners like BDE 154 and 209 accumulate more in terrestrial organism
417 (Luo et al. 2019). Deca-BDE, a mixture of nona-, octa and deca BDEs were used as a
418 replacement for the tetra and penta- BDE mixtures after they got banned (Stockholm
419 Convention 2021). However, deca-BDE debrominates to lower brominated and more persistent
420 BDE congeners, such as BDE-47 (Stapleton et al. 2004). Half-life of BDE-209 may vary in
421 different compartments. Luo et al. (2013) found that the dose dependent half-life of BDE-209
422 in the muscle of rice fish was from 17 to 19.4 days. Recent studies showed that there is a
423 significant amount of gaseous BDE-209 in the global atmosphere, which is subject to long-
424 range atmospheric transport (LRAT) (Li et al. 2017). The occurrence of BDE-209 in nearly

100 % of the fish samples from Lake Kariba may therefore be explained by precipitation of atmospheric transported BDE-209, although the high median BDE-209 levels in site 2 (7.8 ng/g lw) may suggest additional exposure from a local source. Median levels of BDE-47 and -99 were significantly higher in wild fish from site 1 and 2, compared to the farmed fish, strengthening the hypothesis that wild fish has been exposed to historic precipitation of atmospheric transported BDE-209. $\sum_{10}$PBDE levels in wild tilapia sites 1 and 2 were from four to thirteen-fold lower than in tilapia muscle from Lake Victoria but comparable to findings in the other lakes in Tanzania (Polder et al. 2014). They were in the same range as in tilapia from Ghana (Asante et al. 2013) and African tigerfish (*Hydrocynus vittatus*) from South Africa (Wepener et al. 2012), and tilapia in Uganda (Ssebugere et al. 2014), higher than in Milkfish (*Chanos chanos*) from Tanzania (Mwakalapa et al. 2018) but much lower than in trout (*Salmo trutta*) in Norway (Lyche et al. 2018) (Table 5). A global deca-BDE ban was adopted under the UN Stockholm Convention in 2017 and BDE-209 levels and its debromination products are thus expected to decrease in the environment.

Levels and pattern of PFAS

This is the first time PFASs are detected in fish from Zambia. PFASs is a large group of perfluorinated substances produced since the 1950 and because of their water repellant properties used in various consumer products such as impregnated outdoor textiles, shoes, food containers, kitchen ware and firefighting foam. They are very persistent to degradation and may cause adverse health effects in living species. In contrast to the lipophilic POPs, PFASs 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 PFASs were only detected in wild tilapia in site 1 and 2, and not in site 3 and in farmed fish. PFDA and PFOS were both detected in levels >LOD in 100 % of the tilapia from site 1, and in 80 % and 40 % in site 2, respectively (Fig. 3). PFNA was only found in one sample from site 1 and 2. The occurrence of PFASs in the wild fish from Sinazongwe (site 1), Gwembe (site 2) may be related to mining industries in the area. There are to our knowledge only two studies on PFASs in South African fish. Verhaert et al. (2017), who 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 in muscle of fish from the Olifants River basin, while Groffen et al. (2018) found PFOS in fish liver from Vaal River in much higher levels similar or higher than in USA, Europe and Asia. than in European studies. PFOS levels in trout from the isolated large and deep inland Lake Femund, Norway, were 3.96 ng/g ww,

458 thus 10 times higher than in tilapia from the present study (Lycke et al. 2018). The PFASs
459 found in this study were long chained (>6C), of which PFOS are regulated under Stockholm
460 Convention since 2009. These compounds are therefore expected to decline in the future.
461
462 *Possible implications for fish and human health*
463 In the present study, levels of PBDEs exceeded the European standard (EQS) for these
464 contaminants in fish and may harm fish health (Table 4). Follow up studies are needed to ensure
465 that international regulations result in decrease of these and other contaminants that threaten
466 the aquatic environment. Dioxin-like (DL) PCB-118 TEQ levels (pg/g ww) were below EQS
467 for DL-PCPs (Table 4). Liver tissues were used in this study because lipophilic POPs would
468 show highest levels in the lipid rich liver. Results from this study can, therefore, not directly
469 be used in a risk assessment for humans since humans consume fish muscle. However, the
470 percentage of lipid in tilapia liver (range 3-19 %) (present study) is higher than in its muscle,
471 (0.4-4 %) (Polder et al. 2014), one can assume that POP levels in wild and farmed tilapia liver
472 from the present study are much higher than in the muscle tissue. In 2011, the EU set an MRL
473 for Σ non-dioxin like (NDL) PCBs (PCB-28, -52, -101, -138, -153, and -180) in fish filet to 75
474 ng/g ww. The highest sum of ΣNDL-PCBs in the present study (sum of PCB 118, 138, 153,
475 180) was 0.2 ng/g ww in liver, and thus far below this EU MRL. POP levels varied sometimes
476 much between samples from the same area. This may have consequences when calculating risk
477 in future studies, therefore analyzing of individual samples is recommended to get a better view
478 on the variation in the specified areas.
479
480 **5. Conclusion**
481 The present study shows that levels of OCPs, PCBs, BFRs and PFAS are in general, lower in
482 farmed fish compared to wild fish within the same lake. This indicates less risk for fish health
483 in farmed than in wild fish. Farmed fish from this study is also considered safe for human
484 consumption. There was a geographical trend with higher levels of DDTs, PCBs, PBDEs and
485 PFASs from west to east of Lake Kariba. However, levels of HCB and HCHs were also higher
486 in fish that had foraged near the farms, and this need further investigation to elucidate possible
487 sources. The contribution of *p,p'*-DDD to ΣDDTs increased eastwards, possibly due to higher
488 environmental impact of anaerobic processes. PCB levels were low, and PCB profiles
489 dominated by the most persistent PCB congeners (PCB-118, -138, -153, -180), indicated
490 exposure to historical used PCB, but to a different PCB mixture than in some other East African

countries. The finding of the now banned PFOS and BDE-209 in the wild tilapia from the western part of the lake, warrant further research for determination of the possible sources.

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Figures

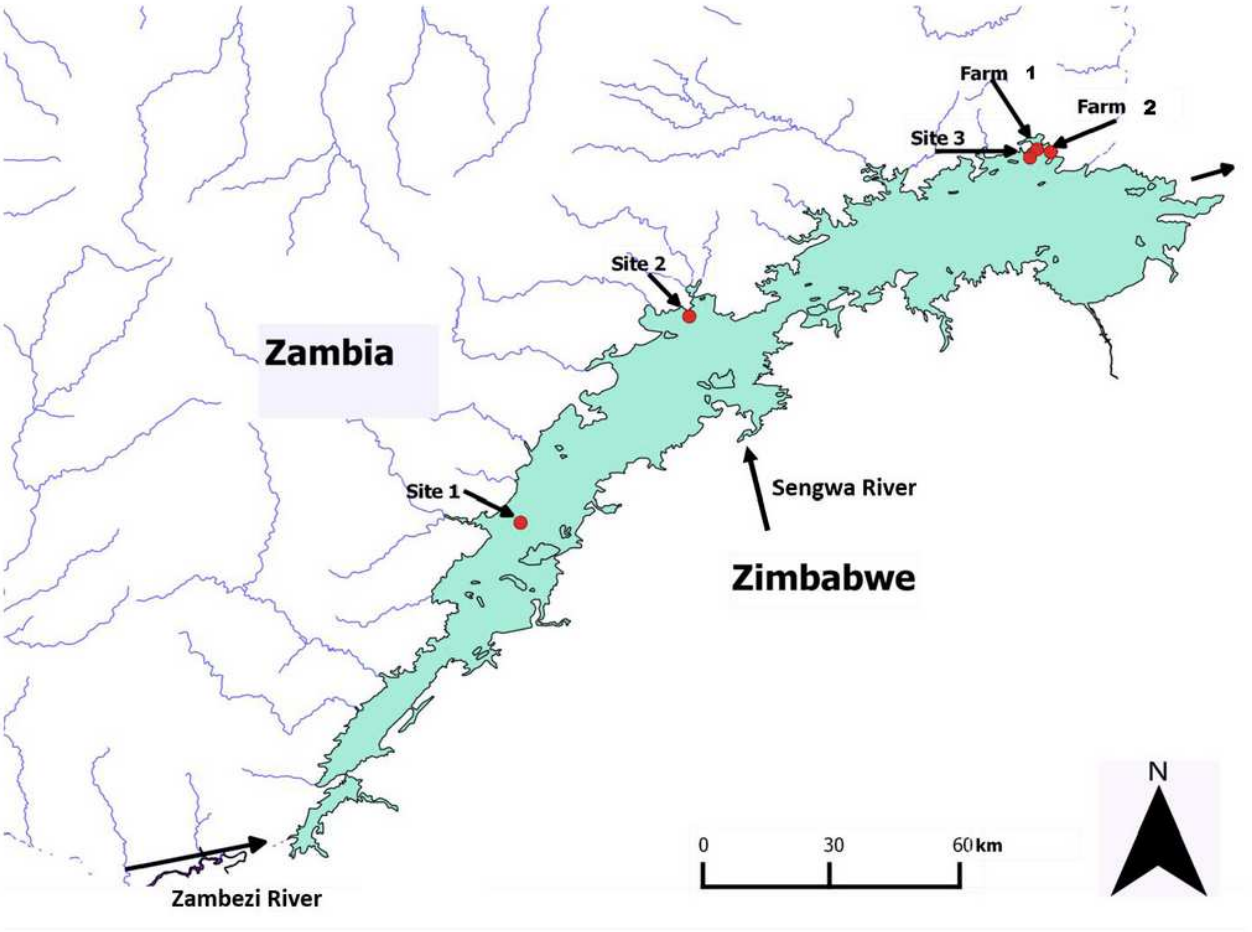

Figure 1

Map of lake Kariba, showing the 5 sampling locations (sites1-3 and farms 1 and 2)

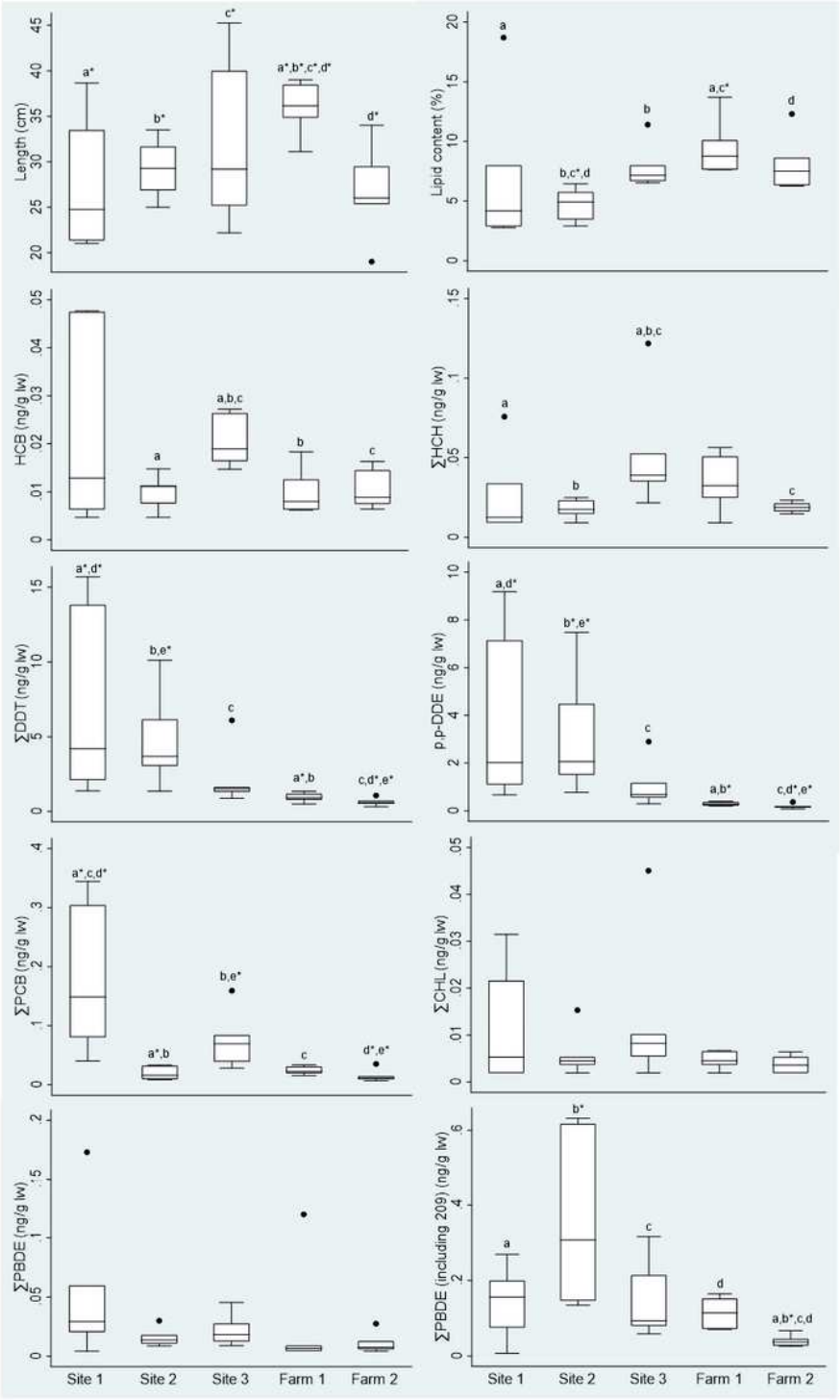

Figure 2

Fish length, liver lipid content and contaminant concentrations in livers from wild fish (site 1-3) and farmed fish (farm 1-2) in Lake Kariba, Zambia. Fish length is given in cm, liver lipid content in % and liver concentrations are presented as ng/g lipid weight. 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) indicate statistically

significant difference ($p<0.05$) between the sites and farms. Asterisk (*) indicates statistical significance ($p<0.05$) after Bonferroni's corrections for multiple testing.

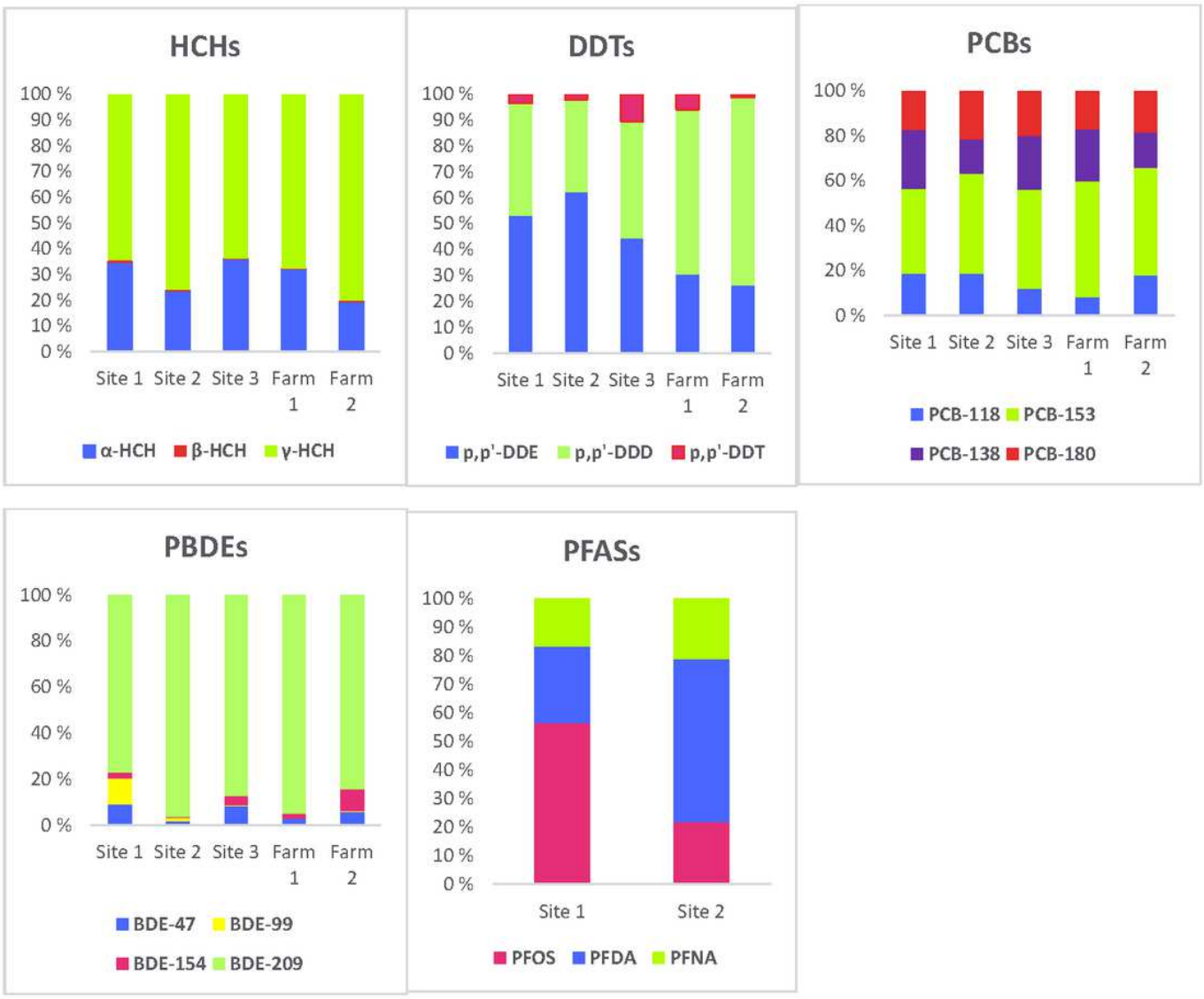

Figure 3

Percent contribution of individual congeners to ΣHCHs, ΣDDTs, ΣCHLs, ΣPCBs, ΣPBDEs and PFASs

Supplementary Files

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