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Seasonal Bioaccumulation of Organohalogens in Tigerfish, *Hydrocynus vittatus* Castelnau, from Lake Pongolapoort, South Africa

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Abstract The study determined current exposure of tigerfish in Lake Pongolaqpoort to organohalogens. Levels of DDT, PCB, HCB, HCH, PBDE and CHLs were measured in tigerfish muscle on a seasonal basis. Historical use of DDT was reflected in the bioaccumulation patterns (5,400–6,000 ng/g lipid) as well as current use of HCBs (7.7–15.7 ng/g lipid) in the agricultural areas. External factors, i.e. increased flow did not play a role in organic pollutant exposure. Levels are a function of the lipid content of the muscle tissue, i.e. 3.8% during low and 9% during high flow, implying that organohalogen exposure remains fairly constant throughout the year.

Keywords Organochlorine pesticides · Fish · DDT · Hexachlorobenzene

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Lake Pongolapoort, which was built in 1972 is situated to the west of the Lebombo Mountains, lies on the border between Swaziland and South Africa and has a holding capacity of 2,492 million m^3 of water. It is the fifth largest impoundment in South Africa and was originally constructed to meet the irrigation needs of the large-scale agricultural activities in the surrounding area, which is dominated by sugarcane (Singels et al. 2005). The surrounding area of the impoundment is classified as an endemic intermediate to low risk malaria area and vector control in the form of dichlorodiphenyltrichloroethane (DDT) spraying has been applied almost uninterrupted since 1946 (Sereda and Meinhardt 2005).

Measurements of pollutants by direct chemical analysis in water and sediment are limited in reliability (Smolders et al. 2004). Many studies now utilize aquatic organisms such as fish to monitor exposure to metal and organic pollutants in the environment (van der Oost et al. 2003). There are limited studies that have been conducted on levels of pollutants in the Phongolo system with only one known study on DDT bioaccumulation in fish of the Phongolo floodplain below the lake (Bouwman et al. 1990). Of the three freshwater fish species (*Hydrocynus vittatus*, *Oreochromis mossambicus* and *Eutropius depressirostris*) collected by Bouwman et al. (1990), the tigerfish (*H. vittaus*) had three times higher levels of DDT than the other two species. This clearly demonstrated the degree to which this predatory species was able to biomagnify DDT. In recent years there has been concern regarding the decline in tigerfish populations in South Africa and Steyn et al. (1996) attributed this to amongst others the increased risk to pollutant exposure as a result of this species' ability to biomagnify pollutants. As a result, *H. vittatus* have been placed on the threatened and protected species list in South Africa.

The aims of this study were three-fold. Firstly to obtain baseline concentrations of organohalogenated pollutants such as organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in tigerfish from the Lake Phongolapoort, secondly to determine if there are seasonal fluctuations in pollutant exposure and subsequent uptake and lastly to compare the values obtained in this study with findings from other studies in African lakes.

Materials and Methods

Tigerfish were caught using artificial lures and bait in the southern regions of the Lake Pongolapoort (27° 26′ 33″S 32° 01′ 12″E) during the summer, high flow (February 2009), winter low flow (July 2009) and spring low flow (September 2009) periods. Following capture they were placed into aerated tanks and transported to the field laboratory. The fish were weighed, measured, sacrificed by severing the spinal cord, and dissected. Approximately 15 g axial muscle tissue were placed in pre-cleaned polypropylene tubes and aluminium wrapping and frozen for OC analyses. All dissection apparatus were rinsed with 99.8% ethanol between dissections.

Analyses were undertaken in pooled muscle samples with five replicates for each survey. Samples were freeze dried prior to analyses. The following compounds were included in the analysis: *p,p′*-DDE, *o,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT, *o,p′* and *p,p′*-DDT (the sum expressed as ΣDDTs), hexachlorobenzene (HCB), α-, *β*-, and *γ*-hexachlorocyclohexane (HCH) isomers (the sum expressed as ΣHCHs), 23 PCB congeners (IUPAC no: 28, 31, 52, 74, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 163, 170, 177, 180, 183, 187, 194, 196, 199), 7 PBDEs congeners (no: 28, 47, 99, 100, 153, 154, 183) and the chlordanes (CHLs), i.e. oxychlordane (OxC), trans-nonachlor (TN) and cis-nonachlor (CN). All sample preparation and analyses were carried out according to the methods described by Covaci et al. (2006) and Voorspoels et al. (2003).

Prior to analysis, samples were grinded to a fine powder with a mortar and pestle and stored at room temperature. Muscle tissues were dried to ensure a high efficiency during the Soxhlet extraction. The lipid content was expressed in g fat per 100 g dry fish whole weight. Dried fish muscle (typically between 1 and 2 g) was accurately weighted into an extraction thimble and spiked with internal standards (20 ng CB 143, 4 ng ε-HCH and 4 ng BDE 77). Samples were extracted for 2 h by hot Soxhlet with 100 mL mixture of acetone/hexane (1/3, *v/v*). For the lipid determination, the residue (after solvent evaporation) was dried for 20 min at 60°C, allowed to cool down and then weighed. The extract was evaporated and cleaned by passing through 8 g of acid silica (H_2SO_4, 44% w/w), from which pollutants were eluted with 20 mL hexane and 15 mL DCM. The elute was evaporated to dryness and re-dissolved in 100 µL iso-octane (Voorspoels et al. 2003; Covaci et al. 2008). Minor adaptations were required as PCBs, HCB and DDTs were analysed by GC-EI/MS, while PBDEs, HCHs and CHLs were analysed by GC-ECNI/MS.

The determination of PBDEs, HCHs and CHLs was performed with an Agilent 6890GC-5973MS equipped with a 30 m × 0.25 mm × 0.25 µm DB-5 capillary column and operated in electron capture negative ionisation (ECNI) mode. The ion source, quadrupole and interface temperatures were 250, 150 and 300°C, respectively. The electron multiplier voltage was set at 2,200 V. Dwell times were set to 40 ms. Helium was used as carrier gas at constant flow (1.0 mL/min), while methane as moderating gas. One µL of the extract was injected in cold pulsed splitless (initial injector temperature at 90°C, stay 0.03 min, then heated at 700°C/min to 300°C, pressure pulse 25 psi, pulse time 1.50 min, split-less time 1.50 min). The temperature of the DB-5 column was programmed from 90°C, kept for 1.5 min, then increased with 15°C/min to 300°C, kept for 15 min.

For the analysis of PCBs, HCB and DDTs, extracts were injected into a GC/MS operated in electron ionization (EI) mode and equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300°C, respectively. One µL of the extract was injected in cold pulsed split-less mode (injector temperature 90°C (0.03 min) then to 300°C at 700°C/min, pressure pulse 25 psi, pulse time 1.50 min, split-less time 1.50 min). Helium was used as carrier gas at constant flow (1 mL/min). The temperature of the HT-8 column was kept at 90°C for 1.50 min, then increased to 180°C at a rate of 15°C/min, further increased to 280°C at a rate of 5°C/min and finally raised to 300°C at a rate of 40°C/min, holding for 20 min.

Quality assurance and quality control were performed through the analysis of procedural blanks, and a standard reference material (SRM 1945, OCPs, PCBs, and PBDEs in whale blubber). For the replicate and SRM 1945, the relative standard deviations (RSD) were <10% for most analytes. Additionally, the method performance was assessed through successful participation to interlaboratory studies organized by NIST (OCPs, PCBs and PBDEs in biological tissues). Procedural blanks were consistent (RSD <20%) and therefore the mean value of each analyte in the procedural blanks was used for subtraction. Method quantification limits (LOQs) for individual OCPs, PCB and PBDE congeners were based on procedural blanks (3 × SD) and the amount of sample taken for analysis.

For results below detection limits the value was substituted with the frequency of detection multiplied by the LOQ. Statistical analyses were conducted using PASW Statistics version 18. The significant seasonal differences in metal and OC concentrations were tested by one-way analysis of variance (ANOVA; $p < 0.05$). Data were tested for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests (Zar 1996), respectively, prior to applying *post-hoc* comparisons. *Post-hoc* comparisons were made using the Scheffé test for homogeneous or Dunnett's-T3 test for non-homogenous data. The use of either one of the two tests resulted in the determination of significant differences ($p < 0.05$) between variables.

Results and Discussion

Although the levels of the organohalogens were also measured in sediment samples from the three surveys, we do not report them in this paper as they were very low, i.e. <5 ng/g dry mass for both ΣDDTs and ΣPCBs. The lipid content in tigerfish muscle (expressed as % muscle mass) was significantly higher in the tigerfish sampled during the summer (February 2009) survey and lowest during the winter (July) survey (Table 1). These differences are related to the lower metabolic and reproductive condition of the fish during the winter period (Steyn et al. 1996). The influence of condition and therefore total lipid reserves in the muscles were identified by Covaci et al. (2006) as a major factor in determining OC bioaccumulation in fish.

The ΣDDTs (defined as the sum of *o,p′*- and *p,p′*-DDE, DDD, DDT) were the most abundant organochlorine pesticides (Table 1). All the isomers were detected in all of the samples, with the highest levels recorded were for DDE followed by DDT and DDD. There were no seasonal differences in DDT levels when comparing the results based on lipid content. However, the levels were significantly lower during the winter low flow period (July) when based on dry muscle mass (data not shown). Even though there is a seasonal difference in DDT bioaccumulation, the bioavailable fractions remained the same between the three surveys as was evident from the similar concentrations when they were normalized for lipid content. The ΣDDTs concentrations exceeded the European Union maximum residue level in edible fat (1,000 ng/g fat) (EC 2005). The high DDE/DDT ratio (>4) indicates historical use of DDT and subsequent breakdown in the system (Strandberg and Hites 2001). Due to the piscivorous nature of tigerfish the higher levels of *p,p′*-DDE may be indicative of biomagnification of the DDT from their the prey and hence the internal biotransformation of DDT to DDE by the tigerfish (Ssebugere et al. 2009). When compared to historical data from the Phongolo River floodplain, the concentrations of ΣDDTs in the muscle tissue of *H. vittatus*, recorded during this survey were similar to those measured in *H. vittatus* by Bouwman et al. (1990). These levels of total DDTs were also similar to levels recorded by Matthiessen (1985) in

Table 1 Mean ± standard error of organochlorine pesticides (ng/g lipid) in tigerfish muscle from the Lake Phongolapoort

Compound	LOD (ng/g)	February 2009 (n = 5)	July 2009 (n = 5)	September 2009 (n = 5)
HCB	4	82.2 ± 40.6	117.5 ± 74.4	355.5 ± 230
o,p′-DDD	4	32.5 ± 6.7^{a}	42.0 ± 10.4^{b}	20 ± 3.5^{ab}
p,p′-DDD	4	333.1 ± 76. 8	316.7 ± 77.8	245 ± 45.9
o,p′-DDE	4	7.5 ± 1.2	7.8 ± 2.4	7.1 ± 2.7
p,p′-DDE	4	4,129.8 ± 957.8	4,161.7 ± 1,478.6	5,600 ± 2,499.3
o,p′-DDT	4	59.6 ± 6.7^{a}	72.6 ± 20.7^{b}	36 ± 7.9^{ab}
p,p′-DDT	4	841.4 ± 107.4^{a}	936.7 ± 263.3^{b}	535.9 ± 113.4^{ab}
ΣDDTs	4	5,403.9 ± 1,156.5	5,537.4 ± 1,677.1	6,443.9 ± 2,635
p,p′-DDE/DDT		4.9	4.4	10.5
OxC	2	ND	ND	ND
TN	2	2.63 ± 0.7	2.04 ± 0.89	2.75 ± 0.96
CN	2	0.86 ± 0.5	0.86 ± 0.46	1.05 ± 0.65
ΣCHLs	4	3.49 ± 1.2	2.9 ± 1.35	3.8 ± 1.61
α-HCH	2	3.10 ± 0.69	6.6 ± 2.0	11.01 ± 8.50
β-HCH	2	0.95 ± 0.45^{a}	2.2 ± 0.2^{ab}	0.97 ± 0.57^{b}
γ-HCH	2	3.69 ± 1.41	4.1 ± 0.8	3.77 ± 2.35
ΣHCHs	4	7.73 ± 2.55	12.9 ± 3.0	15.75 ± 11.43
Lipid content (%)	n = 5	8.97 ± 0.04^{a}	3.83 ± 0.01^{ab}	6.38 ± 2.74^{b}

LOD level of detection, *ND* below detection limits, means with common superscript indicate a significant difference ($p < 0.05$) between the sampling surveys

Table 2 Mean ± standard error of PBDEs and PCBs in tigerfish muscle (ng/g lipid; n = 5) from the Lake Pongolapoort

Compound	LOD (ng/g)	February 2009	July 2009	September 2009
PCB 18	5	ND	ND	ND
PCB 28	5	ND	ND	ND
PCB 47	5	ND	ND	2.6 ± 1.6
PCB 49	5	4.0 ± 1.3[a]	12.8 ± 3.5[a]	7.60 ± 2.45[b]
PCB 52	5	11.5 ± 3.8[a]	44.4 ± 12.1[ab]	19.62 ± 8.82[b]
PCB 95	2	20.1 ± 6.8[a]	77.3 ± 28.2[a]	41.59 ± 18.93[b]
PCB 99	2	7.2 ± 2.5[a]	31.8 ± 12.2[a]	13.51 ± 5.64[b]
PCB 101	2	19.7 ± 6.6[a]	92.8 ± 36.5[a]	39.21 ± 16.93[b]
PCB 105	2	4.0 ± 1.4[a]	17.4 ± 7.2[a]	7.55 ± 3.38[b]
PCB 110	2	16.8 ± 5.3[a]	82.7 ± 34.4[a]	36.6 ± 16.9[b]
PCB 118	2	8.4 ± 2.6[a]	44.2 ± 18.9[a]	15.3 ± 6.7[b]
PCB 128	2	1.0 ± 0.6[a]	5.9 ± 2.8[a]	3.1 ± 1.7[b]
PCB 138	2	6.7 ± 1.5[a]	22.1 ± 10.5[a]	12.5 ± 6.3[b]
PCB 146	2	1.1 ± 0.7[a]	12.2 ± 9.4[a]	3.1 ± 1.4[b]
PCB 149	2	7.9 ± 2.2[a]	34.3 ± 16.4[a]	17.5 ± 8.2[b]
PCB 151	2	1.9 ± 1.0[a]	8.3 ± 3.7[a]	5 ± 2.1[b]
PCB 153	2	7.6 ± 1.6[a]	24.3 ± 12.7[a]	13.5 ± 6.6[b]
PCB 156	2	ND	ND	ND
PCB 170	2	2.3 ± 0.5[a]	4.8 ± 2.3[b]	3.2 ± 0.8[c]
PCB 171	2	ND	ND	ND
PCB 172	2	ND	ND	ND
PCB 174	2	ND	ND	ND
PCB 177	2	0.8 ± 0.4	1.2 ± 0.8	1 ± 0.6
PCB 180	2	4.8 ± 1.1	8.7 ± 4.9	7.3 ± 4
PCB 183	2	0.8 ± 0.4	1.6 ± 1.2	1.5 ± 1.1
PCB 187	2	2.5 ± 0.3	3.8 ± 1.9	3.2 ± 2
PCB 194	2	ND	ND	ND
PCB 195	2	ND	ND	ND
PCB 199	2	0.9 ± 0.5	1.4 ± 1.0	1.8 ± 1.4
PCB 205	2	ND	ND	ND
PCB 206	2	ND	ND	ND
PCB 209	2	ND	ND	ND
ΣPCBs	2	129.9 ± 41.1[a]	531.8 ± 220.5[a]	257.3 ± 114.6[c]
BDE 28	0.5	ND	0.2 ± 0.1	0.34 ± 0.24
BDE 47	0.5	2.0 ± 0.3	2.9 ± 1.3	2.07 ± 1.01
BDE 49	0.5	ND	ND	ND
BDE 99	0.5	0.2 ± 0.1	0.7 ± 0.6	ND
BDE 100	0.5	0.9 ± 0.2	1.0 ± 0.3	1.47 ± 0.79
BDE 153	0.5	0.12 ± 0.02	0.2 ± 0.1	ND
BDE 154	0.5	1.0 ± 0.2	0.9 ± 0.3	1.15 ± 0.65
ΣBDEs	0.5	4.1 ± 0.8	5.8 ± 2.8	4.68 ± 2.45

LOD level of detection, *ND* below detection limits, means with common superscript indicate a significant difference ($p < 0.05$) between the sampling surveys

muscle tissue of *H. vittatus* from Lake Kariba, Zimbabwe. The main difference being that the *p,p′*-DDT isomer was dominant during the previous studies while the *p,p′*-DDE isomer was highest during this study.

The ΣDDTs recorded during this study was five-fold higher that levels recorded in seven fish species (i.e. 68–909 ng/g lipid) from Lake Tanganyika in Burundi (Manirakiza et al. 2002). Their study also revealed that the

DDT levels are a result of historical exposure. Because most results on DDT levels in fish tissue from other African lakes reported in literature are expressed in ng/g dry weight and this study in ng/g lipid, comparison should be made with caution. However, since we recorded levels based on both dry tissue mass and lipid content it was possible to make comparisons. Once again the levels recorded during this study were few folds higher (i.e. 179–389 ng/g dry mass) than concentrations recorded in edible tissue and whole fish from the Lake Edward (five species; below detection to 23 ng/g dry mass; Ssebugere et al. 2009), Lake Malawi (20; 150–872 ng/g dry mass; Kidd et al. 2001) and Lake Victoria (two species; 3,600–3,800 ng/g lipid; Henry and Kishimba 2006). Studies on three lakes in Ghana also revealed that DDT was the most prevalent OCP in the two species sampled (Adu-Kumi et al. 2010; Darko et al. 2008). The levels of DDT in these lakes were due to recent exposure as seen from the high levels of *o,p′*-DDT compared to DDE. The total DDTs in the two species (28–2,200 ng/g lipid) were still lower than the levels recorded in tigerfish during this study. It is therefore clear that even though it does not appear that there has been recent use of DDT in the study area, the very high DDT levels measured in tigerfish can be attributed to their position at the apex of aquatic food chains in African freshwater systems.

Hexachlorbenzene (HCB) concentrations were the next highest (Table 1) in tigerfish muscle. Levels in fish sampled during the winter survey are higher than during the summer and spring sampling periods. The levels reported by Adu-Kumi et al. (2010) for HCB levels in tilapia and catfish from Ghanaian lakes were an order of magnitude lower than those reported in this study. When compared to levels reported in fish from the Danube River (2–33 ng/g lipid – Covaci et al. 2006) and Lake Van in Turkey (14.4 ng/g lipid – Aksoy et al. 2011) the results obtained for tigerfish are very high. This is indicative of large scale use of this fungicide in the study area. The levels of chlordane and HCHs were very low and were only measured in 10% and 28% of the samples respectively, (Table 1). When considering that the bioaccumulation persistence of the HCH isomers is $\beta > \gamma > \alpha$ (Kouras et al. 1998) it is evident that there has been a relatively limited use of pure γ-HCH (lindane, the most toxicological active HCH isomer) in the upper catchment of the impoundment. Similar low levels of HCH were reported in fish from lakes in Ghana (Adi-Kumi et al. 2010).

When the CHL levels (Table 1) are compared to fish from Lake Tanganyika (i.e. 23–288 ng/g lipid; Manirakiza et al. 2002) it clearly demonstrates the low levels of exposure and thus bioaccumulation in tigerfish from Lake Pongolapoort and the ubiquitous and long range transport reported for OCPs (Liu et al. 2009). These are similar to the findings by Adu-Kumi et al. (2010) for tilapia and catfish in Ghana.

As expected, the levels of PCBs found in tigerfish from Lake Pongolapoort were very low indicating the very limited extent of industrial activities in the catchment (Table 2). Twenty-one PCB congeners were quantified in all fish during both surveys. The highest levels were recorded during the winter survey. The penta-CBs, PCB 95, 101 and 110 were the principal contributors to total PCBs during all the surveys. The PCB levels were significantly higher during the winter period and this is attributed to the lower lipid content and thus the increase when converting concentrations as a function of concentrations per g lipid. The total PCBs were much lower than PCBs measured in fish from industrialised regions of Europe (Covaci et al. 2006). The PBDE levels were also very low, with a maximum of 5.8 ng/g lipid (Table 2). These low concentrations are also probably related to a low usage of PBDEs in the catchment. PBDE 47 showed the highest levels but is still much lower than the levels of PBDEs observed in fish from Europe (Covaci et al. 2006).

The use of bioaccumulation studies allows for the determination of the extent of environmental pollutant exposure in aquatic organisms and possible transfer of pollutants along the food chain. In this study we demonstrated that tigerfish of the Lake Pongolapoort are subjected to limited organohalogenated pollutant exposure. It is only the large scale historical use of DDT in the catchment that is still reflected in the bioaccumulation patterns of tigerfish as well as current use of HCBs as pesticide in the upstream agricultural areas. The seasonal variation in the levels of OCPs could be attributed to the lipid reserve status of tigerfish rather than changes in organic pollutant run off from the surrounding catchment due to increased or decreased rainfall.

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