Persistent organic pollutants in the Olifants River Basin, South Africa : bioaccumulation and trophic transfer through a subtropical aquatic food web

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Abstract

This study investigates the trophic transfer of persistent organic pollutants (POPs: PCBs, PBDEs, OCPs and PFASs) in the subtropical aquatic ecosystem of the Olifants River Basin (South Africa) by means of trophic magnification factors (TMFs). Relative trophic levels were determined by stable isotope analysis. POP levels in surface water, sediment and biota were low. Only ∑DDTs levels in fish muscle (<LOQ-61 ng/g ww) were comparable or higher than values from other temperate and tropical regions. Significant positive relationships between relative trophic level and PCB, DDT and HCH concentrations were observed so trophic levels play an important role in the movement of contaminants through the food web. TMFs were > 1, indicating biomagnification of all detected POPs. Calculated TMFs for PCBs were comparable to TMF values reported from the tropical Congo River basin and lower than TMFs from temperate and arctic regions. For p,p'-DDT, a higher TMF value was observed for the subtropical Olifants River during the winter low flow season than for the tropical Congo river. TMFs of DDTs from the present study were unexpectedly higher than TMFs from temperate and arctic aquatic food webs. The fish species in the aquatic ecosystem of the Olifants River can be consumed with a low risk for POP contamination.

Keywords

Persistent organic pollutants; Bioaccumulation; Trophic Magnification Factors; Subtropical, Olifants River Basin

1. Introduction

Persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs: DDT, chlordanes, hexachlorobenzene), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and per- and polyfluoroalkyl Substances (PFASs), have been indicated as hazardous chemicals because of their persistence, toxicity, global distribution and their bioaccumulative and biomagnificative potential. POPs can be transferred across different levels of the aquatic food web with toxic effects frequently manifested most explicitly at the level of top-predators, including human consumers of contaminated fish (Covaci et al., 2005). To effectively mitigate the impact of POP contamination on ecosystems and human health, an understanding of POP exposure in aquatic ecosystems is needed. For this, models and factors, such as trophic magnification factors (TMFs), are used which consider bioavailability, bioaccumulation and biomagnification of contaminants including POPs and evaluate/predict their impact on aquatic ecosystems. TMFs represent the average food web biomagnification and if TMFs are above 1, biomagnification through the food web occurs (Borgå et al, 2012). POP levels in aquatic biota are determined by (1) the amount of POP which is available for uptake into the organisms and (2) the efficacy with which POPs are taken up at the base of the food web and are transferred across different trophic levels (Walters et al., 2016). It can be expected that climatic factors can have an impact on these parameters at different levels. Borgå et al. (2012) predicted lower TMFs in (sub)tropical aquatic ecosystems due to higher biomass dilution of contaminants, because temperature, primary productivity, growth rates and biomass- and tissue turnover are higher and food webs are generally shorter. The majority of the existing TMF studies have been conducted in the Northern hemisphere, and studies from the Southern hemisphere are underrepresented. In this way, it is difficult to investigate global patterns of TMFs and the effect of climate on biomagnification (Borgå et al, 2012; Walters et al., 2016). This large data gap highlights the need for thorough assessment on biomagnification of POPs in (sub)tropical aquatic ecosystems.

The present study evaluates the pollution status of the Olifants River Basin (ORB) in South Africa and investigates the trophic magnification of POPs through this subtropical aquatic food web.

Since South Africa has ratified the Stockholm Convention in 2004, the production, import and use of POPs is banned, yet potential sources are still present. Previous studies on the OR basin report alarming levels of OCPs (Gerber et al. 2015, 2016). OCPs have been used in agriculture and public health to control pests and diseases. Although the use of OCPs is prohibited according to the Stockholm Convention, the use of indoor residual spraying with DDT is allowed by the World Health Organization (WHO, 2006) to control malaria in vector disease risk regions. Nevertheless, the restricted use makes these pesticides available on the market which facilitates illegal usage. PCBs were widely used in a broad range of applications (e.g. dielectric and hydraulic fluids, lubricating and cutting oils). PBDEs were used as flame retardants (Voorspoels et al., 2004). Although PCBs and PBDEs are banned, they are still present in the environment due to their persistent character and the use of contaminated material (the shipping of old electrical equipment containing PCBs and PBDEs from Europe to Africa) (El-Kady et al., 2007). PFASs are used as surfactants and surface protectors in water and oil repellents, firefighting foams and food contact material due to their surface-active properties such as repelling both water and oil (Becker et al., 2010; Lindstrom et al., 2011). Currently, very little data exist on the presence

and pollution status of POPs in the South African Olifants River Basin. Moreover, this is the first study which monitors PFASs in fish in South Africa. It is imperative to address this gap and establish baseline levels. In Africa, fish from inland water bodies are considered to be an important food source. Since it is an important nutrition source, the consumption of contaminated fish is an important route of human exposure to POPs (Gerber et al., 2016).

Specific objectives of the present study are (1) to produce a baseline dataset on selected POPs in surface water, sediment and biota from the Olifants River Basin; (2) to investigate trophic transfer of POPs through a subtropical freshwater food based on trophic magnification factors (TMFs); (3) to compare TMFs from the present study with other TMFs from different climate regions, and (4) to determine the potential human health risk by consumption of POP-contaminated fish. In this way, the mentioned knowledge gap on biomagnification of POPs in (sub)tropical aquatic ecosystems is addressed.

2. Materials and methods

2.1 Study area

The Olifants River basin (ORB) is situated in the North-East of South-Africa (Figure 1). The ORB is situated in a mining, agricultural and urban region. The ORB has been described as one of the most threatened rivers in southern Africa (De Villiers and Mkwelo, 2009), although it has a key role in nature conservation, since the ORB is one of the main water sources for the Kruger National Park (KNP) and delivers important goods and services to the residing communities. Samples were collected from four locations: the Flag Boshielo Dam (FBD), the Phalaborwa Barrage (PB), Mamba Weir (MW) and the Olifants Gorge (OG). The locations were selected upon their position in the ORB: two locations in the mining, agricultural and urban region and two locations in the KNP. The Flag Boshielo Dam (FBD) is situated in the middle sub area of the OR basin and is fed by the Olifants and Elands River (Figure 1). The dam was constructed as a multipurpose dam to provide water for mining, urban, agricultural and recreational sectors (IWMI, 2008). This middle subarea is characterized by wide-scale irrigated agriculture. These commercial agricultural activities are reflected in the ecological status of the Elands River, which is poor to unacceptable (De Villiers and Mkwelo, 2009). The other three sampling points are situated downstream in the lower sub area of the ORB. The Phalaborwa Barrage (PB) is situated just upstream the Kruger National Park (KNP) and was built to provide the Phalaborwa district of potable water for human usage as well as to provide unpurified water for urban, industrial and mining functions (Buermann et al., 1995). Next to the PB, a largescale copper and phosphorus mine is situated (De Villiers and Mkwelo, 2009). An important aspect of the barrage construction is that the sluice gates open at the bottom of the barrage allowing deposited silt to be washed through which means high concentrations of suspended soils are released into the KNP (Buermann et al., 1995). The two river sample points Mamba Weir (MW) and Olifants Gorge (OG) are both situated in the lower ORB within the KNP, where no contamination or discharge into the river system is allowed. MW is situated on the western boundary of the KNP, within close proximity to Phalaborwa, while OG is further east into the park, close to the border with Mozambique. Major crocodile and fish mortalities at OG demonstrated that the ecosystem functions of the river are disturbed, even within the KNP (Ashton, 2010; Van Vuuren,

2009). The ORB is characterized by a humid subtropical climate with high flow rates during hot and wet summers and low flow rates during mild to cool and dry winters. The average annual temperature is 22.4°C and the average annual rainfall is 561mm. More detailed information on geological, hydrogeological and climate regimes in the ORB is reported byeWISA (2010) and IWMI (2008).

2.2 Sample Collection

At each location samples of surface water, sediment, invertebrates and fish were collected during the summer, high flow (April 2012) and the winter, low flow season (September 2012). Physiochemical water quality variables including temperature (°C), pH, oxygen saturation (%), dissolved oxygen (mg/L) and conductivity (µS/cm^2) were measured in situ via a handheld WTW 340i multimeter at each location preceding sampling (Table S1). POPs, such as PCBs, PBDEs and OCPs have very low aqueous solubility and accumulate preferentially in the sediment (Voorspoels et al., 2004). Therefore, these pollutants were not analyzed in surface water samples. For PFASs however, numerous studies have shown that the dominating fraction of PFASs can be found within the surface waters of the ecosystem (Li et al., 2010; Yang et al., 2011). For PFASs surface water analyses, a 1L polypropylene (PP) bottle was filled per location.

At FBD and PB locations, sediment samples were taken with a Van Veen Grab from a boat. At the river locations (MW & OG), sediment was collected with a 50mL vial from the shallow river banks due to boat and wildlife constraints. At each location, three (sediment) grabs were pooled to 1 sample per location and frozen to prevent loss of organic material. In the laboratory, sediment samples were subdivided for POP and total organic carbon content (TOC) analysis.

Concerning invertebrates, dragonfly larvae (Gomphidae, Odonata) and the snail species *Tarebia granifera* (Thiaridae, Gastropoda) were collected with an invertebrate net (mesh size: 0.5mm) and kept at -20°C until POP and stable isotope analysis.

Fish from FBD and PB were collected with gill nets (70 to 120 mm stretched mesh size). In MW and OG, *Clarias gariepinus* and *Hydrocynus vittatus* were collected with artificial lures and bait method and for the other fish species an electrofishing unit was used. The following species were selected based on their distribution throughout the study area: *Labeo rosae* (Rednose labeo), *Labeo congoro* (Purple labeo)*, Synodontis zambesensis* (Plain squeaker)*, Schilbe intermedius* (Silver catfish), *Labeobarbus marequensis* (Largescale yellowfish)*, Hydrocynus vittatus* (Tiger fish), *Clarias gariepinus* (Sharptooth catfish) and *Oreochromis mossambicus* (Mozambique tilapia). Fish standard length (0.1cm) and weight (0.001g) were measured upon being filleted and skinned. Muscle tissue was collected for POP and stable isotope analysis. Liver samples were collected for POP analysis. Samples were stored at -20°C prior to extraction.

2.3. Total Organic Carbon (TOC)

For TOC, 3 replicates of the pooled sediment sample were analysed. TOC was determined through Loss on Ignition (LOI). To this, the sediment samples (5-10g ww) were incinerated at 550 °C for 4 h, weight loss was determined and LOI was calculated with the following formula (Heiri et al., 2001):

$$LOI\ (\%) = (m_b – m_c / m_b - m_a) * 100$$

with m_a = weight empty crucible (g), m_b = weight crucible + sediment sample before heating in muffle furnace(g), m_c = weight crucible + sediment sample after heating in muffle furnace

To calculate the total amount of organic carbon a conversion factor of 1.724 is used, assuming that organic carbon makes up 58% of the total organic matter content (Nelson and Sommers, 1996).

2.4. PCBs, PBDEs and OCPs

2.4.1. Chemicals and sample preparation

The following compounds were included in the analysis: 33 PCB congeners (IUPAC numbers: CB 18, 28, 44, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 151, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, 206, 209), 7 PBDEs (IUPAC numbers: 28, 47, 99, 100, 153, 154, and 183), DDT and metabolites (o,p′-DDD, o,p′-DDE, o,p′-DDT, p,p′-DDD, p,p′-DDE, and p,p′-DDT), chlordanes-CHLs (trans-chlordane (TC), cis-chlordane (CC), cis-nonachlor (CN), trans-nonachlor (TN), and oxychlordane (OxC)), HCHs (α-, β-, and γ-hexachlorocyclohexanes) and hexachlorobenzene (HCB). BDE 209 was only analyzed in sediment samples. All solvents and chemicals were purchased or prepared as described previously (Chu et al., 2002; Covaci et al., 2002). The methods used for the determination of POPs in sediment and biota samples have been previously described and validated (Covaci et al., 2005) and are summarized below. Per season, analyses were conducted on one pooled sediment sample per location, one pooled invertebrate sample per species and per location and three individual fish replicates per species and per location (POP analysis in liver samples was limited to two species: *C. gariepinus* and *L. marequensis*). For the biota samples, fresh fish muscle and liver (2.5-4.0 g wet weight (ww)) and invertebrates (2.0-3.5 g ww) were homogenized with anhydrous Na_2SO_4, spiked with internal standards (CB 143, BDE 77, ε-HCH) and extracted for 2 h by hot Soxhlet with 100 mL hexane/acetone (3/1, v/v). After lipid determination on an aliquot of the extract, the remainder of the extract was cleaned-up on 8 g acidified silica (44%, w/w) and analytes were eluted with 20 mL hexane and 15 mL dichloromethane. The cleaned extract was then concentrated and reconstituted in 100 µL iso-octane. To obtain sufficient tissue mass required for the analytical analysis, individuals per invertebrate species and per location were pooled. For the sediment (3 g), the same procedure was followed, but 5 g of activated copper powder was added and mixed with the sample. The sediment samples were spiked with internal standards (CB 143, BDE 77, ^{13}C-BDE 209, and ε-HCH). For the clean-up step, 2 g of copper powder was added on top of the acid silica column.

2.4.2. PCB, PBDE and OCP analysis

PBDEs, HCHs and CHLs were measured with an Agilent 6890-5973 gas chromatograph coupled to a mass spectrometer (GC–MS) and equipped with a 30 m × 0.25 mm × 0.25 µm DB-5 capillary column. The MS was operated in electron capture negative ionization (ECNI) mode and was used in the selected ion-monitoring (SIM) mode with ions *m/z* = 79 and 81 monitored during the entire run and specific ions for OCPs acquired in well-defined windows. PCBs, DDTs, and HCB were measured with a similar GC–MS system as for the PBDE determination, operated in electron ionization (EI) mode and equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column. The MS was used in the SIM mode with two ions

monitored for each PCB homologue group or for each OCP. More detailed information on the GC-MS analysis is given in the supplementary information (text + Table S2).

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

Retention times, ion chromatograms and relative abundance of the monitored ions were used as identification criteria. A deviation of ion abundance ratios within 15% of the mean values for calibration standards was considered acceptable. Quantification was based on five-point calibration curves. The peaks were positively identified as target compounds if: (1) the retention time matched that of the standard compound within ±0.1 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. One procedural blank was analyzed for each batch of 10 samples and this for each type of samples (fish, invertebrates and sediments). The blank values were for most compounds not detectable, while for compounds with detectable (but very low) blanks, the variation between the blanks was <30%. For each analyte detected in the blanks, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank, which ensures >99% certainty that the reported value originates from the sample. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio S/N equal to 10. LOQs depended on the sample intake and on the analyte and ranged between 1 and 4 ng/g lipid weight (lw) for biota and 10 and 50 pg/g dry weight (dw) for sediments. QC was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (OCPs, PCBs, and PBDEs in whale blubber) and CRM 536 (PCBs in harbour sediment) was used to test the accuracy of the method. Obtained values were not deviating more than 10% from the certified values (Table S3). The QC scheme is assessed through successful participation to the Interlaboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissue organized by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

2.5. PFASs

2.5.1. Chemicals and sample preparation

The following compounds were included in the analysis: PFBS, PFHxS, PFOS, PFDS, PFBA, PFHxA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrA, and PFTeA. The extraction method to determine carboxylate and sulfonate PFASs in fish is primarily based on the procedure developed by Powley et al. (2005). PFASs analyses were restricted to fish samples, not in invertebrates due to limited biomass and was conducted on muscle tissue of three replicates per fish species, per season and per location (for liver tissue, only 1 species, *C.gariepinus*, was selected). Muscle tissues (1 g, ww) were homogenized with an Ultra-Turrax dispersing tool and placed within individual 50 mL PP tubes. The samples were then spiked with 80 µL of internal standard mixture (MPFAS = 125 pg/µL concentration consisting of $^{13}C_8$-PFOS, $^{13}O_2$-PFHxS, $^{13}C_4$-PFBA, $^{13}C_2$-PFHxA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUdA), 10 mL of acetonitrile (ACN) was added, followed by proper mixing via vortex. After sonication (3 x 10 min with vortex mixing in between), mixing (at 230rpm, 16h.) and centrifugation (2400 rpm for 10 min), the supernatant was transferred to new pre weighted 15 mL PP tubes. The acquired extract was evaporated to 0.5 mL using a Speedvac and the weight of the tubes was determined. For sample purification,

Eppendorf tubes were firstly prepared with 25 mg Envi-Carb and 50 µL glacial acetic acid (100%) followed by the transfer of the evaporated extract. Rinsing of the empty 15 mL tubes was performed twice by adding 250 µL ACN, vortexed and transferred to the Eppendorf tube. Then samples were vortexed for 1 min, centrifuged at 10 000rpm for 10 min and the cleaned supernatant was transferred to new tubes. For filtration 195 µL HPLC grade water, 2 mM ammonium acetate and 105 µL of the extract was added to an empty Eppendorf tube and vortexed thoroughly. The resulting 300 µL was filtrated (0.2 µm, syringe filter, OASIS Medical Inc. USA) using a syringe into polypropylene injection vials and samples were ready for UPLC analysis.

One surface water sample per location and per season was analyzed. The extraction method of PFASs from surface water was based on the Taniyasu et al. (2005) procedure. First, the water sample was filtrated through Whatman 47 mm filters. A volume of 500 mL of the sample was spiked with the 80 µL internal standard (See Biota extraction) and shaken well. For the solid phase extraction, a vacuum glass pump was set up with a Waters Oasis Wax cartridge (60 mg, 3cc) and an approximately flow of 2 drips/sec. Preconditioning of the cartridge with 4 mL 0.1 % NH_4OH in ACN and thereafter with 4 mL HPLC grade water took place after which the spiked 500 mL water was added systematically. The columns were washed with 2 mL 40% ACN in HPLC grade water. When completely dry, cartridges were removed and eluded with 1 mL of 2 % NH_4OH in ACN. Before analysis the extract was filtrated (See biota filtration). The only difference is that 105 µL extract is diluted with 195 µL HPLC grade water.

2.5.2. PFASs analysis

PFASs were quantified using an AQUITY Ultra Performance Liquid Chromatography (UPLC) coupled to a tandem quadrupole mass spectrometer (TQD, Waters, USA) with electrospray interface operating in negative ion mode (ES-MS/MS). Separation was performed on an ACQUITY BEH C18 column (1.7µm particle size; 50 x 2.1 mm, Waters, USA). The injection rate proceeded with a volume of 10 µL with a flow rate of 450 µL/min. Mobile phases were conveyed by a gradient program consisting of ACN with 0.1 % formic acid and water with 0.1% formic acid. The following analytes and internal standards with mass transition were monitored and used for detection [precursor ion (m/z) → product ion (m/z)]: 213 → 169 (*PFBA*), 313 → 269 (*PFHxA*), 217 → 172 (*$^{13}C_4$-PFBA*), 315 → 270 (*$^{13}C_2$-PFHxA),* 413 → 369 (*PFOA*), 463 → 419 (*PFNA*), 513 → 469 (*PFDA*), 563 → 519 & 269 (*PFUdA*), 663 → 619 (*PFTrA*), 713→ 669 (*PFTeA*), 299 → 99 (*PFBS*), 403 → 84 (*$^{13}O_2$-PFHxS*), 399 → 99 (*PFHxS*), 499 → 80,99 (*PFOS*), 599 → 80 (*PFDS*), 313→296 (PFHxA), 421→376 ($^{13}C_8$PFOA), 472→427 ($^{13}C_9$PFNA), 519→474 & 270 ($^{13}C_6$PFDA), 570→525 ($^{13}C_7$PFUdA), 507→80 ($^{13}C_8$PFOS). For quantification, an external calibration curve was used. Non-labelled standards of PFBS, PFHxS, PFOS, PFDS, PFBA, PFHxA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrA and PFTeA were used to construct ten-level calibration curves (r^2>0.98) The internal standards (MPFAS) were added to the samples prior to extraction and the same amount of these standards was added to each calibration point. The calibration curves were created preceding the analysis and were analyzed in the same run. The results are corrected for matrix effects and recovery was based on the response area of the internal standards. Concentrations of sulfonates are based on the ion, not on the salt. At least two calibration curves were analysed for each run and pure ACN was injected for every ten injections to avoid carry-over effects.

2.5.3. Quality Assurance and Quality Control (QA/QC)

Duplicates for each 1 g of wet tissue and 500 mL water sample were incorporated and each sample was injected twice. For each lot of approximately ten samples, a routine procedural blank of HPLC grade water was spiked with the internal standard mixture and analyzed simultaneously. These measures were taken to assure correctness and quality of the routine method. The response of the internal standard in the samples provides information on possible matrix effects, suppression or enhancement of the signal. The analyte limits of quantification (LOQ) were calculated as 10 times the signal to noise ratio which were 100 pg/g for PFOA, 120 pg/g for PFOS and 65pg/g for PFNA and for surface water 0.86 ng/L, 0.73 ng/L and 0.55 ng/L, respectively.

Samples with reference material, sterilized fish muscle tissue (pike perch; *Stizostedion lucioperca*) obtained from QUASIMEME Laboratory Performance Studies (Van Leeuwen *et al.,* 2011) were added. The mean recoveries ranged from 67% to 106% for the analyzed PFASs.

2.6. Stable Isotope analysis

Fish and snail samples were dried at 60 °C, homogenized with a mortar and pestle into a fine powder, weighed to the nearest 0.001 mg and encapsulated in pre-weighed 5 × 8 mm Sn capsules to determine $\delta^{13}C$ and $\delta^{15}N$. A similar procedure was used for the *gomphidae* larvae, but Ag cups were used because HCl was added to remove traces of non-dietary carbonates due to the presence of an exoskeleton (Verhaert et al. 2013). Stable isotope measurements were performed using a Thermo Flash HT/EA coupled to a Thermo DeltaV Advantage IRMS with a Conflo IV interface at the Department of Earth and Environmental Sciences, KULeuven (Belgium). Stable isotope results are expressed using the following formula:

$\delta^{13}C$; $\delta^{15}N = [(R_{sample}/R_{reference}) - 1] \times 1000$

with $R = {}^{13}C/{}^{12}C$ for carbon and ${}^{15}N/{}^{14}N$ for nitrogen.

Data were calibrated using a combination of IAEA-C6, IAEA-N1, and acetanilide, which was calibrated in house for $\delta^{13}C$ and $\delta^{15}N$. Estimated precision is generally better than 0.15 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Relative trophic levels were derived from animal $\delta^{15}N$ values using the following equation (Post, 2002):

$TL_{consumer} = [(\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/\Delta\ \delta^{15}N] + 2$

where $TL_{consumer}$ is the trophic level of the organism, $\delta^{15}N_{consumer}$ is $\delta^{15}N$ of the organism, $\delta^{15}N_{primary\ consumer}$ is the mean $\delta^{15}N$ of a local long-lived primary consumer, 2 is the trophic level of the primary consumer and $\Delta\ \delta^{15}N$ is the trophic enrichment factor, or the shift in $\delta^{15}N$ between two consecutive trophic levels (Post, 2002). In the present study, the primary consumer used as a baseline was the snail species *Tarebia granifera.* A $\Delta\delta^{15}N$ trophic fractionation of 3‰ was used, as this is the most adequate estimate for non-acid treated muscle tissue (McCutchan et al., 2003; Vanderklift and Ponsard 2003). TMFs were based on lipid-normalized contaminant POP concentrations and relative trophic levels, and were calculated from the slope of the regression of the log-transformed concentrations of pollutants versus trophic level calculated based on $\delta^{15}N$ (Borgå et al., 2012) (Figure S1).

$\text{Log [contaminant]} = a + b\ TL + \varepsilon \qquad TMF = 10^{b}$

2.7. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 5 (GraphPad Software, Inc) and the SPSS 15.0 statistical package. The level of statistical significance was defined at $p < 0.05$. For POP concentrations <LOQ, the value of LOQ * f (detection frequency) was incorporated. Prior to analyses, data was tested for normality (Shapiro-Wilk) and homogeneity of variance (Levene's tests) after which log transformation was applied to data sets when necessary. Since invertebrates were pooled per species and per location for POP analysis the ability to perform comparative statistical analysis for these data sets was limited. In addition, POP analysis was performed on one pooled sediment sample per location and per season and therefore statistical analysis was again limited. Differences in contamination levels per fish species for locations and seasons were tested with two-way ANOVA, followed by Post-Hoc Tukey test. One-way ANOVA was used for the comparison of POP contamination between species. A paired sampled T-test was performed for the identification of any significant differences in means between corresponding liver and muscle samples. Pearson's correlation coefficients were calculated for (1) TOC content in sediment and sediment contamination levels; (2) the association between fish biological characteristics (lipid content, length, weight) and POP levels in biota tissue; (3) the relation between POP levels in sediment and biota tissue POP levels.

To investigate differences in TMFs between different climate regions, ANCOVA was used by comparing slopes of the regression between trophic level and the log of the contaminant concentration for the different climates by R (Version 2.15.3).

2.8. Human health risk assessment

The Agency for Toxic Substances and Disease Registry (ATSDR, 2013) and the European Food Safety Authority (EFSA, 2008) have determined Minimum Risk Levels (MRL) for oral intake of POPs. With these MRLs, the maximum amounts of fish (kg) which can be consumed by a person of 60 kg without potential human health risks is calculated taken into account the 50^{th} and 95^{th} percentile of observed concentration of ∑PCBs, ∑DDTs, ∑HCHs, ∑CHLs, PFOA, and PFOS for all fish species sampled in Olifants River, South Africa. The following formula is used:

$Y = W \times M$; $Q = Y / C / 1000$; $Q = W \times M / C / 1000$

with:

Y (ng/day) = maximum amount of POPs a 60 kg person can consume per day without posing health risk; M (ng/kg body weight/day) = Minimum Risk Level (MRL) for oral intake of POPs; W (kg) = weight of an average person of 60 kg; Q (kg) = maximum amount of contaminated fish muscle a 60 kg person can consume per day without posing health risks; C (ng/g ww) = 50th and 95th percentiles of the observed concentration of POPs in the fish muscle

3. Results

3.1. Surface water

PFASs concentrations in surface water were all < LOQ in the present study (0.86 ng/L for PFOA, 0.73 ng/L for PFOS and 0.55 ng/L for PFNA).

3.2. Sediment

3.2.1. POPs

PCBs

ΣPCB concentrations for all locations ranged between 0.16 and 2.0 ng/g dw and from 0.056 to 0.33 ng/g dw for the summer high and winter low flow season, respectively (Figure S2 and Table 1). The following PCB congeners were not detected in the sediment samples: CB 28, 52, 49, 74, 151, 138, 171, 156, 199, 196/203, 194, 206, and 209. Four of the seven indicator PCBs (CB 101, 118, 153, and 180) were present and ranged from 0.10 to 0.86 ng/g dw for the summer high flow and from 0.06 to 0.22 ng/g dw for winter low flow season (Table 1). The main contributing PCB congeners in the sediment were CB110 (18%), CB101 (17%), CB153 (15%), and CB118 (15%). A trend in seasonal difference was observed for the river sampling points (MW & OG) with the high flow season having higher PCB levels than the low flow season.

PBDEs

ΣPBDEs levels ranged from 0.017 to 1.5 ng/g dw and from <LOQ to 0.011 ng/g dw in summer high flow and winter low flow season, respectively (Table 1). PBDE congeners 99, 100, 153, 154, and 183 were not detected in the sediments. The most dominant congeners of ΣPBDEs for all locations were BDE 209 (94 %) and BDE 47 (6%). Again, a trend in seasonal difference is observed with higher levels in the summer high flow season compared to the winter low flow season.

OCPs

ΣDDTs ranged from 0.094 to 2.4 ng/g dw for the summer high flow season and from <LOQ to 4.3 ng/g dw in winter low flow (Figure 2 and Table 1). The observed metabolite profile for DDTs was p,p'-DDE (85%); p,p'-DDD (9.9%) and p,p'-DDT (4.9%). Metabolites o,p'-DDE, o,p'-DDD and o,p'-DDT were not detected. The DDT/(DDE+DDD) ratio ranged from 0.01 to 0.97. For both seasons, FBD had the highest ΣDDTs sediment levels.

For ΣHCHs, the summated levels throughout all locations ranged between <LOQ and 0.049 ng/g dw and from <LOQ to 0.069 ng/g dw in summer high flow and winter low flow, respectively (Table 1). The principal contributors to the ΣHCHs abundance were isomers γ-HCH (66 %) and β- HCH (34 %). Isomer α-HCH could not be detected in the sediment samples.

ΣCHLs ranged from <LOQ to 0.046 ng/g dw in summer high flow and from <LOQ to 0.15 ng/g dw in the winter low flow season. OxC was absent and CC (62%) was the predominant component overall. HCB was detected at low levels ranging from <LOQ to 0.08 ng/g dw and from <LOQ to 0.03 ng/g dw in high flow and low flow seasons, respectively.

3.2.2. TOC

The mean total organic carbon content (TOC in %) in the sediment samples ranged from 0.61±0.04% to 8.9±1.1% in the high flow season and 0.31±0.03 to 3.1±0.09% in winter low flow (Table 1). Significant differences were found among locations and between seasons ($F_{6,14}=168$, $p<0.0001$). TOC values in sediment from the dams FBD and PB were significantly higher than TOC in sediment from the river points MW and OG. In addition, TOC was higher in FBD than in PB and TOC in the summer season of FBD was higher than in the winter season.

Significant positive correlations between TOC (%) and OCP concentrations were observed for ΣDDT ($r(5)=0.86$, $r^2=0.74$, $p=0.013$, $N=7$); p,p'-DDE ($r(5)=0.87$, $r^2=0.76$, $p=0.011$, $N=7$) and p,p'-DDD ($r(5)=0.93$, $r^2=0.87$, $p=0.002$, $N=7$) (Figure S3).

3.3. Invertebrates

The lipid content varied between 0.82% and 1.6% for *Gomphidae* larvae and between 1.1% and 1.3% for the snail *T. granifera,* with no significant difference among locations or between seasons.

PCBs, PBDEs, HCHs and CHLs were close to or < LOQ for all invertebrate species at all sites (Table 2). The ΣDDT values throughout all locations for *Gomphidae* ranged between <LOQ and 1.7 ng/g ww (20 to 206 ng/g lw). For *T. granifera,* the ΣDDT levels ranged between <LOQ and 1.2 ng/g ww (15 to 135 ng/g lw) (Figure 2). Metabolites o,p'-DDE, o,p'-DDD, o,p'-DDT and p,p'-DDD were not detected in invertebrates for any of the locations. The predominant metabolites were p,p'-DDE contributing to 94 % of ΣDDT and p,p'-DDT 6%. HCB could be detected with levels close to the LOQ throughout all locations ranging between <LOQ and 0.21 ng/g ww (1.3 to 3.1 ng/g lw) for *Gomphidae* larvae and between <LOQ and 0.22 ng/g ww for *T. granifera*.

3.4. Fish

3.4.1. POPs

PBDEs and HCB were < LOQ in all muscle tissues.

PCBs

The ΣPCBs concentrations ranged from <LOQ to 3.0 ng/g ww (<LOQ to 1278 ng/g lw) for all species (Table 3, Figure S2). The Σ7PCBs (congeners 28, 52, 101, 118, 138, 153, and 180) varied from <LOQ to 1.7 ng/g ww. PCB congeners 28, 52, 49, 74, 95, 151, 156, 183, 174, 177, 171, 194, and 209 were not quantifiable in the fish species. The congener profile was dominated by CB 153 (32%); CB 180 (21%); CB 138 (11%); CB 118 (8 %); CB 187 (7%); and CB 170 (6%). No significant differences among species, locations and seasons were observed.

To investigate tissue specific contamination, ΣPCB levels in liver tissue from *C. gariepinus* and *L. marequensis* were analyzed. ΣPCB levels in the liver ranged from <LOQ to 71 ng/g lw (Table S4) and for Σ7PCBs the range was from <LOQ to 36 ng/g lw. Tissue specific accumulation was observed in the fish with higher PCB levels in the liver than in the muscle tissue ($4.4 \leq t \leq 14.1$; $0.0001 \leq p \leq 0.001$). No correlation was found between liver and muscle for all individual PCB congeners.

436 *OCPs*

437 The ΣDDTs levels in the muscle fish tissue varied from <LOQ to 61 ng/g ww (28 to 27891 ng/g lw) (Table
438 3, Figure 2). Except for o,p'-DDE, all analyzed DDT metabolites were detected. p,p'-DDE isomer entails
439 91% of the overall contamination burden. The DDT/(DDE+DDD) ratio ranged between 0.01 and 0.25.
440 The ΣDDTs metabolite profile in liver from *C. gariepinus* and *L. marequensis* corresponded to the muscle
441 composition (p,p'-DDE also dominant metabolite contamination contributor in liver), with levels ranging
442 from 1 to 4326 ng/g lw (Table S4). A positive significant correlation for p,p'-DDE ($r(13)=0.79$, $r^2=0.63$,
443 $p<0.001$, N=14) between levels in the muscle and levels in the liver as well as for isomer p,p'-DDD
444 ($r(13)= 0.65$, $r^2=0.43$, $p=0.011$, N=14) was observed. DDT and metabolites accumulated more in liver
445 than in muscle tissue ($3.7 \leq t \leq 11.1$; $0.0001 \leq p \leq 0.003$).
446 The other detected OCPs in the fish tissues were HCHs and CHLs. ΣHCHs concentrations varied from
447 <LOQ to 0.89 ng/g ww (<LOQ to 87 ng/g lw) (Table 3). While isomer γ-HCH is the most dominant
448 metabolite in the sediment, this metabolite is absent in the fish. α-HCH and β-HCH constituted 28% and
449 72% respectively in all samples analyzed. ΣHCH levels in liver ranged from <LOQ to 0.42 ng/g lw (Table
450 S4) and were significantly higher than in the muscle tissue ($t1,13=4.2$, $p=0.001$). The HCH pattern in the
451 liver was dominated by γ-HCH (84%), β-HCH (16%) and no α-HCH, in contrast with the muscle where
452 γ-HCH was not detected. In the sediment TC and CC were dominant CHLs while in fish TC and OxC
453 were absent with TN (64%), CN (26%) and CC (11 %) encompassing the majority of the isomers. The
454 ΣCHLs ranged between <LOQ and 0.81 ng/g ww (<LOQ to 87 ng/g lw) (Table 3). ΣCHL levels in the
455 liver tissue ranged from <LOQ to 8.6 ng/g lw with similar compositional profiles than muscle tissue (Table
456 S4). No significant correlation between liver and muscle tissue was observed.
457
458 *PFASs*

459 Regarding ΣPFASs concentrations measured in the fish muscle and liver, only 3 out of 13 PFAS
460 compounds analyzed could be detected (Figure 3, Table S5 and S6). PFOS, PFOA and PFNA in muscle
461 tissue ranged from 0.15 to 2.7 ng/g ww, from <LOQ to 0.42 ng/g ww and from <LOQ to 0.14 ng/g ww
462 respectively with PFOS>PFOA>PFNA (Table S5). Values were significantly higher in liver compared to
463 muscles for PFOA ($T5=3.08$, $p=0.027$), PFOS ($T5=3.04$, $p=0.029$) and PFNA ($T5=4.16$, $p=0.009$). No
464 significant correlation between liver and muscle tissue was observed.
465
466 3.4.2. Relation between POP levels and biological characteristics

467 The lipid content percentage in fish studied varied from 0.18±0.09% for *S. intermedius* to 2.9±0.87% for
468 *L. marequensis* (Table 3). No significant difference in lipid content for the same species at different
469 locations or seasons were identified.
470 No clear trend was observed between lipid content, weight and length and POP levels in fish. For *C.*
471 *gariepinus, L. rosae*, *L. marequensis, S. intermedius* and *S. zambesensis* no significant correlation
472 between biological parameters and POP levels was observed. However, for *L. congoro* and *H. vittatus*
473 a significant negative correlation was observed between length and CB118, 153,187, 183, 128, 174,
474 177,180, 170, 199, HCB, p,p'-DDE and p,p'-DDT (Table S7).
475

3.5. Relationships between POP levels in the environment and biota tissues

Weak or no significant correlations were found for POPs in TOC normalized sediments correlated with lipid normalized POP levels in invertebrate tissue samples and fish muscle tissue. Additionally, no or weak negative correlations were found between individual fish species and respective invertebrates Gomphidae or *T. granifera.*

3.6. Trophic transfer of POPs through a subtropical food web

3.6.1. Trophic level

Ranges and mean (±SD) levels of nitrogen stable isotopes are presented in Table 2 and 3. Trophic levels ranged from 2.0±0.1 for *T. granifera* to 4.0±0.6 for *S. intermedius*. On average, trophic levels increased from detrivores to omnivores to piscivores (Figure S4).

3.6.2. Trophic transfer and trophic magnification factors

TMFs are based on the relation between the trophic level (TL) and the log contaminant concentration. For POPs, the log lipid normalized concentrations were used. For POPs which could be detected in both invertebrates and fish (CB153, CB187, CB180, CB170, CB199, CB206, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, α-HCH, and β-HCH), the relationship between TL and logPOP concentrations were tested for FBD, MW and OG. At FBD, invertebrates could not be collected in the winter low flow season, so trophic transfer and TMFs were not calculated for this season. For PFASs, no significant relation between TL and PFASs were found. . In the summer high flow season at FBD, significant relations between TL and CB153, CB187, CB180, CB170, CB206, *p,p'*-DDE and *p,p'*-DDD were observed. At MW, CB170, CB199 and CB206, *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT and α-HCH were significantly related to TL in both seasons. In addition, TL and logCB153, CB180 and β-HCH were also significantly related in the summer high flow. For OG, a significant relationship between TL and CB153, 187, 180, 170, 199 and 206, *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT, α-HCH and β-HCH was observed in both seasons (Figure 4). Based on the slopes of these relationships, TMFs were calculated (Table S8). TMFs ranged from 1.3 for α-HCH at OG to 9.0 for p,p'-DDE at OG, with the highest TMF values for DDXs, followed by PCBs and finally HCHs. POPs with a log K_{ow} < 5, such as HCHs have lower TMFs than POPs with 5 < log K_{ow} < 7 (PCBs, DDTs) (Table S8 and Figure 5). For all POPs, no significant difference in slopes of the regression between TL and logPOP for the two seasons was observed.

3.7. Minimum Risk Levels for Human Health

Table 4 represents the maximum amounts of fish muscle tissue (kg) which can be consumed by a person of 60 kg without potential human health risks based on MRLs (ATSDR, 2010; EFSA, 2008) and taken into account the 50th and 95th percentile of observed concentration of ∑PCBs, ∑DDTs, ∑HCHs, ∑CHLs, PFOA and PFOS for all fish species sampled in Olifants River, South Africa The highest potential health risk is found in *S. zambesensis* because of elevated ∑DDTs levels. A person of 60 kg can consume a daily amount of 640 g of *S. zambesensis* without posing a potential human health risk.

4. Discussion

4.1. Surface water

For PFASs, levels were all < LOQ. Studies on PFASs in South Africa are scarce, but levels of PFOA and PFOS were detected in rivers in the Western Cape Province (314 and 182 ng/l in Diep River, 390 and 47 in Salt River and 146 and 23 in Eerste River for PFOA and PFOS respectively) (Mudumbi et al. 2014). Therefore, these pollutants are present in South Africa, but apparently to a lesser extent in the ORB.

4.2. Sediment

4.2.1. POPs

One pooled sample was analyzed for POPs, so differences in sediment levels among locations and between seasons can only be expressed as trends, but not statistically tested. Both for PCBs and PBDEs, a trend of higher levels in the summer season was observed. ΣPCB and ΣPBDE levels in the sediment of the Olifants River are low when compared to the literature. The Σ7PCBs levels in the present study were comparable to values reported from the Congo River Basin (Verhaert et al., 2013), the Nile River, Egypt (El-Kady et al., 2007) and Lake Victoria (Ssebugere et al., 2014), but lower than in the industrialized, urban and agricultural area of the Vaal River (South Africa) (Quinn et al., 2009) or several industrialized European rivers (Waszak & Dabrowska, 2009; Van Ael et al, 2012). ΣPBDE sediment concentrations were comparable to levels detected in the pristine Congo River Basin (Verhaert et al., 2013), lower than levels determined in the Juksei River, South Africa (Olukunle et al., 2012) and up to 1000 fold lower than in sediment samples from the more industrialized Scheldt river in Belgium, Europe (Covaci et al., 2005, Van Ael et al., 2012).

For both seasons, FBD had the highest ΣDDTs sediment levels. FBD is located in the Middle ORB where agricultural practice is more extensive compared to the Lower ORB. However, this DDT pollution originates from historical use when referred to the DDT/(DDE+DDD) ratio (Quinn et al. 2011). To determine the magnitude of DDT contamination, the results were compared to ΣDDTs observed in sediments from other studies. Gerber et al. (2015) collected samples from the Olifants River at MW and OG during high and low flow seasons in 2009-2011. Levels ranged from <LOQ to 1.97 ng/g dw which is in the same range as the observed concentrations in the present study. Levels were higher than those detected in the Congo River by Verhaert et al. (2013) and Tana- and Sabaki Rivers in Kenya (Lalah et al., 2003), while analogous to the range (0.27 to 4.62 ng/g dw) identified by Quinn et al. (2009) in the Vaal River, South Africa and to sediment samples analyzed from Lake Bosomtwi in Ghana (Darko et al., 2008).

ΣHCHs, ΣCHLs and HCB sediment levels were all low compared to the literature (Verhaert et al., 2013; Covaci et al., 2006; Darko et al., 2008; Getenga et al., 2004; Quinn et al., 2009). ΣHCHs and ΣCHLs levels at MW and OG from the present study are lower than levels found in 2009-2011 at MW and OG which indicates a possible decrease of the pollution in these environments. For HCB, levels were comparable (Gerber et al. 2015).

4.2.2. TOC

Sediment characteristics, such as total organic carbon (TOC) are imperative in the fate and retention of POPs in the sediment with expected higher sediment POP levels when TOC content increases (Miglioranza et al., 2002; Munn and Gruber, 1997). This relationship was determined for p,p'-DDE, p,p'-DDD and ∑DDT.

4.3. Invertebrates

Similar as for sediment, DDT and metabolites were the predominant pollutants. The presence of p,p'-DDE metabolite in greater excess compared to p,p'-DDT confirms the historical use. For all sampling points, FBD showed the highest ΣDDT accumulated levels for both invertebrate species which is in line with the fact that FBD is situated in a more developed area with agricultural, urban and industrial practices compared to the other sampling points. Compared to other studies in Africa, levels are similar to slightly higher (Kidd et al. 2001; Verhaert et al. 2013). Although, DDT can be used for residual indoor spraying in South Africa, the concentrations found in the invertebrates do not show evidence of problematic or recent pollution by DDT in the middle and lower Olifants River.

4.4. Fish

4.4.1. POPs

PCB concentrations in the current study were considerably lower than in the freshwater fish from studies in more industrialized European countries such as Belgium (Van Ael et al., 2012), Italy (Viganò et al., 2008), and the USA (Monosson et al. 2003). Also the ΣPCBs levels in *H. vittatus* at Lake Pongolapoort, South Africa, with a mean concentration of 257±115 ng/g lw, are higher than ΣPCBs levels from the present study (Wepener et al., 2012). Comparably, ΣPCBs levels in fish from Lake Tanganyika, and Congo River, were higher compared to the current study (Manirakiza et al., 2002; Verhaert et al. 2013). DDT levels in *H. vittatus* from OG in the present study are lower when compared with previously reported DDT levels of *H. vittatus* from OG in the low flow season of 2010 and the high flow season of 2011 (9037±3221 and 647±261 ng/g lw, respectively) (Gerber et al., 2015). Gerber et al. (2015) stated that concentrations of the majority of the OCPs in fish from OG were the highest levels ever recorded from South African freshwater systems and in many cases the concentrations were higher than most contaminated areas from around the world. This observation indicates a decrease in DDT levels between 2010-2011 and 2012. The DDT/(DDE+DDD) ratio was < 1 which indicates that the ΣDDTs within the aquatic ecosystem originates mainly from historical usage. Also DDT levels in *H. vittatus* in Lake Pongolapoort (South Africa) reflected a larger scale historical use of DDT (Wepener et al., 2012). The DDT profiles of the fish closely resemble those of the sediment with the main differences occurring with o,p'-DDT and o,p'-DDD being none detectable in the sediment samples.

ΣHCH and ΣCHL levels in the present study were low and in the same range as levels found in fish from Lake Tanganyika (Burundi) and Congo River Basin (Manirakiza et al., 2002; Verhaert et al., 2013). Regarding ΣPFASs concentrations in fish, only 3 out of 13 PFAS compounds analyzed could be detected. An important determinant of PFASs bioaccumulation potential is the functional group attached

to the carbon chain. It is known that for example the functional group 'sulfonate' has a greater bioconcentration factor, half live and uptake rate in comparison with carboxylate perfluoroalkyl of the same carbon chain length (Lindstrom et al., 2011; Martin et al., 2003). Furthermore, the largest bioaccumulation concern pertains to the PFASs long-chain lengths C ≥ 8 which generally bioaccumulate more than those with C ≤ 7 (Martin et al., 2003; Labadie and Chevreuil, 2011; Lindstrom et al., 2011). These facts can be extrapolated to the current study where PFOS, with long chain length and a sulfonate group, is the predominant contributor of ΣPFAS in the different fish tissues. Furthermore this pattern is in agreement with findings reported in other studies (Kannan et al., 2005; Bossi et al., 2008; Berger et al., 2009; Becker et al., 2010). In terms of comparative studies, the current study was the first survey done on PFASs delineation in fish of the ORB and to our knowledge, the first study on PFASs in fish in South Africa. Generally, a lack of data and studies in the African continent for PFASs in fish was observed. The reported concentrations of PFOS in crocodile plasma from the ORB (0.776 to 118 ng/g ww) largely exceed the levels in fish from the present study (Christie et al., 2016). Compared to other studies around the world, PFOS levels in muscle tissue and liver in fish from the Olifants River are low (Hoff et al., 2005; Kannan et al., 2005; Berger et al., 2009; Becker et al., 2010; Labadie and Chevreuil, 2011; Malinsky et al., 2011; Pan et al., 2014) (Table S9).

For ∑PCBs, ∑DDTs and ∑HCHs and PFAAs, higher levels in liver tissue than muscle tissue were detected. As toxicants are metabolized in the liver, high pollutant levels are expected in this organ (Barni et al., 2016). In addition, given that PFASs are protein-bound (Li et al., 2010; Martin et al., 2003), the liver is considered a major organ for PFAS storage (Labadie and Chevreuil, 2011). In the literature, it was reported that PFASs preferentially accumulates in blood, followed by liver, muscle and kidney (Houde et al., 2011; Kannan et al., 2005; Martin et al., 2003; Suja et al., 2009). Muscle tissue however, is also frequently used in biomonitoring assessments being an edible component and thus of interest from a human health perspective (Labadie and Chevreuil, 2011). No clear trends in species, geographical and temporal variations of PCBs, PBDEs, OCPs and PFASs concentrations in biota were observed.

4.4.2. Relation between biological characteristics and POP levels

Biological characteristics, including fish length, fish weight and lipid content were previously reported as determining factors for POP bioaccumulation. But in the present study, no clear trends were observed between lipid content, weight and length and POP levels in fish with exception for a significant negative relation between length and POPs in *L. congoro* and *H. vittatus*.

4.5. Relationships between POP levels in the environment and biota tissues

To investigate the relationship between POP levels in sediment and biota, POP levels in biota tissue were related to POP concentrations in the sediment. TOC and lipid normalization is required since POP levels are prone to accumulate in organic fractions of sediments and lipids of biota (Kafilzadeh et al., 2012). No significant relationships were found between POP levels in surface water and sediment and biota. Surface water and sediment POP levels were observed to be poor indicators of the real exposure and bioavailability for biota of subtropical freshwater food webs. This is possibly due to lesser retention

in surface water and sediment and more dominant dissipation processes, like volatilization and faster rates of degradation with higher temperatures in (sub)tropical climates

Previous research determined that low absolute levels of POPs in sediments from (sub)tropical regions are not necessarily an indication of low exposure to POPs. The semi-volatile character of POPs combined with their low aqueous solubility and elevated ambient temperatures, leads to higher atmospheric concentrations and lower aquatic ecosystem concentrations in (sub)tropical regions relative to temperate regions (Verhaert et al., 2013; Iwata et al., 1994; Kannan et al., 1995; Larsson et al., 1995).

4.6. Trophic transfer of POPs through a subtropical food web

4.6.1. Trophic level

The ratio of ^{15}N to ^{14}N ($\delta^{15}N$) is a powerful tool for estimating the trophic position of organisms since stable nitrogen isotope ratios of consumers are typically enriched by 2-4‰ relative to their diet (Layman et al., 2012). Trophic levels corresponded well with the levels reported on Fishbase (www.fishbase.org) for *L. marequensis, C. gariepinus, S intermedius and H vittatus*. According to Fishbase, *S. zambesensis* and *L. rosae* have a trophic level of 2.7±0.34 and 2.4±0.18 respectively, which is slightly lower than trophic levels determined in the present study (3.3±0.39 and 3.5±0.29, respectively). But on average, trophic levels increased from detrivores to omnivores to piscivores.

4.6.2. Trophic transfer and trophic magnification factors

For CB153, CB187, CB180, CB170, CB199, CB206, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, α-HCH, and β-HCH, significant correlations between TL and log POP were observed and TMFs were > 1, thus biomagnification of POPs occurs in the food web of the ORB. No significant relation between TL and PFASs were found which means no trophic transfer of PFASs in the ORB.

One of the key predictors of biomagnification is the octanol-water partition coefficient (log Kow). Organic compounds with log K_{OW}<5 have lower potential for biomagnification and POPs with 5<log K_{OW}<7 have the highest potential. These findings were observed across modeling studies, laboratory experiments, and field studies (Walters et al., 2011). The present study confirms these findings (Figure 5).

Challenges remain in identifying general patterns in biomagnification among ecosystems and food webs (Walters et al., 2016). Studies on these patterns are mainly conducted in freshwater systems of temperate or cold climates and TMF studies on POPs in the Southern hemisphere are scarce which makes comparison of the present study with other data difficult. This stresses again the importance of this study in contributing to the significant knowledge gap on biomagnification in (sub)tropical aquatic ecosystems. In addition, comparison among studies is often complicated because TMFs for a given pollutant can vary widely among studies depending on various factors including types of consumers included in the analysis, the range of trophic levels investigated, energetic requirements of organisms, freshwater versus marine systems (Walters et al., 2011; Walters et al., 2016).

Previous studies have shown that latitude is a determining factor for TMFs with higher TMFs in the arctic aquatic ecosystems. The processes behind this observation are not yet well understood but the following

points are mentioned: biodilution, excretion rate and complexity of the food web (Borgå et al., 2012). When comparing TMFs with data from the tropical Congo River from Verhaert et al. (2013) significant differences could be observed for the compounds which were detected in both regions (CB153, CB187, CB180, p,p'-DDE and p,p'-DDT). Data from the Itimbiri river for the CRB and OG for the ORB were used. For PCBs no significant interaction was observed which means that slopes are the same for the tropical, subtropical summer high flow and subtropical winter low flow (CB153: $F_{2,47}$=0.41, p=0.67, CB187: $F_{2,49}$=0.50, p=0.61, CB180: $F_{2,49}$=0.10, p=0.91) (Figure 6). However, a significant difference in PCB levels between tropical and subtropical was determined with higher PCB levels in the tropical region than in the subtropical region and no difference between subtropical summer and winter (CB153: $F_{2,49}$=50, p<0.001, CB187: $F_{2,51}$=17, p<0.001, CB180: $F_{2,51}$=6.3, p=0.003). In conclusion, although PCB background concentrations differ between the climate regions, no significant differences in TMFs were observed for CB153, CB187 and CB180 so the average food web biomagnificantion of these PCBs occurs in the same way in the tropical and subtropical regions.

For p,p'-DDE and p,p'-DDT, a significant interaction was found so a significant difference between the slopes occurs and thus the TMFs (p,p'-DDE: $F_{2,49}$=3.62, p=0.03, p,p'-DDT: $F_{2,47}$=0.41, p=0.02) (Figure 5). The average biomagnification of p,p'-DDE and p,p'-DDT is higher in the subtropical region during winter than in the tropical region. However, no significant difference in biomagnification between the subtropical region during summer and the tropical region was observed.

Compared to TMFs from the subtropical lake Taihu (Yu et al. 2012 and Wang et al., 2012), TMFs from the present study were higher for both PCBs and DDTs. But when compared to mean TMFs from marine and freshwater systems all over the world (Walters et al. 2016) and freshwater lakes across Canada and US (Houde et al., 2008), TMFs for PCBs from the present study were lower and for DDTs higher (Table S10).

4.7. Minimum Risk Levels for Human Health

Fish are a pivotal food source for rural communities inhabiting the ORB (Gerber et al., 2016). Since POPs are accumulated and biomagnified in the food web of the Olifants River, also human consumers which are on the top of the food chain can be at risk by consuming contaminated fish (Du Preez et al., 2003; Afful et al., 2010). A broad range of adverse health effects are associated with POPs. POP health effects include endocrine disrupting capacities, reproductive impairment, immune system damage and documented or suspected cancers (Afful et al., 2010; Bordajandi et al., 2003; Jones and de Voogt, 1999; Munn and Gruber, 1997).

Based on the MRLs (ATSDR, 2010), the maximum amounts of fish (kg) which can be consumed by a person of 60 kg without potential human health risks is calculated. The FAO (2010) estimated the average fish consumption rate of the South African population on 21 g per day. So taken into account the observed concentrations of the present study, the fish species in the aquatic ecosystem of the ORB can be consumed without a risk for the analyzed POP contamination.

5. Conclusions

The overall detection frequency and detected concentrations of PCBs, PBDEs, OCPs and PFASs in surface water, sediment, invertebrates and fish were low. As a result, fish from the ORB can be consumed without a risk for POP contamination. DDT and metabolites are predominant, but the detected DDT pollution originates from historical use. Significant positive relationships between relative trophic level and PCB, DDT and HCH concentrations were observed and TMFs were > 1, indicating POP biomagnification in the ORB food web. Previous research predicted lower POP TMFs in (sub)tropical aquatic ecosystems when compared to temperate and arctic ecosystems. In the present study, this prediction was confirmed for PCBs but for DDTs, TMFs were unexpectedly higher than TMFs from temperate and arctic aquatic food webs.

6. Acknowledgements

Samples were taken during a fieldtrip in the frame of the VLIR-Institutional University Cooperation Programme with the University of Limpopo, South Africa (The impact of land-use on functions of the Limpopo and Olifants river systems ZIUS2016AP021). We would like to thank VLIR for the financial support, Ruan Gerber, Russell Tate, Steven Osmond and Kris Bal for the help in the field and Prof. Steven Bouillon for the stable isotope analysis. Partial financial support for this research was obtained from the Research Foundation Flanders, FWO (grant 1.5.182.13N).

Figures

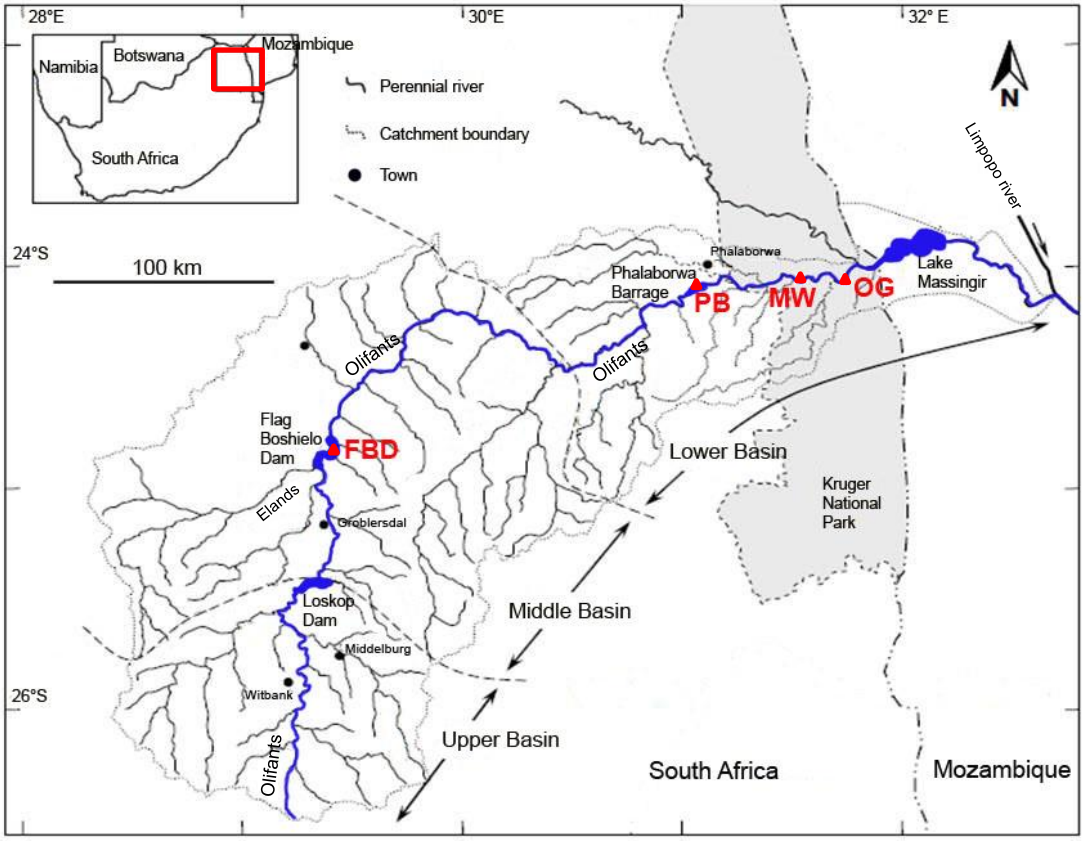


Figure 1: Adapted from Ashton (2010) illustrating the Upper, Middle and Lower Sub Basins of the Olifants River Catchment, South Africa. Sampling locations are indicated in bold: Δ FBD: Flag Boshielo Dam, Δ PB: Phalaborwa Barrage, Δ MW: Mamba Weir, Δ OG: Olifants Gorge.

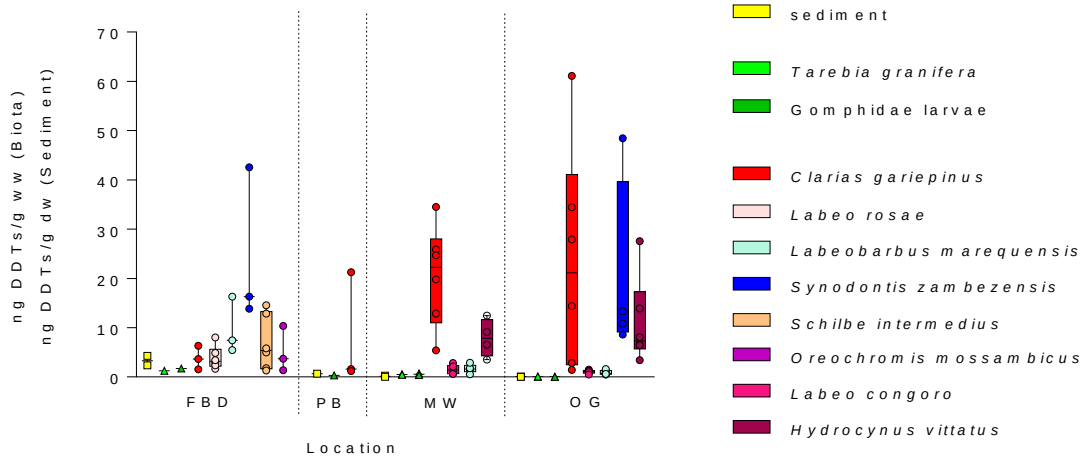


Figure 2: Boxplot of ∑DDT in sediment(ng/g dw) and invertebrate and fish species (ng/g ww) per location (Box: 25-75 percentiles, line: median, whiskers: minimum and maximum, each individual value was plotted)

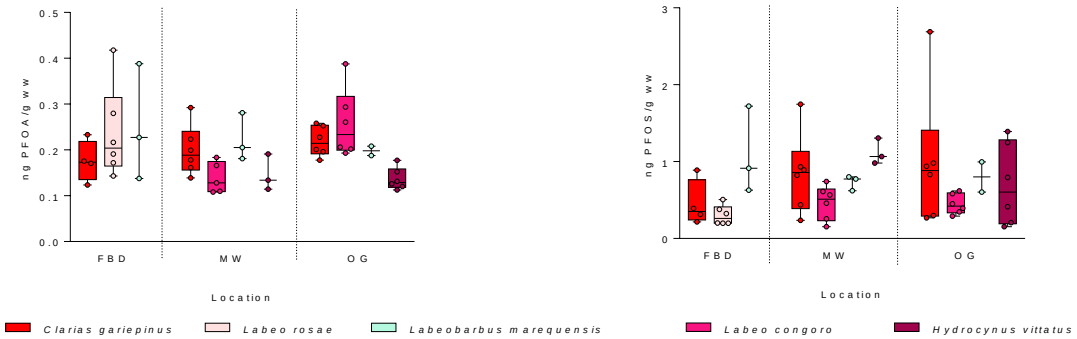
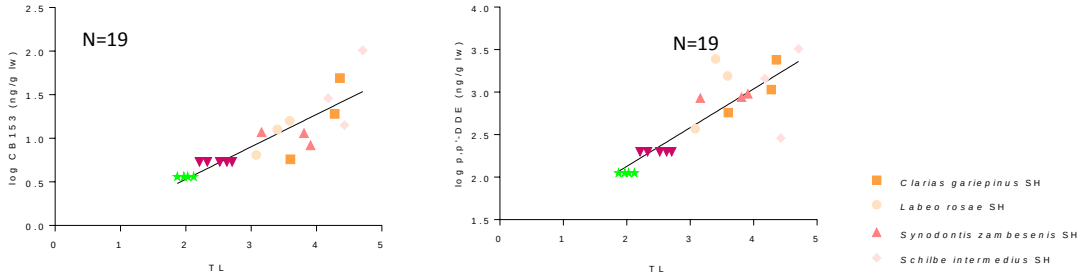
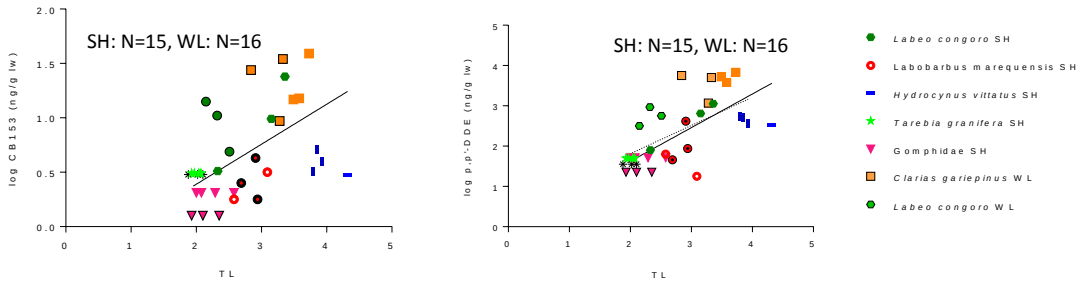


Figure 3: Boxplots of PFOA (left) and PFOS (right) for different fish species (ng/g ww) per location (Box: 25-75 percentiles, line: median, whiskers: minimum and maximum, each individual value was plotted)

A.



B.



C.

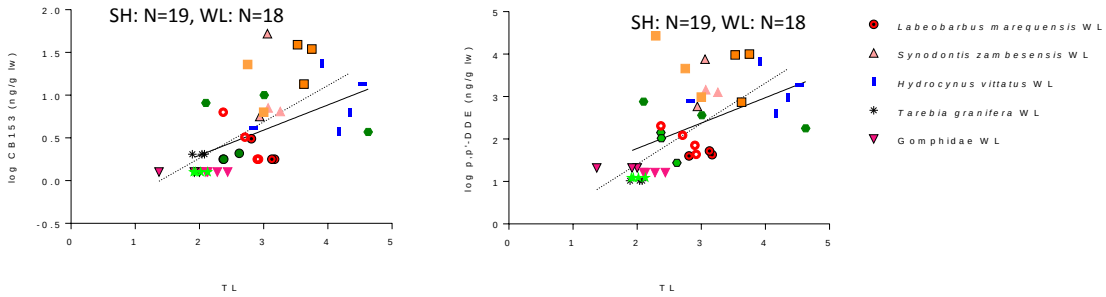


Figure 4: Relationship of trophic level (TL) and log concentrations of CB153 and p,p'-DDE of different food webs in the summer high flow SH (———) and winter low flow WL season (- - - -) at FBD (A), MW (B) and OG (C).

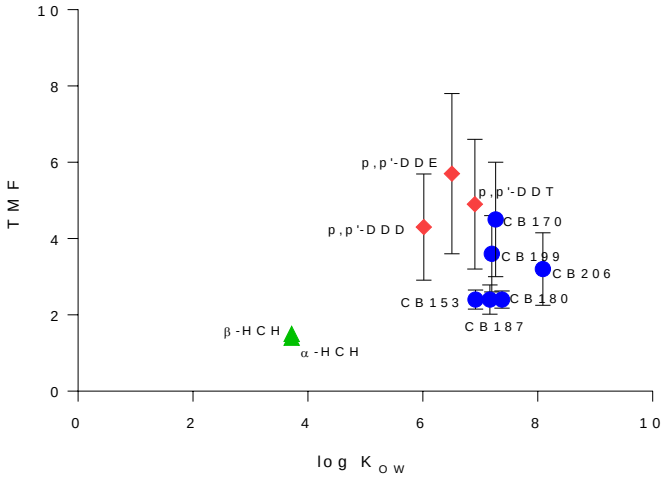
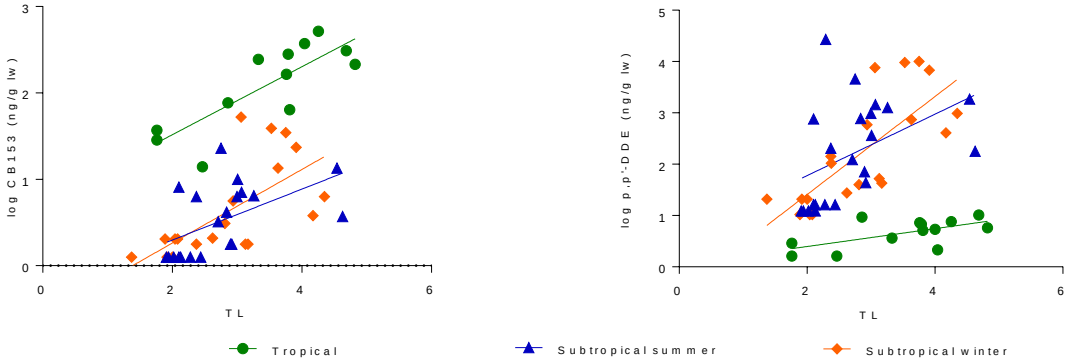


Figure 5: Average TMFs (different sampling locations and seasons were pooled since no significant differences were observed) versus log Kow for the different measured POPs (Error bars represent SD)



TL*location: $F_{2,47}$=0.41, p=0.67
TL: $F_{1,49}$=38, p<0.001
Location: $F_{2,49}$=50, p<0.001

TL*location: $F_{2,49}$=3.62, p=0.03

Figure 6: Comparison of slopes for the relationship between TL and log CB153 and ppDDE for the tropical region (Itimbiri river, Congo River Basin: Verhaert et al., 2013), the subtropical summer high flow (OG of ORB) and subtropical low flow winter (OG of ORB).

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