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Distribution of perfluorinated compounds (PFASs) in the aquatic environment of the industrially polluted Vaal River, South Africa

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Abstract

Perfluorinated alkyl substances (PFASs) are highly persistent chemicals, which have a bioaccumulative potential and can be found in wildlife around the world. Although multiple studies have been performed on PFASs pollution of the aquatic environment, little is known on PFASs pollution on the African continent and their possible risks for human health. In the present study, we examined the distribution of 15 PFASs in fish, invertebrates, sediment and water, collected at three sites, representing a gradient of industrial and mining pollution, along the Vaal River, South Africa. Furthermore, possible risks for human health through consumption of contaminated fish were examined.

Perfluorooctane sulfonate (PFOS) was the most dominant PFAS measured in biota, whereas Perfluoropentanoic acid (PFPeA) was measured in higher concentrations in water. Mean PFAS concentrations in water ranged from < LOQ to 38.5 ng/L. PFAS concentrations in water decreased along the gradient and were similar or lower compared to other studies in Europe, Asia and America. PFAS measurements in sediment were <LOQ, with the exception of PFOS at Thabela Thabeng (2.36 ng/g dry weight (dw)). Average ∑PFAS concentrations in biota increased along the gradient and ranged from < LOQ to 34.5 ng/g wet weight (ww) in invertebrates, <LOQ to 289 ng/g ww in liver and <LOQ to 34.0 ng/g ww in muscle tissue. Although PFOS concentrations were relatively high compared to literature, concentrations of other PFASs were rather low.

A potential risk for humans through consumption of PFAS-contaminated fish was assessed. Tolerable daily intake values (grams of fish that can be eaten daily without risking health effects) were much lower than the average South African fish consumption per day, implying a potential risk for human health through consumption of PFAS contaminated fish.

Keywords: perfluorinated compounds, South Africa, aquatic environment, PFASs, human health

Capsule: Concentrations of perfluorinated compounds in water, sediment, fish and invertebrates from the Vaal River were low or intermediate and posed a potential risk for human health through consumption of contaminated fish.

Introduction

Perfluorinated alkyl substances (PFASs) are a diverse class of highly persistent substances. The high energy of the C-F covalent bonds make PFASs thermally and chemically stable and resistant to biodegradation (Liu et al., 2014). Therefore, PFASs are commercially and industrially used for many purposes and can be found in many objects, including fast-food packaging, paper plates, fire-fighting foams, etc. (Domingo, 2012; Mudumbi et al., 2014a). Perfluorinated substances are used to make material stain, oil and water resistant (Xia et al., 2013) and are also commonly found in adhesives, cosmetics, pharmaceuticals, electronics, cleaning products, polishes and waxes, insecticides and paints (Domingo, 2012; La Rocca et al., 2012; Mudumbi et al., 2014a; Xia et al., 2013).

Most PFASs are extremely persistent as they do not hydrolyze, photolyse or biodegrade under various environmental conditions (Mudumbi et al., 2014a). PFASs are considered to be bioaccumulative in the environment (Domingo, 2012; Mudumbi et al., 2014a), which they may enter either directly or indirectly from environmental degradation of precursor compounds (Buck et al., 2011; Prevedouros et al., 2006). This indicates that these compounds may be found throughout the entire food chain, including humans. Intake of drinking water and food are considered to be among the most important exposure pathways for humans (D'Hollander et al., 2010; Haug et al., 2011; La Rocca et al., 2012), with fish being the main contributor to PFASs in humans (D'Hollander et al., 2010; Ericson et al., 2008; La Rocca et al., 2012; Squadrone et al., 2014, 2015). Possible negative effects of PFASs on humans include a lower ponderal index (Olsen et al., 2009), reduced fertility, difficult pregnancies (Fei et al., 2009; La Rocca et al., 2012) and reduced semen quality causing a higher male infertility (Governini et al., 2015; La Rocca et al., 2012).

Although multiple studies on PFASs in the aquatic environment have been conducted in countries on the northern hemisphere, including North America (e.g. Collí-Dulá et al., 2016; Levengood et al., 2015; Sinclair et al., 2006), Asia (e.g. Guo et al., 2015; He et al., 2015; Pan et al., 2015; Zhang et al., 2012; Zhou et al.,

2012) and Europe (e.g. Hoff et al., 2005; Giari et al., 2015; Renzi et al., 2013; Squadrone et al., 2014, 2015), little is known on PFASs pollution on the African continent. Two studies on PFASs have been performed on water and sediments in the Diep River, Salt River and Eerste River in South Africa (Mudumbi et al., 2014a; 2014b), but both these studies did not test for concentrations of PFASs in biotic compartments of the ecosystem. To our knowledge, only four studies have been performed on aquatic biota in Africa, with only one in fish. Verhaert et al. (2017) detected relatively low PFAS concentrations in liver and muscle tissue of fish from the Olifants River basin in South Africa. In addition, a study on PFASs in South African crocodiles in Kruger National Park detected multiple PFASs in relatively high concentrations and suggested a point source for PFOS contamination of this area (Christie et al., 2016). A similar study on crocodile eggs from the Kruger National Park has been conducted by Bouwman et al. (2014) and reported mean concentrations between 12 and 27 ng/g in eggs. Finally Bouwman et al. (2015) detected mean PFAS concentrations of 12 ng/g in eggs of African penguins. In addition, Lesch et al. (2017) measured PFASs in the terrestrial environment (adult Odonata) from selected sites in central and northern South Africa with concentrations up to 21 ng/g in Bloemhof Dam situated in the highly polluted Vaal River system.

Only a few studies investigated the relationships between PFAS concentrations in water and sediment with concentrations in biota (Campo et al., 2016; Hong et al., 2015; Kwadijk et al., 2014; Lorenzo et al., 2016). Hong et al. (2015) concluded that the bioaccumulation of PFASs in aquatic organisms is strongly dependent on PFAS concentrations in water, regardless of species. However, differences in bioaccumulation between species may occur (Kwadijk et al., 2014) and sometimes no relationship between PFAS concentrations in sediment or water and biota can be found (Lorenzo et al., 2016). Furthermore, only a few studies deal with the possibility of negative effects on humans due to consumption of PFAS polluted fish (He et al., 2015; Pan et al., 2015; Renzi et al., 2013; Zhao et al., 2011).

In the present study, the distribution of multiple PFASs in an aquatic food web in the Vaal River, South Africa, has been investigated. Additionally, biomagnification and trophic transfer within the food chain was assessed using stable isotope analysis. Finally, the possible risks for human health through consumption of PFAS-contaminated fish were determined. It was hypothesized that PFASs, present in the Vaal River system, were bio-accumulated and biomagnified in the aquatic ecosystem. These accumulated concentrations of PFASs in fish muscle tissue may pose a risk to human health, especially to local communities that rely on this fish as a main protein source.

Materials and method

Sample collection

The Vaal River is considered to be of major importance for South Africa, as it serves major economic activities, agriculture, industrial and mining activities, and a population of around 12 million people (Tempelhoff, 2009; Van Vuuren, 2008).

During the high flow period in September 2014, sediment, water, invertebrates and fish species (Table S1) were collected from three sampling sites (Fischgat, Vaal Barrage and Thabela Thabeng; Figure 1), representing a gradient of industrial and mining pollution, in the upper basin of the Vaal River, South Africa.

At each location three separate grab water (1 L) and sediment (100 mL; stainless steel hand shovel) were pooled in PFASs-free polyethylene and polypropylene (PP) bottles. General water characteristics, including temperature, pH, oxygen saturation, dissolved oxygen, conductivity and total dissolved solids (TDS) were measured using an Extech DO610 multimeter (Eutech, Thermo Scientific). Invertebrates were collected from instream rocks by hand with stainless steel tweezers, using sweep nets or shovels. Zooplankton was caught with a zooplankton net. Two groups of invertebrate taxa were collected at each site, i.e. Baetidae (Ephemeroptera, N = 10 – 20) and *Caridina nilotica* (Decapoda, N = 20 – 30) at Fischgat, zooplankton (50 mL) and Hirudinea (N = 10 – 20) at Barrage and *C. nilotica* (N = 20 – 30,) and Gyrinidae (adults, N = 50 – 100) at Thabela Thabeng. Individuals of the same species were pooled to obtain sufficient material for PFASs analysis. Fish were collected in a reach of approximately 100 m at each site using fyke nets and an electrofishing unit (Samus 300 Fish Shocker). Fish were identified, measured and weighted, and sacrificed with a blow on the head followed by the severing of the spinal cord. Axial muscle samples were collected, skin was discarded and liver samples were removed and placed into PFASs-free PP tubes. At each location at least three individuals of two species were captured: smallmouth yellowfish (*Labeobarbus aeneus*) and Orange River mudfish (*Labeo capensis*). In addition, the African sharptooth catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*) were caught at only two of the locations. All samples were transported to the laboratory in a field freezer at -20°C. Sediment samples were analyzed for particle size (1g; Malvern Mastersizer 2000 and Hydro 2000G) and TOC (Loss on Ignition as described by Heiri et al., 2001) in the laboratory.

Chemical analysis

PFAS abbreviations are according to Buck et al. (2011). Target analytes included perfluorobutanoic acid (PFBA), PFPeA, perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), PFOS and perfluorodecane sulfonate (PFDS). The isotopically mass-labelled internal standards (ISTDs, MPFAS mixture), containing $^{13}C_4$-PFBA, [1,2-$^{13}C_2$]PFHxA, [1,2,3,4-$^{13}C_4$]PFOA, [1,2,3,4,5-$^{13}C_5$]PFNA, [1,2-$^{13}C_2$]PFDA, [1,2-$^{13}C_2$]PFUdA, [1,2-$^{13}C_2$]PFDoA, $^{18}O_2$-PFHxS and [1,2,3,4-$^{13}C_4$]PFOS,were purchased at Wellington

Laboratories (Guelph, Canada). HPLC-grade water and acetonitrile (ACN; Acros Organics, New Jersey, USA) were used.

Sample extraction

Biotic samples were homogenized with an Ultra Turrax mixer (T25, Staufen, Germany) prior to extraction. Both biotic and abiotic samples were divided into duplicates (1g ww for biota, 2g dw for sediment, 500 mL water). Subsamples from biota and sediment were taken for stable isotope analysis.

The extraction procedure for water was based on a method described by Taniyasu et al. (2005). Samples (0.5 L) were filtrated through a glass fiber filter (Whatman),spiked with 80 μL of a 125 pg/μL MPFAS mix containing 125 pg/μL of each of the previously described mass-labelled internal standards, loaded into a Oasis Wax (Waters, 3cc) cartridge, preconditioned with 4 mL 0.1% ammonium hydroxide (NH_4OH) in acetonitrile (ACN) and HPLC grade waterand filtered over the cartridge. Hereafter the cartridge was washed with 40% ACN in HPLC grade water and eluted with 1 mL 2% NH_4OH in ACN.

The pretreatment method for sediment was based on a procedure described by Powley et al. (2005), whereas the rest of the extraction method was based on the procedure described by Powley et al. (2004). Prior to the analysis, sediment samples were air-dried in aluminum foil containers. Hereafter, 1 g of sediment was spiked with 80 μL of the previously described MPFAS mix (125 pg/μL) and mixed thoroughly. To each sample 1 mL 200 mM sodium hydroxide (NaOH) in methanol (CH_3OH) was added. After 30 minutes, 100 μL 2M hydrochloric acid (HCl) in methanol and 9 mL methanol were added. After vortexing, samples were extracted for 30 min on a shaking plate. Samples were centrifuged (4°C, 10 min, 2400 rpm, Eppendorf centrifuge 5804R) and concentrated in a rotational-vacuum-concentrator at 20°C (Martin Christ, RVC 2-25, Osterode am Harz, Germany). After weighting, the samples were transferred into a 1 mL Eppendorf tube, containing 25 mg graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Belgium) and 50 μL 100% acetic acid (CH_3COOH). The empty tubes were rinsed twice with 250 μL ACN, which was transferred to the same Eppendorf tube. After centrifugation (4°C, 10 min, 10000 rpm, Eppendorf centrifuge 5415R), the supernatant was transferred into a new Eppendorf tube and ready for filtration. For biota (1 g ww, in duplicate), samples were pretreated according to Powley et al. (2004). After spiking with 80 μL of the 125 pg/μL MPFAS mix, 10 mL ACN was added. Samples were then sonicated for 3x10 min and left overnight on a shaking plate. The rest of the procedure follows the method previously described for sediment.

Before PFASs analysis, 105 μL of the water extract was diluted with 195 μL HPLC grade water. For sediment and biotic samples 105 μL extract was added to an Eppendorf tube containing 195 μL HPLC grade water with 2 mM ammonium acetate (CH_3COONH_4). The entire volume was filtrated through an Ion Chromatography Acrodisc 13 mm Syringe Filter with 0.2 μm Supor (PES) Membrane into a polypropylene auto-injector vial.

PFASs were analyzed with an ACQUITY UPLC (Waters, Milford, MA, USA) linked to a tandem quadrupole mass spectrometer (ACQUITY, TQD, Waters, USA). Electrospray interface operated in negative ion mode. An ACQUITY BEH C18 column (2.1 x 50 mm; 1.7 μm, Waters, USA) was used to separate PFASs. To retain any PFAS originating from the UPLC system, a pre-column was inserted between the solvent mixer and injector. The injection volume was 10 μL and the flow rate was 450 μL/min. ACN and water, both with 0.1% formic acid, were used as mobile phases. All samples were injected twice and acquisition was performed by multiple reaction monitoring (MRM). The diagnostic transitions (precursor ion (m/z) → product ion (m/z)) used for identification and quantification are displayed in Table S2.

Calibration

A 10-point linear ($R^2 > 0.99$) calibration curve in ACN and HPLC-grade water (concentrations 0, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 pg/μL) was made with non-labelled standards of all target analytes. To each calibration point 125 pg/μL MPFAS mix was added. Concentrations were corrected for matrix effects and recovery losses by using the internal standards of the corresponding compounds. Recoveries ranged from 4 – 88.5% in biota and 0 – 88.5% in abiotic samples.

Quality assurance

The quality control was performed by a regular analysis of procedural blanks (one per 10 samples). As a blank for water HPLC-grade water was used, whereas empty 50 mL PP tubes were used for sediment and biota. PFOA concentrations detected in blanks (53.5 pg/g in all matrices with exception of water, in which no contamination was detected) were subtracted from the correspondent concentrations found in the samples. In addition, one sample of sterilized fish muscle tissue (pike perch: *Stizostedion lucioperca*, QUASIMEME Laboratory Performance Studies; Van Leeuwen et al., 2011) was included per ten samples as reference material. The limit of quantifications (LOQs) were calculated according to a signal-to-noise ratio of 10 and ranged from 0.01 to 0.95 ng/g. The recoveries of the ISTDs were calculated for all samples based on the ratio of internal standards in the sample.

Stable isotope analysis

Stable isotope analyses were performed on 31 fish, 6 invertebrate and 6 sediment samples. The procedure was based on the method described by Verhaert et al. (2013). Samples were oven-dried at 60°C, homogenized into a fine powder, weighted to the nearest 0.5 mg and encapsulated in pre-weighted 5 x 8 mm tin (Sn) or silver (Ag) capsules to determine nitrogen (N) concentrations, as well as $\delta^{15}N$. For sediment 15 mg was used. Samples in the Ag capsules were acidified by adding 3M HCl (Vafeiadou et al., 2013). A Thermo Flash HT/EA coupled to a Thermo DeltaV Advantage IRMS with a Conflo IV interface was used for stable isotope measurements. Stable isotope results are expressed in the standard notation as defined by:

$$\delta^{15}N = \left[\left(\frac{R_{sample}}{R_{reference}}\right) - 1\right] \times 1000$$

With R = $^{15}N/^{14}N$. A combination of IAEA-C6, IAEA-N1 and acetanilide was used for calibration of the data. The estimated precision was better than 0.15‰ for $\delta^{15}N$.

Risk to human health

The maximum edible amount (MEA) of fish for a person weighting 70 kg has been calculated using tolerable daily intake (TDI) values. The TDI for PFOS and PFOA are 30 ng/kg/day and 20 ng/kg/day respectively (ATSDR, 2016). The following formula was used:

MEA = (TDI * W)/C

With MEA = maximum edible amount in g/d; TDI = tolerable daily intake of a specific compound (ng/kg body weight); W = body weight (kg); C = average PFAS concentration (ng/g).

A worst case scenario was calculated based on the maximum PFAS concentrations.

Statistical analysis

Statistical analyses were conducted using R 3.1.3. (R Core Team, 2012). Concentrations below the LOQ were given a value of LOQ/2 (Bervoets et al., 2004; Custer et al., 2000). Samples that were not quantifiable due to low recoveries of the internal standards, were excluded from further analysis.

All data was tested for normality and homogeneity of variances. In case of non-normality non-parametric alternative tests were used. Differences in concentrations between locations and among fish species were detected using two-way ANOVA followed by the Tukey HSD test or a Pairwise Wilcox test. To test for differences between locations and species for invertebrates, two-sample t-tests were used. Pairwise t-tests were used to test for differences in liver and muscle tissue, as well as a Spearman rank correlation test to test for the correlation between liver and muscle tissue. The Wilcox rank sum test or bootstrap and permutation tests were used in case of non-normality. Linear regression was used to detect biomagnification of PFASs within the food web.

Results

Abiotic environment

An overview of mean concentrations and ranges of PFASs in water is given in Table 1. The PFBA, PFDA, PFUdA, PFDoA, PFTrA, PFTeA, PFDS were not detected or quantifiable and are therefore not displayed in the table.

Significant differences were observed between sampling sites for PFPeA, PFBS, PFHxA, PFOA and PFOS concentrations in water. Post hoc analysis revealed that concentrations were significantly lower at Thabela Thabeng compared to Barrage and Fischgat (all $p < 0.001$). In addition, PFHpA and PFHxS concentrations in water were significantly lower at Thabela Thabeng than at Fischgat ($p = 0.010$ and 0.003 respectively) and at Barrage ($p = 0.010$ and 0.037 respectively). PFOS concentrations in water were significantly ($p < 0.001$) higher at Fischgat compared to Barrage.

Recoveries for the sediment ranged between 10% and 60%.However, with exception of a PFOS concentration of 2.36 ng/g dw at Thabela Thabeng, all other concentrations were <LOQ.

Table S3 gives an overview of the physicochemical properties of water and sediment. Median grain size was significantly higher at Fischgat compared to Barrage and Thabela Thabeng ($p = 0.003$ and $p = 0.005$ respectively). The TOC was significantly higher at Thabela Thabeng than at Fischgat ($p = 0.048$). Conductivity, pH, temperature, oxygen saturation and dissolved oxygen were all significantly higher at Thabela Thabeng compared to the other locations (all $p < 0.001$). The pH, oxygen saturation and dissolved oxygen were lower at Barrage than at Fischgat ($p = 0.001$, <0.001 and 0.04 respectively), whereas conductivity and temperature were lowest at Fischgat (all $p < 0.001$). Salinity at Fischgat was significantly lower compared to both locations ($p < 0.001$). We observed the following significant ($p < 0.05$) correlations between physicochemical properties of the water: temperature was positively correlated with pH (R^2=0.70), conductivity (R^2 = 0.69), salinity (R^2 = 0.61), oxygen saturation (R^2 = 0.55) and dissolved oxygen (R^2 = 0.36). Furthermore, positive correlations were observed between pH and oxygen saturation (R^2 = 0.94), between pH and dissolved oxygen (R^2 = 0.79), salinity and conductivity (R^2 = 0.99) and between saturation oxygen and dissolved oxygen (R^2 = 0.81).

Biotic environment

Tables 2 and 3 show the mean PFASs concentrations and ranges in invertebrates and fish, respectively. The PFHxA, PFHpA and PFTeA were not quantifiable or detected in invertebrates, whereas PFBA and PFHpA were not quantifiable or detected in fish. All of these compounds were therefore removed from Tables 2 and 3.

As different invertebrate taxa were collected at each site, accurate comparisons in PFASs concentrations between locations were only possible for *C. nilotica* at Thabela Thabeng and Fischgat. Only PFOS concentrations were significantly higher ($p < 0.001$) at Thabela Thabeng compared to Fischgat. When comparing the average invertebrate concentration (both species combined at each location) between locations, PFBA, PFNA concentrations were significantly higher at Fischgat compared to Barrage (all $p < 0.05$) and Thabela Thabeng (all $p < 0.05$), PFOA concentrations were significantly higher at Fischgat compared to Barrage ($p = 0.014$), but not to Thabela Thabeng ($p = 0.054$) and PFOS concentrations were significantly higher at Thabela Thabeng compared to the other locations (both $p < 0.001$). Comparison of PFASs concentrations between species within each location showed significantly higher PFDoA and PFOS concentrations (both $p < 0.001$) in Hirudinea compared to zooplankton at Barrage. PFDoA ($p = 0.013$) and PFOS ($p < 0.001$) concentrations were higher in *C. nilotica* than in Gyrinidae adults from Thabela Thabeng.

At Fischgat higher concentrations of PFNA ($p = 0.002$), PFOA ($p < 0.001$) and PFOS ($p < 0.001$) were detected in Baetidae.

Only two fish species were collected from all three sites, i.e. *L. capensis* and *L. aeneus*. Therefore, comparisons of PFASs concentrations between locations has only been performed on these species. The PFOS concentrations in liver of both species were significantly higher at Thabela Thabeng (all $p < 0.025$). Although a similar result was obtained for muscle tissue, with higher PFOS concentrations at Thabela Thabeng compared to Fischgat (for both species $p < 0.001$), no differences with Barrage were observed. However, PFOS concentrations in muscle tissue of *L. aeneus* were significantly higher at Barrage than at Fischgat ($p = 0.004$). The PFDA concentrations were also higher in liver tissue of both species at Thabela Thabeng compared to Fischgat ($p = 0.002$ and $p = 0.011$ for *L. aeneus* and *L. capensis* respectively), but compared to Barrage this was only the case for *L. aeneus* ($p = 0.008$). The PFOA concentrations in muscle and liver of *L. capensis* were significantly lower at Thabela Thabeng compared to Barrage ($p = 0.014$ and $p = 0.042$ for muscle and liver respectively), and Fischgat ($p = 0.002$ for liver).

The PFHxS and PFOA concentrations in *C. carpio* were higher than those in *L. aeneus* (both $p < 0.05$), *L. capensis* (both $p < 0.05$) and *C. gariepinus* ($p < 0.001$ for PFOA).

Significant differences between concentrations in liver and muscle are illustrated in Figure 2a. Liver concentrations of PFOS ($p < 0.001$), PFDoA ($p = 0.002$), PFHxS ($p = 0.023$), PFNA ($p = 0.022$), PFTeA ($p = 0.011$) and PFTrA ($p < 0.001$) were all higher than concentrations in muscle. However, a significant positive correlation ($p < 0.001$, $R^2 = 0.73$) between concentrations in liver and muscle has only been observed for PFOS (Figure 2b).

Significant positive correlations were only observed between body weight and liver concentrations of PFOS ($p < 0.001$, $R^2 = 0.55$) and PFHxS ($p < 0.001$, $R^2 = 0.52$) for all species together and are illustrated in Figure 3.

Trophic transfer through the food web

As no primary consumers were caught at all three locations, it was impossible to calculate trophic levels (TLs) and trophic magnification factors (TMFs). However, it was still possible to look at the food web structure, trophic transfer and biomagnification based $\delta^{15}N$ values.

Figure 4 shows the significant relationships between $\delta^{15}N$ and PFASs concentrations. At Fischgat a significant increase of PFBA ($p = 0.022$, $R^2 = 0.96$) and PFTrA ($p = 0.022$, $R^2 = 0.61$) concentrations were observed with increasing $\delta^{15}N$. However, as PFBA was <LOQ in all fish samples, this correlation is only based on sediment and invertebrates. The PFDA concentrations were positively related to $\delta^{15}N$ values at Barrage ($p = 0.012$, $R^2 = 0.98$). A negative relationship between PFOS concentrations in water and invertebrates (Figure 5; $p < 0.001$, $R^2 = 0.52$) was observed.

Risks to human health

Maximum edible amounts (MEA) of fish muscle tissue per day were calculated for an average person weighting 70 kg and are shown in Table S4. As PFOS concentrations were higher than PFOA concentrations, the MEA was lower when looking at PFOS. MEAs were higher at Fischgat (0.29– 0.43 g/d for PFOS; 0.95 – 1.43 g/d for PFOA) compared to the other locations (PFOS: 0.01 – 0.09 g/d and 0.02 – 0.05 g/d at Barrage and Thabela Thabeng, respectively; PFOA: 0.95 g/d at both Barrage and Thabela Thabeng). The lowest MEA values were observed for *C. carpio* at Barrage (0.01 g/d) and Thabela Thabeng (0.02 g/d).

Discussion

Abiotic environment

The Vaal River Barrage acts as a reservoir of sewage and waste water, coming from the Suikerbosrant or Klip River, which both are contaminated due to mining, heavy industry and waste water treatment works (Tempelhoff, 2009; Wepener et al., 2011). It was therefore expected that PFAS concentrations upstream were lower than those at the Barrage, which was not the case. Possible explanations are that water from these tributaries gets pushed upstream, due to back-up of water, or that smoke emissions from SASOL, a large-petro-chemical plant in Sasolburg that also produces waxes (Tempelhoff, 2009), are mainly deposited upstream due to prevailing wind in the northeasterly direction towards the Vaal River (Kruger et al., 2010). Another possible explanation is that bioavailability of PFASs differs among locations due to differences in physicochemical properties of water and sediment (Chen et al., 2012; Jia et al., 2010; Milinovic et al., 2015; You et al., 2010; Zhou et al., 2010). However, this is all speculative and more research is necessary to examine the factors that affect the environmental concentrations of PFAS in the Vaal River or elsewhere.

Data on PFAS concentrations in water of aquatic systems in Africa are very scarce. Only two studies could be found in South African river water (Mudumbi et al., 2014a; Verhaert et al., 2017), in which PFOA and PFOS were detected in concentrations up to 314 and 182 ng/L in the Diep River, 390 and 47 ng/L in the Salt River and 146 and 23 ng/L in the Eerste River, respectively (Mudumbi et al., 2014a). In the Olifants River basin all surface water concentrations of PFAS were <LOQ (Verhaert et al., 2017).

The PFOS, PFHxS and PFOA concentrations in the Niagara River (3.3–6.7 ng/L PFOS; 1.2 – 1.4 ng/L PFHxS; 18 – 22 ng/L PFOA), Erie Canal (5.7 – 13 ng/L PFOS; 2.5 – 5.6 ng/L PFHxS; 25 – 59 ng/L PFOA) and Hudson River (1.5 – 3.4 ng/L PFOS; 0.7 – 1.6 ng/L PFHxS; 22 – 173 ng/L PFOA) were all higher or comparable to those measured in the present study (Sinclair et al., 2006). Compared to PFAS concentrations in water of the Yangtzi River Estuary (36.3 – 703.3 ng/L for PFOS; Pan & You, 2010), Yangtze River (<0.01 – 14 ng/L PFOS; So et al., 2007), Taihu Lake (ΣPFASs 17.8 – 448 ng/L; Yang et al., 2011), Pearl River Delta (0.02 – 730 pg/mL for PFOS; So et al., 2004), Haihe River (PFOS concentrations of 2.0 – 7.6 ng/L; Li et al., 2011), Dianchi Lake (ΣPFASs 30.98 ng/L; Zhang et al., 2012) and Baiyangdian Lake (PFOS 0.1 – 17.5 ng/L; Zhou et al., 2012), concentrations in the Vaal River were also comparable or lower. At Baiyangdian Lake, PFPeA was also the most abundant PFAS (Zhou et al., 2012). In the Orge River in France similar PFOS (17.4 ng/L) and PFOA (9.4 ng/L) concentrations were detected (Labadie & Chevreuil, 2011).

Sediment PFOS concentrations at Thabela Thabeng were comparable to the maximum concentrations found in China, where concentrations ranged from 0.13 to 6.95 ng/g in Taihu Lake (Guo et al., 2015) and <LOQ – 3.69 ng/g in the Yellow River (Pan et al., 2015). However, Mudumbi et al. (2014b) detected higher PFOS concentrations up to 121 ng/g in the Diep River, South Africa.

Variation in physicochemical properties of sediment and water might explain variations amongst sites in PFAS concentrations in the abiotic environment. As TOC is the dominant parameter affecting sorption of PFASs to sediments (Milinovic et al., 2015; You et al., 2010), a higher TOC at Thabela Thabeng could be the explanation of the higher PFAS concentrations in sediment and possibly also the lower PFAS concentrations in water at Thabela Thabeng. This could also be explained by the higher temperature of the water at Thabela Thabeng, as sorption of PFOS on humic acid is known to increase with temperature (Jia et al., 2010). Furthermore, Zhou et al. (2010) mention a higher sorption due to adsorption of microorganisms in the sediment, meaning that lower activities of microorganisms at lower water temperatures might cause a decreased sorption capacity of the sediment. Dissolved calcium and magnesium are responsible for the sorption-enhancing effect of salinity for PFOS (Chen et al., 2012), indicating that PFASs might be less available in areas with higher salinity such as Thabela Thabeng and Barrage. However, this is all speculative and causal relationships have not been tested for. Therefore, more research is necessary to examine the bioavailability of PFASs and to determine the influence of the tributaries of the Vaal River.

Biotic environment

Mean PFOS concentrations in *C. nilotica* were higher downstream than upstream, most likely due to either influences from polluted tributaries, which has been observed for metals and organic pollutants in the Vaal River (Wepener et al., 2011), or differences in bioavailability.

PFAS concentrations are low to intermediate compared to literature. PFAS concentrations in South African Odonata (terrestrial environment) are comparable to those in the aquatic biota at Thabela Thabeng and ranged up to 21 ng/g (Lesch et al., 2017). Mean PFOS concentrations in all invertebrate species from the Vaal River where higher than those in the Namhan River, South Korea (Lam et al., 2014), where mean PFOS concentrations of 3.21 ng/g ww were found, whereas they were comparable to those reported from Gaobeidian Lake, China (4.18 ng/g ww). However, PFOA concentrations at the Barrage were 6 times higher than those in China (0.05 ng/g ww; Li et al., 2008). Lescord et al. (2015) detected differences between pelagic and benthic invertebrates in multiple lakes in the Canadian High Arctic, with higher PFOS concentrations in benthic invertebrates (5.3 – 445 ng/g ww) compared to pelagic species (0.12 – 60 ng/g ww). This could possibly also explain the difference between the benthic *C. nilotica* and hypopleustonic Gyrinidae at Thabela Thabeng and zooplankton and Hirudinea at Barrage. Differences between *C. nilotica* and Baetidae could possibly be explained by differences in diet.

As far as we know, only one study has been performed on PFASs in South African fish. PFOS, PFOA and PFNA concentrations ranging from 0.15 to 2.7, <LOQ to 0.42 and <LOQ to 0.14 ng/g ww have been detected in muscle of fish from the Olifants River basin (Verhaert et al., 2017). The PFOA and PFNA concentrations were comparable with those measured in the present study. However, PFOS concentrations in the Vaal River were much higher.

Comparison with concentrations found in literature (Table 4) revealed that PFOS concentrations in liver of fish from the Vaal River were in the same range compared to those in smallmouth (*Micropterus* *dolomieu*) and largemouth (*Micropterus salmoides*) bass from New York State (9 – 315 ng/g; Sinclair et al., 2006). Lower PFOS concentrations, ranging between 7.4 and 30.8 ng/g, have been detected in bighead carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) fillets and whole fish from the Illinois River, USA (Levengood et al., 2015), whereas Collí-Dulá et al. (2016) detected much higher PFOS concentrations, with means up to 834.4 ng/g, in largemouth bass (whole fish) from five lakes in the US.

Mean PFOA (0.83 ng/g), PFNA (0.92 ng/g) and PFDA (1.15 ng/g) concentrations in muscle tissue of multiple fish species, including catfish and carp, from the Danjiang reservoir and the Xiangyang and Zhongxiang sections of the Hanjiang River in China were higher compared to the Vaal River (He et al., 2015). However, PFOS concentrations were higher in the Vaal River compared to the Hanjiang River in China (5.03 ng/g; He et al., 2015) and concentrations in common carp from the Pearl River in China (8.7 ng/g in muscle and 150 ng/g in liver; Pan et al., 2014). Compared to PFAS concentrations in muscle tissue from freshwater fish, including catfish and multiple carp species, from Hong Kong and Xiamen, PFOS concentrations in the Vaal River were higher, whereas PFOA and PFNA concentrations were lower (Zhao et al., 2011).

PFAS concentrations were also determined in multiple studies across Europe (Table 4). Renzi et al. (2013) detected mean concentrations ranging from <0.40 to 2.90 ng/g for PFOA and < 0.40 to 3.11 ng/g for PFOS in multiple lagoon taxa from different trophic levels in Orbetello Lagoon, Italy. In Comacchio Lagoon mean PFOS concentrations of 1.73 ng/g and 1.10 ng/g in liver and muscle have been observed (Giari et al., 2015). Giari et al. (2015) also detected mean PFOS concentrations of 1.76 ng/g and 0.72 ng/g in liver and muscle of fish from the Po River. The PFOA concentrations at Comacchio lagoon and the Po River were 5.08 ng/g and 9.12 ng/g for liver tissue and 3.55 ng/g and 0.90 ng/g for muscle tissue (Giari et al., 2015). Compared to both these Italian studies, PFOS concentrations were higher in the Vaal River, whereas PFOA concentrations were lower. However, PFOS concentrations in *C. carpio* from the Vaal River were low compared to a study in Belgian *C. carpio* near a PFASs hotspot (11.3 – 1822 ng/g; Hoff et al., 2005).

Despite the absence of a known direct industrial source of PFASs in South Africa, concentrations are still relatively high compared to countries in Europe, Asia and the USA, where direct industrial sources of PFASs have been identified. This suggests that there might be a point source for PFASs in the basin of the Vaal River or its tributaries.

In most of these studies and in the present study PFAS concentrations in liver tissue were higher than in muscle, which could be explained by the higher preference to concentrate in liver tissue (Sinclair et al., 2006). It has been suggested that PFASs are *proteinophilic*, as protein-rich tissues, such as blood and liver, usually contain higher concentrations than other biological compartments (Conder et al., 2007). The small sampling size in the present study resulted in a lower variation in PFAS concentrations, with exception of PFOS, which might explain that only PFOS concentrations were significantly correlated between liver and muscle. In addition, no significant correlation was observed between liver and muscle tissue for PFOS, PFOA and PFNA in fish from the Orange River Basin in South Africa (Verhaert et al., 2017).

Multiple studies investigated the effect of contaminants on growth in fish. Growth suppression was observed with increasing PFOS concentrations in smallmouth bass (Sinclair et al., 2006) and zebrafish fry (Du et al., 2009). As growth suppression can be explained by growth dilution, in which the rate of tissue growth exceeds the rate of PFOS accumulation (Sinclair et al., 2006), a positive correlation can be explained by a higher accumulation rate compared to growth rate. In addition, changes in diet with increasing body size resulted in higher metal concentrations in larger and heavier fish (Farkas et al., 2003). Squadrone et al., 2015 also observed a positive correlation between PFOS concentrations in muscle tissue and weight of the perch (*Perca fluviatilis*) from lake Varese, Italy. However, stable isotope analysis showed no difference between the fish species. Increasing the sample number might change the patterns found in the stable isotope analysis and might consequently provide more information on the positive correlations between PFOS and PFHxS and weight.

Trophic transfer through the food web

Based on $\delta^{15}N$ values, Baetidae showed the highest trophic position, followed by the fish species and *C. nilotica*. All studied fish species are omnivorous, which explains these results (Bloomer et al., 2007; Jimoh et al., 2011; Mondol et al., 2013). The high trophic position of Baetidae was not expected, as Palmer et al. (1993) showed that the gut content of mayfly larvae consisted mainly of amorphous detritus.

It is critical to understand the trophic transfer, i.e. the movement of chemicals from lower to higher trophic levels (Verhaert et al., 2013), of PFASs to evaluate the influence of PFASs on the ecosystem. Trophic magnification, the increase in concentrations from one trophic level to the next (Verhaert et al., 2013), of PFOS, PFDA, PFUdA and PFDoA has been observed in a subtropical food web in the Mai Po Marshes Nature Reserve in Hong Kong (Loi et al., 2011). However, at Baiyangdian Lake in China, no biomagnification or trophic transfer of PFASs occurred (Zhou et al., 2012). Verhaert et al. (2017) observed no significant relationships between trophic levels and PFAS concentrations in fish from South Africa. Although in the present study trophic transfer and biomagnification occurred for PFBA, PFDA and PFTrA, not enough individuals from each species were sampled to get a reliable investigation of the relationship. Furthermore, the negative relationship between PFOS concentrations in water and invertebrates suggests that PFASs uptake by these invertebrates does not occur only through water, but possibly also via sediment and food. We expected similar patterns with other PFASs, but due to small sampling sizes and low variation in concentrations of these PFASs, no relationships were observed. Increasing the sampling size would result in a more reliable investigation of possible relationships between different environmental matrices. Unfortunately, relationships with sediment could not be tested due to the low recoveries.

Risks to human health

In most countries only the muscle tissue of the fish is consumed. However, sometimes people eat the livers of the fish as well (D'Hollander et al., 2010). As liver concentrations are higher than those in muscle, the advised MEA of fish per day will be lower when people also consume liver. In the present study, the MEA of fish was calculated only for muscle tissue. The MEA was lower than the average daily fish consumption in South Africa (7.4 kg/capita/year, which is approximately 20g/capita/day; Speedy, 2003). These results indicate a potential risk for human health through the consumption of PFASs-contaminated fish.

Conclusion

PFASs have been detected in both the abiotic as well as the biotic compartments of the Vaal River. Highest concentrations in water were found upstream, showing a gradient to downstream parts, whereas PFAS concentrations in biota showed an inverse trend, possibly due to differences in bioavailability. Water concentrations of PFOS, PFHxS and PFOA were low or similar compared to literature. Although only PFOS has been detected in sediment from Thabela Thabeng, concentrations were relatively high compared to other non-African countries, whereas other studies on PFASs in South African sediments showed even higher concentrations. PFOS concentrations in fish are higher or comparable to those detected in the US, Asia or Europe. Biomagnification was only observed for PFBA, PFTrA and PFDA. A negative relationship was observed between PFOS concentrations in water and invertebrates, whereas no relationship was detected between water and fish. Therefore, it was suggested that contamination of fish is mainly due to bottom foraging and exposure to PFASs in sediment and invertebrates. However, more research is necessary to confirm this. Adverse health effects through consumption of PFAS-contaminated fish are expected, as the daily fish consumption in South Africa is much higher than the tolerable maximum consumption calculated in the present study.

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Figures

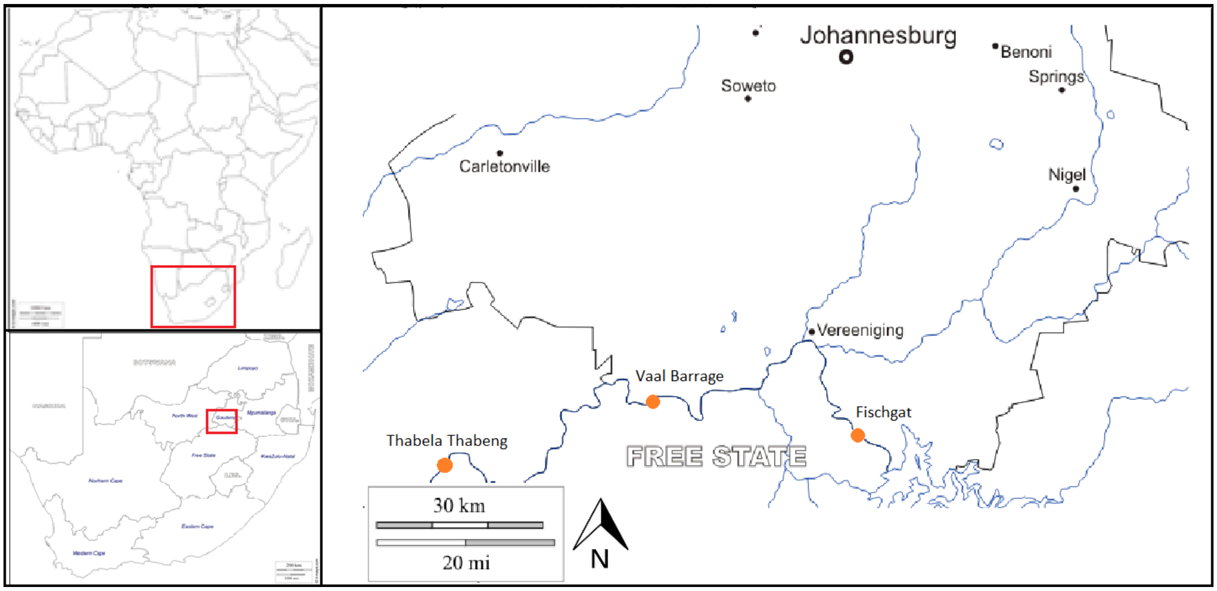

Figure 1. Situation of the study area and location of the three sampling points (Thabela Thabeng, Vaal Barrage and Fischgat) in the upper basin of the Vaal River, South Africa.

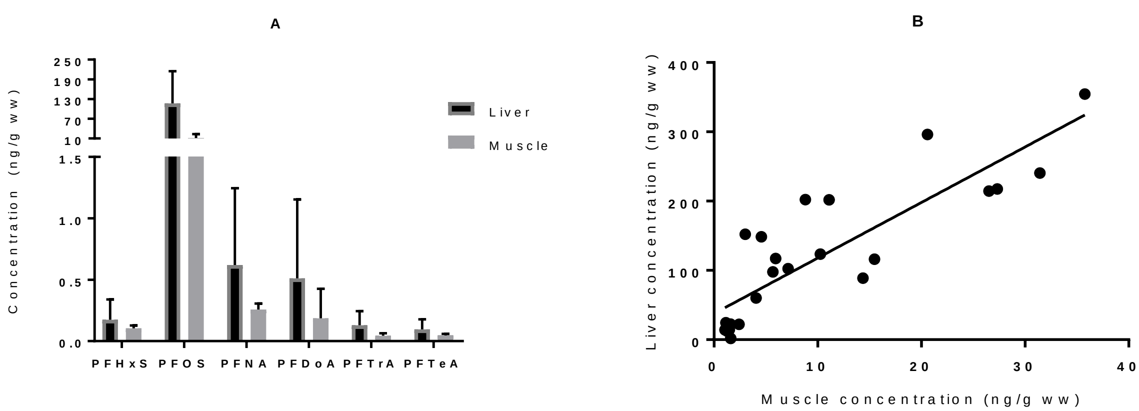

Figure 2. Concentrations of PFAS in liver and muscle tissue of fish from the Vaal River. A) Comparison of significant differences in concentrations of liver and muscle of fish from all three locations. B) Correlation between PFOS concentrations in muscle and liver tissue of fish from all three locations ($p < 0.001$, $R^2 = 0.73$).

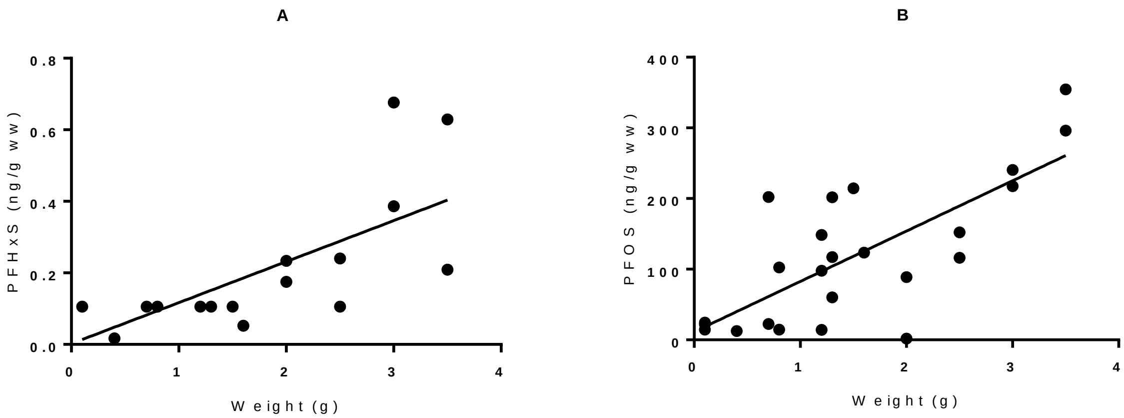

Figure 3. Concentrations of PFAS (ng/g ww) in the liver correlated with the body weight of the fish for A) PFHxS ($p < 0.001$, $R^2 = 0.52$) and B) PFOS ($p < 0.001$, $R^2 = 0.55$)

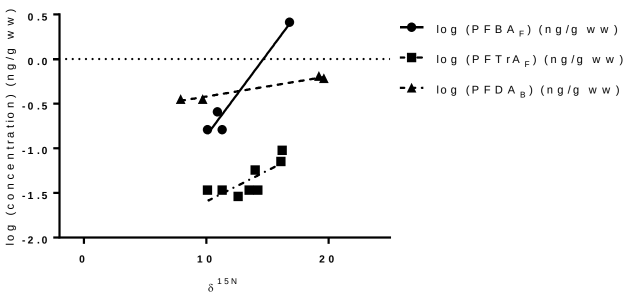

Figure 4. Biomagnification of PFBA ($p = 0.022$, $R^2 = 0.96$) and PFTrA ($p = 0.022$, $R^2 = 0.61$) at Fischgat and PFDA ($p = 0.012$, $R^2 = 0.98$) at Barrage.

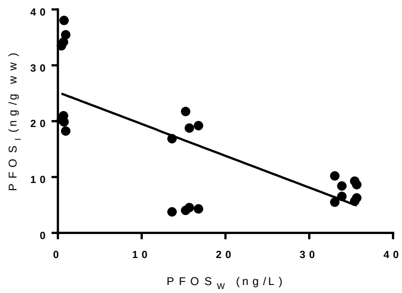

Figure 5. Correlation ($p < 0.001$, $R^2 = 0.52$) between PFOS concentrations in invertebrates ($PFOS_I$) and water ($PFOS_W$).

Tables

Table 1. LOQs, Mean concentrations and ranges (between brackets) in ng/L of PFASs in water from the Vaal River, South Africa. Concentrations below the limit of quantification are displayed as <LOQ. PFBA, PFDA, PFUdA, PFDoA, PFTrA, PFTeA and PFDS were not quantifiable or detected and are therefore not displayed in the table.

Compound	LOQ	Fischgat (N = 3)	Barrage (N = 3)	Thabela Thabeng (N = 3)
PFPeA	0.14	38.5 (32.3 – 45.0)	31.8 (26.5 – 37.9)	7.2 (5.7 – 9.6)
PFHxA	0.40	17.4 (15.6 – 20.3)	15.4 (12.4 – 18.9)	1.8 (<LOQ – 3.2)
PFHpA	0.25	1.2 (<LOQ – 2.5)	1.1 (<LOQ – 1.7)	<LOQ
PFNA	0.54	1.6 (1.3 – 1.8)	1.1 (0.7 – 1.5)	1.0 (<LOQ – 1.5)
PFOA	0.07	4.2 (4.1 – 4.3)	4.2 (3.9 – 4.6)	0.7 (0.6 – 0.9)
PFBS	0.37	19.7 (14.0 – 24.7)	14.8 (12.5 – 15.6)	<LOQ
PFHxS	0.21	4.9 (3.0 – 7.6)	3.2 (1.4 – 5.3)	<LOQ
PFOS	0.12	34.5 (33.1 – 35.7)	15.3 (13.6 – 16.8)	0.7 (0.4 – 0.9)

Table 2. LOQs, Mean concentrations and ranges (between brackets) in ng/g ww of multiple PFAS in invertebrates from the Vaal River, South Africa. Concentrations below the limit of quantification are displayed as <LOQ. Compounds that were not detected are displayed as ND. PFHxA, PFHpA and PFTeA were not quantifiable or detectable and are therefore not displayed in the table.

		Fischgat		**Barrage**		**Thabela Thabeng**	
Compound	**LOQ**	**Baetidae (N = 10 – 20)**	***Caridina nilotica* (N= 20 – 30)**	**Zooplankton (50 mL)**	**Hirudinea (N = 10 – 20)**	***Caridina nilotica* (N = 20 – 30)**	**Gyrinidae (N = 50 – 100)**
PFBA	0.32	1.4 (1.1 – 1.6)	<LOQ	<LOQ (<LOQ – 0.6)	<LOQ	<LOQ (<LOQ – 0.6)	<LOQ
PFPeA	0.14	<LOQ	<LOQ	<LOQ	0.5 (<LOQ – 2.1)	<LOQ	<LOQ
PFNA	0.54	0.8 (0.7 – 0.9)	0.63 (0.62 – 0.64)	<LOQ	<LOQ	0.6 (<LOQ – 0.6)	ND
PFOA	0.07	0.9 (0.7 – 1.0)	0.2 (0.2 – 0.3)	0.3 (0.26 – 0.32)	<LOQ	0.3 (0.2 – 0.4)	0.3 (0.2 – 0.3)
PFDA	0.71	<LOQ	<LOQ	<LOQ	<LOQ	0.9 (<LOQ – 1.8)	<LOQ
PFUdA	0.95	<LOQ	1.0 (<LOQ – 2.0)	<LOQ	<LOQ	<LOQ	<LOQ
PFDoA	0.07	<LOQ	0.2 (0.1 – 0.2)	<LOQ	0.3 (0.2 – 0.3)	0.2 (0.2 – 0.2)	0.1 (0.1 – 0.1)

PFTrA	0.07	<LOQ	<LOQ	<LOQ	0.1 (0.1 – 0.2)	<LOQ	<LOQ
PFBS	0.37	<LOQ (<LOQ – 0.4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFHxS	0.21	<LOQ (<LOQ – 0.4)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ (<LOQ – 0.3)
PFOS	0.12	9.2 (8.4 – 10.2)	6.0 (5.5 – 6.6)	4.3 (4.1 – 4.5)	<LOQ	34.5 (33.6 – 35.5)	19.9 (18.3 – 21.0)
PFDS	0.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.03 (<LOQ – 0.06)

Table 3. LOQs, Mean concentrations and ranges (between brackets) in ng/g ww of multiple PFAS in liver (L) and muscle (M) tissue of fish (LC = *Labeo capensis*, LA = *Labeobarbus aeneus*, CC = *Cyprinus carpio* and CG = *Clarias gariepinus*) from Fischgat (F), Barrage (B) and Thabela Thabeng (T). PFBA and PFHpA were not detected or quantifiable and are therefore not displayed in the table.

Loc			**PFPeA**	**PFHxA**	**PFNA**	**PFOA**	**PFDA**	**PFUdA**	**PFDoA**	**PFTrA**	**PFTeA**	**PFBS**	**PFHx**
	LOQ		0.14	0.40	0.54	0.07	0.71	0.95	0.07	0.07	0.10	0.37	0.21
F	**LC (N = 4)**	**L**	<LOQ	<LOQ	1.1 (0.8 – 1.4)	0.5 (0.4 – 0.7)	1.1 (<LOQ – 2.4)	<LOQ	0.4 (0.2 – 0.7)	0.2 (0.1 – 0.6)	<LOQ	<LOQ	<LO
		M	<LOQ	<LOQ	<LOQ	0.2 (0.2 – 0.3)	<LOQ	<LOQ	0.1 (<LOQ – 0.2)	<LOQ (<LOQ – 0.1)	<LOQ	<LOQ	<LO
	LA (N = 3)	**L**	<LOQ	<LOQ	<LOQ	0.5 (0.2 – 1.5)	<LOQ	<LOQ	0.3 (<LOQ – 0.5)	0.3 (<LOQ – 0.4)	<LOQ (<LOQ – 0.1)	<LOQ	<LO
		M	<LOQ	<LOQ	<LOQ	0.2 (0.2 – 0.3)	<LOQ	<LOQ	<LOQ (<LOQ – 0.1)	<LOQ (<LOQ – 0.1)	<LOQ	<LOQ	<LO
	CG (N = 2)	**L**	<LOQ	<LOQ	<LOQ	0.6 (0.2 – 1.1)	<LOQ	<LOQ	0.5 (0.2 – 0.9)	0.2 (0.1 – 0.2)	<LOQ	<LOQ	<LO
		M	<LOQ	<LOQ	<LOQ	0.3 (0.2 – 0.3)	<LOQ	<LOQ	0.1 (<LOQ – 0.1)	<LOQ (<LOQ – 0.1)	<LOQ	<LOQ	<LO

B	**LC (N = 2)**	**L**	<LOQ	<LOQ	<LOQ	0.5 (0.4 – 0.5)	3.3 (1.8 – 4.8)	1.3 (<LOQ – 2.3)	0.9 (0.8 – 1.1)	0.1 (0.1 – 0.2)	<LOQ	0.6 (0.5 – 0.8)	<LO (<LO – 0.8
		M	0.2 (<LOQ – 1.3)	<LOQ	<LOQ	0.3 (0.2 – 0.3)	<LOQ	<LOQ	0.2 (0.1 – 0.2)	<LOQ	<LOQ	<LOQ	<LO
	LA (N = 3)	**L**	<LOQ	<LOQ	<LOQ	0.3 (0.3 – 0.4)	1.2 (<LOQ – 1.7)	<LOQ	0.6 (0.4 – 0.8)	0.1 (0.1 – 0.2)	<LOQ (<LOQ – 0.1)	<LOQ (<LOQ – 0.6)	0.3 (<LO – 0.6
		M	<LOQ (<LOQ – 0.7)	<LOQ	<LOQ	0.3 (0.2 – 0.4)	<LOQ	<LOQ	0.1 (0.1 – 0.2)	<LOQ	<LOQ	<LOQ	<LO
	CC (N = 3)	**L**	<LOQ	<LOQ	<LOQ (<LOQ – 0.6)	0.4 (0.4 – 0.6)	3.3 (0.9 – 5.1)	1.1 (<LOQ – 2.8)	1.5 (0.9 – 1.8)	0.2 (<LOQ – 0.4)	<LOQ (<LOQ – 0.1)	<LOQ (<LOQ – 0.4)	0.7 (<LO – 1.1
		M	<LOQ	<LOQ	<LOQ	0.3 (0.2 – 0.4)	<LOQ (<LOQ – 1.1)	<LOQ	0.2 (<LOQ – 0.4)	<LOQ (<LOQ – 0.1)	<LOQ	<LOQ	<LO (<LO – 0.5
T	**LC (N = 4)**	**L**	<LOQ	<LOQ	<LOQ	0.3 (0.1 – 0.4)	5.8 (3.3 – 8.7)	<LOQ	0.8 (0.5 – 1.4)	0.2 (0.1 – 0.4)	<LOQ	<LOQ	<LO (<LO – 1.8

		M	<LOQ	<LOQ	<LOQ	0.2 (0.1 – 0.3)	<LOQ	<LOQ	0.1 (<LOQ – 0.2)	<LOQ	<LOQ	<LOQ	<LO
	LA (N = 4)	**L**	<LOQ	1.3 (<LOQ – 6.6)	<LOQ	0.2 (<LOQ – 0.3)	3.4 (1.2 – 4.8)	1.3 (<LOQ – 4.7)	0.7 (0.4 – 1.2)	0.2 (0.1 – 0.3)	<LOQ (<LOQ – 0.2)	<LOQ	<LO (<LO – 0.9
		M	<LOQ – 0.4	<LOQ	<LOQ	0.2 (0.1 – 0.3)	<LOQ	<LOQ	0.1 (<LOQ – 0.3)	<LOQ	<LOQ	<LOQ	<LO (<LO – 0.8
	CC (N = 3)	**L**	<LOQ	<LOQ	1.1 (0.7 – 1.7)	0.4 (0.3 – 0.5)	3.6 (2.0 – 5.4)	1.7 (<LOQ – 3.2)	1.3 (0.8 – 1.9)	0.4 (0.1 – 0.8)	<LOQ (<LOQ – 0.2)	<LOQ (<LOQ – 0.4)	0.5 (<LO – 1.2
		M	0.8 (<LOQ – 5.8)	<LOQ (<LOQ – 0.7)	<LOQ	0.3 (<LOQ – 0.4)	<LOQ (<LOQ – 1.9)	<LOQ	0.2 (<LOQ – 0.3)	<LOQ (<LOQ – 0.1)	<LOQ	<LOQ	<LO
	CG (N = 5)	**L**	<LOQ (<LOQ – 0.5)	3.1 (<LOQ – 11.9)	<LOQ (<LOQ – 0.8)	0.4 (<LOQ – 2.3)	2.2 (<LOQ – 3.2)	<LOQ (<LOQ – 1.4)	0.8 (<LOQ – 1.4)	0.3 (<LOQ – 0.8)	<LOQ (<LOQ – 0.2)	<LOQ (<LOQ – 0.6)	<LO (<LO – 1.2
		M	<LOQ (<LOQ – 1.2)	<LOQ	<LOQ	0.2 (<LOQ – 0.3)	<LOQ	<LOQ	0.1 (<LOQ – 0.3)	<LOQ (<LOQ – 0.2	<LOQ	<LOQ	<LO (<LO – 0.7

Table 4. Comparison of PFAS concentrations in ng/g ww in muscle (M) and liver (L) tissue of fish at multiple aquatic environments. Single values represent mean values, whereas ranges are indicated by '-'.

Species	**Location**	**PFOS**		**PFBA**	**PFHxA**	**PFOA**		**PFNA**	**PFDA**	**PFUdA**	**PFDoA**	**Reference**
		L	**M**	**M**	**M**	**L**	**M**	**M**	**M**	**M**	**M**	
Multiple species (n = 15)	Olifants River basin, South Africa		0.15 – 2.7				<LOQ – 0.42	<LOQ – 0.14				Verhaert et al., 2017
Micropterus salmoides (n = 28)	New York State, USA	9 - 315										Sinclair et al., 2006
Micropterus dolomieu (n = 38)	New York State, USA	10 – 120										Sinclair et al., 2006
Hypophthalmichthys nobilis (n = 10)	Illinois River, USA		1.2 – 10.0									Levengood et al., 2015

Hypophthalmichthys molitrix (n = 10)	Illinois River, USA		1.1 – 5.6									Levengood et al., 2015
Multiple species (n = 15)	Danjiang reservoir and Hanjiang River, China		5.03	0.92	0.22		0.83	0.92	1.15	2.25	2.19	He et al., 2015
Cyprinus carpio (n = 12)	Pearl River, China	150	8.7									Pan et al., 2014
Multiple species (n = 10)	Hong Kong, China		0.27 – 4.5				1.1 – 1.4	0.69 – 0.89				Zhao et al., 2011
Multiple species (n = 8)	Xiamen, China		0.49 – 5.98				1.1 – 1.4	0.65 – 0.87				Zhao et al., 2011

Anguilla anguilla (n = 16)	Comacchio Lagoon, Italy	1.73	1.10			5.08	3.55					Giari et al., 2015
Anguilla anguilla (n = 19)	Po River, Italy	1.76	0.72			9.02	0.90					Giari et al., 2015
Cyprinus carpio (n = 12)	Blokkersdijk, Belgium		11.3 – 1822									Hoff et al., 2005
Cyprinus carpio (n = 6)	Vaal River, South Africa	195.5 – 460.7	<LOQ – 45.7	ND	<LOQ – 0.7	0.3 – 0.6	<LOQ – 0.4	<LOQ	<LOQ – 1.9	<LOQ	<LOQ – 0.4	Present study
Clarias gariepinus (n = 7)	Vaal River, South Africa	<LOQ – 90.33	1.0 – 29.0	ND	<LOQ	<LOQ – 2.3	<LOQ – 0.3	<LOQ	<LOQ	<LOQ	<LOQ – 0.3	Present study
Labeobarbus aeneus (n = 10)	Vaal River, South Africa	13.8 – 429	0.8 – 24.4	ND	<LOQ	<LOQ – 1.5	0.1 -0.4	<LOQ	<LOQ	<LOQ	<LOQ – 0.3	Present study

Labeo capensis (n = 10)	Vaal River, South Africa	12.9 – 245	0.8 – 11.0	ND	<LOQ	0.1 – 0.7	0.1 – 0.3	<LOQ	<LOQ	<LOQ	<LOQ – 0.2	Present study

Supplementary material

Table S1. Overview of samples collected at each location, including the number of individuals (N) and the volume of the samples (mL).

Type of Sample	Species/Taxa	Location	N
Water	-	All	1000 mL, pooled
Sediment	-	All	100 mL, pooled
Invertebrate	Zooplankton	Barrage	50 mL, pooled
	Hirudinae	Barrage	10 – 20, pooled
	Gyrinidae	Thabela Thabeng	50 – 100, pooled
	Caridina nilotica	Thabela Thabeng	20 – 30, pooled
		Fischgat	20 – 30, pooled
	Baetidae	Fischgat	10 – 20, pooled
Fish	*Labeobarbus aeneus*	Barrage	3
		Fischgat	3
		Thabela Thabeng	4
	Labeo capensis	Barrage	2
		Fischgat	4
		Thabela Thabeng	4
	Cyprinus carpio	Barrage	3
		Thabela Thabeng	3
	Clarias gariepinus	Thabela Thabeng	5
		Fischgat	2

Table S2.Abbrevations, chemical formulas, internal standards and diagnostic transitions of the chemicals and internal standards.

Chemical	**Abbreviation**	**Chemical formula**	**Internal standard used for quantification**	**Diagnostic transition (precursor ion (m/z) → product ion (m/z))**	**Diagnostic transition (precursor ion (m/z) → product ion (m/z)) of the internal standard**
Perfluorobutanoic acid	PFBA	C_3F_7COOH	$^{13}C_4$-PFBA	213 → 169	217 → 172
Perfluoropentanoic acid	PFPeA	C_4F_9COOH	[1,2-$^{13}C_2$]PFHxA	263 → 219	315 → 270
Perfluorohexanoic acid	PFHxA	$C_5F_{11}COOH$	[1,2-$^{13}C_2$]PFHxA	313 → 269	315 → 270
Perfluoroheptanoic acid	PFHpA	$C_6F_{13}COOH$	[1,2-$^{13}C_2$]PFHxA	363 → 319	315 → 270
Perfluorooctanoic acid	PFOA	$C_7F_{15}COOH$	[1,2,3,4-$^{13}C_4$]PFOA	413 → 369	417 → 372
Perfluorononanoic acid	PFNA	$C_8F_{17}COOH$	[1,2,3,4,5-$^{13}C_5$]PFNA	463 → 419	468 → 423
Perfluorodecanoic acid	PFDA	$C_9F_{19}COOH$	[1,2-$^{13}C_2$]PFDA	513 → 469	515 → 470 515 → 270
Perfluoroundecanoic acid	PFUdA	$C_{10}F_{21}COOH$	[1,2-$^{13}C_2$]PFUdA	563 → 519 563 → 169	565 → 520
Perfluorododecanoic acid	PFDoA	$C_{11}F_{23}COOH$	[1,2-$^{13}C_2$]PFDoA	613 → 569	615 → 570
Perfluorotridecanoic acid	PFTrA	$C_{12}F_{25}COOH$	[1,2-$^{13}C_2$]PFDoA	663 → 619	615 → 570
Perfluorotetradecanoic acid	PFTeA	$C_{13}F_{26}COOH$	[1,2-$^{13}C_2$]PFDoA	713 → 669	615 → 570
Perfluorobutane sulfonate	PFBS	$C_4F_9SO_3H$	$^{18}O_2$-PFHxS	299 → 99	403 → 103
Perfluorohexane sulfonate	PFHxS	$C_6F_{13}SO_3H$	$^{18}O_2$-PFHxS	399 → 99	403 → 103
Perfluorooctane sulfonate	PFOS	$C_8F_{17}SO_3H$	[1,2,3,4-$^{13}C_4$]PFOS	499 → 80 499 → 99	503 → 80 503 → 99
Perfluorodecane sulfonate	PFDS	$C_{10}F_{21}SO_3H$	[1,2,3,4-$^{13}C_4$]PFOS	599 → 99	503 → 80 503 → 99

Table S3. Mean values for water and sediment quality parameters at each location.

	Fischgat	**Barrage**	**Thabela Thabeng**
pH	8.38	8.26	9.29

Conductivity (µS/cm)	168	668	707
TDS (mg/L)	119	476	468
Temperature (˚C)	15.1	17.1	19.8
Saturation O_2 (%)	84.2	73.4	111
Dissolved O_2 (mg/L)	8.12	6.94	10.5
Median grain size (µm)	919	331	249
TOC (%) sediment	0.58	0.81	0.96

Table S4. Tolerable Daily Intake (TDI; ng/kg body weight/day) values for PFOS and PFOA and maximum edible amounts (g/d) of different fish species calculated for an average person weighting 70 kg. The worst case scenario is based on the highest concentrations measured in the fish tissue.

		Mean concentrations		Worst case scenario	
		PFOS	PFOA	PFOS	PFOA
TDI (ng/kg body weight/d)		30	20	30	20
TDI (ng/d) for a person of 70kg		2100	1400	2100	1400
Maximum edible amount of *C. carpio* per day (g/d) for a person of 70 kg	Barrage	0.01	0.95	0.01	0.71
	Thabela Thabeng	0.02	0.95	0.01	0.71
Maximum edible amount of *L. capensis* per day (g/d) for a person of 70 kg	Barrage	0.09	0.95	0.07	0.95
	Thabela Thabeng	0.05	-	0.04	-
	Fischgat	0.43	-	0.29	-
Maximum edible amount of *L. aeneus* per day (g/d) for a person of 70 kg	Barrage	0.03	-	0.02	-
	Thabela Thabeng	0.02	-	0.02	-
	Fischgat	0.29	1.43	0.19	0.95

Maximum edible amount of *C. gariepinus* per day (g/d) for a person of 70 kg	Thabela Thabeng	0.03	-	0.01	-
	Fischgat	0.36	0.95	0.33	0.95