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Reference:
Groffen Thimo, Rijnders Jet, van Doorn Loïc, Jorissen Cas, Mortier De Borger Seppe, Oude Luttikhuis Dorien, de Deyn Lara, Covaci Adrian, Bervoets Lieven. - Preliminary study on the distribution of metals and persistent organic pollutants (POPs), including perfluoroalkylated acids (PFAS), in the aquatic environment near Morogoro, Tanzania, and the potential health risks for humans
Environmental research - ISSN 0013-9351 - 192(2021), 110299
Full text (Publisher's DOI): https://doi.org/10.1016/J.ENVRES.2020.110299
To cite this reference: https://hdl.handle.net/10067/1723210151162165141
Preliminary study on the distribution of metals and persistent organic pollutants (POPs), including perfluoroalkylated acids (PFAS), in the aquatic environment near Morogoro, Tanzania, and the potential health risks for humans

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Abstract

Metals and persistent organic pollutants (POPs), including perfluoroalkylated acids (PFAS), are chemicals with a bioaccumulative potential that are detected in wildlife around the world. Although multiple studies reported the pollution of the aquatic environment with these chemicals, only limited data is present on the environmental pollution of Tanzania’s aquatic environment and the possible risks for human health through consumption of contaminated fish or invertebrates. In the present study, we examined the distribution of metals and POPs in fish, invertebrates, sediment and water, collected at two different years at multiple important water reservoirs for domestic and industrial purposes, in the aquatic environment near Morogoro, Tanzania. Furthermore, we assessed the possible risks for human health through consumption of contaminated fish and shrimp.

Metal concentrations in the water, sediment, invertebrates and fish appeared to increase in sites downstream from Morogoro city, likely caused by the presence of the city as pollution source. Significant differences in accumulated concentrations of metals and POPs were observed between species, which was hypothesized to be caused by dietary differences. Concentrations of multiple metals exceeded water and sediment quality guidelines values. Only Cu (2.8 – 17 μg/L) and Zn (<LOQ – 151 μg/L) in water exceeded chronic and acute effect values. Furthermore, PFOS, PBDE and HCB concentrations exceeded biota quality standard values, suggesting an ecological risk caused by these metals and POPs in the aquatic environment around Morogoro.

Our results suggest that potential health effects through consumption of contaminated shrimp, and to minor extent fish, are expected. The daily consumption of these proteins (0.016 – 0.027 kg/capita/day) in Tanzania is similar or higher than the tolerable maximum consumption of shrimp for Cu (< 0.02 kg/capita/day), Co (< 0.02 kg/capita/day) and PFOS (<0.01 kg/capita/day). The outcome of this study could be used in future studies on metals and POPs in African aquatic ecosystems.

**Keywords:** Perfluoroalkylated acids ; Tanzania; Fish; Invertebrates; Metals; Persistent Organic Pollutants (POPs)
Funding source

This work was funded by the Research Foundation Flanders (FWO project G038615N and G018119N).
1. Introduction

Human activities, including increasing industrial activities, have led to a global pollution of the environment with many pollutants. Especially since the last century, the development of organic chemical industries led to an increased production of a large number of anthropogenic chemicals, such as metals and persistent organic pollutants (POPs). Due to their global presence in nature, many of these pollutants have received worldwide scientific attention (e.g. Fernández and Grimalt, 2003; Jaspers et al., 2014).

Metals are chemicals that may end up in the environment from both natural and anthropogenic sources (Connell, 2005). They may be distributed by air (EMEP, 2015) and water (Khan et al., 2017) and can accumulate in both the abiotic and biotic compartments of the ecosystem (Mdegela et al., 2009). In the aquatic environment, some metals are adsorbed onto suspended matter and sediments, resulting in lower dissolved concentrations and bioavailability.

Persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs, including hexachlorobenzene (HCB), dichloro diphenyl trichloroethane (DDT) and chlordanes), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and perfluoroalkylated acids (PFAS) are halogenated chemicals that have bioaccumulation and biomagnification potential (Buck et al., 2011; Verhaert et al., 2017). They are often semi-volatile and can hence be transported over long ranges, resulting in contamination of sites far away from where they were used or produced (Polder et al., 2014). Many POPs are persistent and toxic chemicals that are globally distributed in wildlife and humans (Dominguez et al., 2010; Houde et al., 2006).

Sediments are the most important reservoir for metals and POPs in the aquatic environment (Covaci et al., 2005; Zrnčić et al., 2013). Sediment-bound pollutants may be released back into the water column after re-suspension and may hence pose a threat to ecosystems (Mataba et al., 2016; Wasi et al., 2013). Furthermore, these pollutants may enter the aquatic food chain through feeding of benthic and benthopelagic species (Mdegela et al., 2009) and may finally end up in humans through consumption of water (Pawelczyk, 2013) or aquatic organisms, such as fish (Christensen et al., 2017). This might result in negative health effects in biota (Demirak et al., 2006), including humans (Wasi et al., 2013), where they can cause several toxic effects (Ahmed et al., 2016; Christensen et al., 2016; Dalsager et al., 2016).

In Tanzania, several studies have been performed on metal pollution in the aquatic environment, but the majority of these studies only targeted sediment and invertebrates (e.g. Hellar-Kihampa et al., 2014; Mdegela et al., 2009; Rumisha et al., 2017). Studies on metal bioaccumulation in fish from Tanzania are
rather scarce (e.g. Mataba et al., 2016; Mdegela et al., 2009; Mwakalapa et al., 2019). Multiple studies have reported POPs in the Tanzanian environment, as a result of agricultural activities, malaria control and waste disposals (e.g. Müller et al., 2017; Mwakalapa et al., 2018; Polder et al., 2014). However, the majority of these studies on POPs remain restricted to the abiotic environment or do not test for correlations in concentrations between abiotic and biotic compartments of the food web. Due to the nutritional importance of fish and the public health concerns related to the pollution of these fish with metals and other chemicals such as POPs, there is a need to assess the potential human health risks of fish consumption (Mwakalapa et al., 2019).

In the present study, we examined the occurrence and distribution of POPs, including PCBs, PBDEs, DDXs, chlordanes, HCB, and PFAS, and metals in the aquatic environment in the Morogoro region, Tanzania. Additionally, we tested for potential risks for human health through consumption of contaminated fish and invertebrates. It was hypothesized that concentrations of these pollutants were 1) accumulated in the aquatic environment, 2) higher at downstream sites, compared to upstream sites, and 3) posing a potential risk for human health through consumption of contaminated fish and/or invertebrates.

2. Materials and methods

2.1. Study area, species and sample collection

As a study area, one dam and two rivers flowing through the city of Morogoro, Tanzania, were selected (Fig. 1) because of their importance as water reservoirs. They supply the majority of the domestic and industrial water requirements for Morogoro municipality (Ngonyani and Nkotagu, 2007). The Ngerengere River rises southwest of the city, in the Uluguru Mountains and runs through Mindu Dam and further along the western side of the city. The Kikundi River, which rises in the Uluguru Mountains south of the city, subsequently runs along the eastern side of Morogoro. At the northern edge of Morogoro, both rivers merge into the Tzugi River. These watercourses have changed at an accelerating rate due to farming and deforestation (Ashagre et al. 2014).
Two sampling campaigns, i.e. in June 2012 and June 2016, resulted in 12 sampling sites (Fig. 1): four on Mindu Dam (M1 – M4), three on the Ngerengere River (N1 – N3), three on the Kikundi River (K1 – K3) and two on the Tzugi River (T1 – T2). An overview of all collected water, sediment, invertebrate and fish samples at each location and each sampling campaign is displayed in Table A1.

Sediment samples (duplicates or triplicates; Table A1) were collected by pooling three separate sediment grab samples in 50 mL polypropylene (PP) falcon tubes for metal, POPs (only in 2012) and PFAS (only in 2016) analyses. Similarly, three separate grabs of water were pooled in a 50 mL PP tube, 1 L glass jar, or a 1 L polyethylene (PE) bottle, for metals, POPs and PFAS, respectively, but no replicates were collected for water. The general water characteristics, including temperature, conductivity, salinity, pH and dissolved oxygen were measured using a handheld multimeter (Hanna HI 9928) in 2012 (Table A2). In 2016, the conductivity (DIST by Hanna) and pH (pHep by Hanna) were assessed (Table A2).
At each sampling site, at least two groups of invertebrate taxa (when possible) were collected for pollutant assessment using an invertebrate net (mesh size 500 µm). Individuals of the following taxa were collected at one or more of the sites: snails (Gastropoda, in 2012 only from the Planorbidae family (ramshorn snails) and in 2016 a mixture of different families), shrimp (Palaemonidae), crabs (Brachyura), whirligig beetles (Gyrinidae), dragonfly larvae (Odonata), predaceous diving beetles (Dytiscidae), mosquito larvae (Chironomidae) and waterbugs (Hemiptera) (Table A1). Individuals were stored per site per taxon in 50 ml PP tubes. Subsamples (individuals) were taken, prior to homogenization, from these tubes for the analysis of metals (2012 and 2016) or PFAS (2016). Only in 2012, fish were collected, as fishing at Mindu Dam was prohibited in 2016. In 2012 at Mindu Dam (M1), five individuals of two species, i.e. Wami tilapia (*Oreochromis urolepis*) and African sharptooth catfish (*Clarias gariepinus*) were bought from local fishermen. Additionally, one mottled eel (*Anguilla bengalensis labiata*) was bought. These fish were already sacrificed by the fishermen. The length and weight of the tilapia and catfish were measured prior to dissection (Table A3). Of all the species, the gills (after removal of the gill arches), the entire liver and a piece of about 3 gram of axial muscle tissue, were collected and stored in 50 mL PP tubes. In the Ngerengere River, Kikundi River and Tzugi River, an invertebrate net was used to collect seven individuals of the redspot barb (*Barbus kerstenii*). Due to insufficient tissue for POPs and PFAS analysis in *B. kerstenii*, we only analyzed them for metals. Only the fish samples from Mindu Dam were analyzed for POPs and PFAS. All samples were transported to the laboratory on ice and stored at -20°C until further analysis. All samples were extracted and analyzed in the year of sampling (2012 or 2016).

### 2.2 Sample treatment and analysis

#### 2.2.1 Metals

The extraction procedure for metals followed the protocol described by Van Ginneken et al. (2019). A detailed description of the extraction protocols for water, sediment and biota is provided in the SI.

Trace elements in all samples were measured using an inductively coupled plasma mass spectrometer 7700x ICP-MS (Agilent Technologies, Santa Clara, CA, USA) and included silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb) and zinc (Zn). Limits of quantification (LOQs) of the metals were determined based on a signal-to-noise ratio of 10 and ranged between 0.1 and 50 µg/L. The LOQs of the trace elements are displayed in Tables A4, A5, 1, and A6 for water, sediment, invertebrates, and fish, respectively. The quality of the extraction protocol was assured by regular analysis of procedural blanks and certified reference soil (BCR-142R. No. 0682) and mussel tissue (SRM2976) of the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Concentrations in procedural blanks were
subtracted from those in the samples. The measured concentrations in the reference materials deviated less than 10% from the certified values.

2.2.2. POPs, excluding PFAS

The extraction method for POPs was based on the methods described by Voorspoels et al. (2003). A detailed protocol is provided in the SI.

An Agilent 6890-5973 gas chromatograph, coupled with a mass spectrometer (GC-MS), equipped with a capillary column (30 mm x 0.25 mm x 0.25 μm DB-5) was used to analyze the POPs. The GC-MS was operated in electron capture negative ionization (ECNI) mode and used in the selected ion-monitoring (SIM) mode for the analyses of PBDEs and chlordanes. For the detection of PCBs, DDXs and HCB, a GC-MS system was operated in electron ionization (EI) mode and equipped with a HT-8 capillary column (25 m x 0.22 mm x 0.25 μm). Target analytes included 34 PCBs (IUPAC numbers: CB 18, 28, 44, 47, 49, 52, 66, 74, 87, 95, 99, 101, 105, 110, 118, 128, 138, 146, 153, 156, 167, 170, 171, 172, 174, 177, 180, 183, 187, 194, 196/203, 199, 206 and 209), 6 PBDEs (IUPAC numbers BDE 28, 47, 99, 100, 153 and 154), 6 DDXs (o,p'-DDD, p,p'-DDE, o,p'-DDE, o,p'-DDT and p,p'-DDT), 5 chlordanes (TC, CC, CN, TN and OxC) and HCB. Procedural blanks and standard reference materials (SRM 1945 – PCBs, PBDEs and OCPs in whale blubber (NIST)) were included regularly in the analyses. The LOQs ranged between 5 and 30 pg/g ww for the POPs and concentrations above the LOQs in the blanks were subtracted from the concentrations in the samples. The measured concentrations in the reference materials deviated less than 15% from the certified values.

2.2.3. PFAS

The extraction protocol for PFAS in biota was based on a method described by Powley et al. (2005) with minor modifications. Water samples in 2012 were extracted according to a protocol described by Taniyasu et al. (2005). The extraction protocol for water and sediment in 2016 was based on Groffen et al. (2019). Detailed protocols of the different extraction procedures are provided in the SI.

The PFAS were analyzed by UPLC coupled ES(-) mass spectrometry (ACQUITY, TQD, Waters, Milford, MA, USA) using an ACQUITY BEH C18 column (2.1 x 50 mm; 1.7 μm, Waters, USA) to separate the analytes and an ACQUITY BEH C-18 pre-column (2.1 x 30 mm; 1.7 μm, Waters, USA), inserted between the solvent mixer and injector, to retain any PFAS contamination originating from the system. The analytes (4 perfluorosulfonic acids (PFSAs; C4, C6, C8 and C10) and 11 perfluorocarboxylic acids (C4 – C14)) were identified and quantified using multiple reaction monitoring (MRM). Diagnostic transitions and further details regarding instrumental settings are provided in Groffen et al. (2019). The quality was assured by a
regular analysis of procedural blanks and the use of sterilized fish muscle tissue (pike perch; *Stizostedion lucioperca*, QUASIMEME Laboratory Performance Studies (Van Leeuwen et al., 2011)) as reference material. The LOQs ranged between 0.01 and 0.71 ng/g ww for all PFAS, but could not be determined for perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS) and perfluoroundecanoic acid (PFUnDA) due to too low recoveries of the internal standards. The measured concentrations in the reference materials deviated less than 10% from the certified values.

2.3 Risks to human health

The risks to human health through consumption of contaminated fish and shrimp were calculated based on the minimum risk level (MRL). MRLs of most metals, PFOS, PFOA, HCB, PCBs, DDX and PBDEs (Table 3) were obtained from the ATSDR (2019). The maximum edible amount a person can eat without risking an adverse effect on its health was calculated with the following formula (Verhaert et al., 2017):

\[ Q = W \times \frac{M}{C} \times 1000 \]

With \( M \) = MRL for oral intake of the substance (ng/kg body weight/day), \( W \) = weight of a person (kg), \( Q \) (kg/d) = maximum amount of contaminated organisms that an average person can consume per day without risking health effects and \( C \) = observed concentrations of a substance in an organism (ng/g ww).

2.4 Statistical analyses

Statistical analyses were performed using R Studio (3.2.2). The level of significance was set at \( p \leq 0.05 \). Concentrations below the LOQ were substituted with a value of LOQ/2 (Bervoets et al., 2004). Normality assumptions of the used statistical models were examined using the Shapiro-Wilk test and running diagnostic plots. The data were log-transformed when needed to meet the normality assumptions of the residuals. Locations, species or tissues were excluded from the analyses when the detection frequency of a certain compound at that location or in that species or tissue was below 50%.

Two-way ANOVAs, followed by Tukey post-hoc analyses, were used to test for differences in metal concentrations among fish species and fish tissues for metals. Significant differences among locations, and among species for POPs were examined using either one-way ANOVA, followed by Tukey post-hoc analysis (for cases with more than two means), or a two-sample T-test (or non-parametric Wilcoxon Rank test in case of non-normality), in cases with only two means. For invertebrate species that were collected at only two locations (e.g. Gyrinidae in 2012), we used two-sample T-tests (or Wilcoxon Rank tests) to compare the concentrations between different taxa or between different locations. Spearman rank correlation
tests were used to test for correlations between metal concentrations in different fish tissues, and to test for correlations among the different environmental and biotic matrices. In the latter case, the sampling location, species or sampling year was not taken into account due to the unavailability of enough replicates at each location and sampling year, and hence to increase the size of the dataset for these tests.

3. Results and discussion

3.1. Metals

The metal concentrations in water and sediment are displayed in Table A4 and Figure 2, respectively. In 2012, Ag, As, Cd and Ni were not assessed, whereas in 2016 Mg and Na were not assessed. In addition, the concentrations of Ag and Cd were <LOQ in sediment in 2016 (Table A5). Therefore, these metals were excluded from Fig. 2. More details on sediment concentrations are presented in Table A5. As no repeated measurements were taken, we could not compare the concentration in water among sites statistically.

In 2012, the metal concentrations in the sediment from downstream sites (Tzugi River; T1) were in most cases significantly higher (p < 0.05) than those in Mindu Dam (M1 and M2) and the Ngerengere River (N1) (Fig. 2). Similarly, in 2016, the metal concentrations were often also higher (p < 0.05) in the Tzugi River (T2), compared to Mindu Dam (M4) and the Kikundi River (K2 and K3) (Fig. 2). However, no differences between the Tzugi River and Ngerengere River were observed in 2016.
The metal concentrations in the water and sediment appeared to be higher downstream of Mindu Dam in both years. In addition, in 2016 the concentrations in the Ngerengere River were often higher than those in the Kikundi River. These higher concentrations in the Ngerengere River and Tzugi River might be caused by anthropogenic activities along these rivers (e.g. caused by the city of Morogoro). However, the
sediment concentrations are similar to those reported by Mdegela et al. (2009) for the same area, who suggested that the metal concentrations in the sediment are mainly the result of natural background sources, such as weathering of rocks and aerial deposition, rather than anthropogenic sources. The sediment concentrations in the present study were often lower than those reported in the Pangani River Basin (Hellar-Kihampa et al., 2014). Compared to the Thigithe River, the concentrations were within the same range or lower in the present study (Mataba et al., 2016).

Metal concentrations in the invertebrates from 2012 and 2016 are displayed in Table 1. Similar to the metal concentrations in water and sediment, the concentrations in invertebrates appeared to be higher downstream of Mindu Dam in both years. In 2012, Gyrinidae from the Tzugi River (T1) contained significantly higher concentrations of Cu and Fe compared to the Ngerengere River (N1) (p < 0.05). In 2016, the metal concentrations in Odonata from Mindu Dam (M4) were often significantly lower than those in the other sites (p < 0.05). In addition, the concentrations of Al, Co, Fe, Mn and Ni were higher in Palaemonidae from the Ngerengere River (N2) compared to those from M4 (p < 0.05). Furthermore, the concentrations in the Ngerengere River were often higher in Odonata from the Ngerengere River, compared to the Kikundi River (p < 0.05), which was also in agreement with the previously reported concentrations in water and sediment. Again, this supports the hypothesis that the Ngerengere River and Tzugi River are also affected by anthropogenic activities along these rivers, which is likely caused by the presence of Morogoro city.
<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Taxa</th>
<th>Ag (N = 5)</th>
<th>Al (N = 5)</th>
<th>As (N = 5)</th>
<th>Cd (N = 5)</th>
<th>Co (N = 5)</th>
<th>Cr (N = 5)</th>
<th>Cu (N = 5)</th>
<th>Fe (N = 5)</th>
<th>Mg (N = 5)</th>
<th>Mn (N = 5)</th>
<th>Ni (N = 5)</th>
<th>Na (N = 5)</th>
<th>Pb (N = 5)</th>
<th>Zn (N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Planorbididae (N = 4)</td>
<td>NA</td>
<td>75 (41 – 102)</td>
<td>1.62 (0.98 – 2.17)</td>
<td>&lt;LOQ</td>
<td>24 (12 – 27)</td>
<td>809 (451 – 1318)</td>
<td>9204 (4771 – 14652)</td>
<td>459 (186 – 605)</td>
<td>NA</td>
<td>805 (322 – 1071)</td>
<td>NA</td>
<td>34 (12 – 52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>N1</td>
<td>Gyrinidae (N = 5)</td>
<td>NA</td>
<td>&lt;LOQ</td>
<td>NA</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>33 (29 – 42)</td>
<td>246 (225 – 339)</td>
<td>21 (13 – 46)</td>
<td>626 (593 – 664)</td>
<td>NA</td>
<td>23 (17 – 24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemiptera (N = 4)</td>
<td>NA</td>
<td>430 (68 – 694)</td>
<td>1.10 (0.28 – 3.22)</td>
<td>22 (20 – 30)</td>
<td>1008 (149 – 1427)</td>
<td>645 (400 – 961)</td>
<td>246 (27 – 366)</td>
<td>1409 (642 – 1682)</td>
<td>NA</td>
<td>38 (27 – 98)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K1</td>
<td>Hemiptera (N = 4)</td>
<td>NA</td>
<td>201 (157 – 1953)</td>
<td>0.28 (&lt;LOQ – 1.73)</td>
<td>17 (12 – 33)</td>
<td>1584 (429 – 3361)</td>
<td>493 (342 – 1635)</td>
<td>141 (68 – 1164)</td>
<td>1532 (1145 – 5411)</td>
<td>NA</td>
<td>69 (35 – 84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>Gyrinidae (N = 7)</td>
<td>NA</td>
<td>9.19 (4.00 – 31)</td>
<td>&lt;LOQ</td>
<td>0.17 (0.14 – 0.26)</td>
<td>9.22 (7.08 – 15)</td>
<td>52 (38 – 90)</td>
<td>273 (217 – 367)</td>
<td>39 (14 – 86)</td>
<td>NA</td>
<td>27 (18 – 40)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Odonata (N = 5)</td>
<td>NA</td>
<td>184 (78 – 317)</td>
<td>0.29 (&lt;LOQ – 0.33)</td>
<td>61 (45 – 87)</td>
<td>309 (208 – 522)</td>
<td>NA</td>
<td>151 (98 – 198)</td>
<td>0.27 (&lt;LOQ – 0.43)</td>
<td>NA</td>
<td>&lt;LOQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palaemonidae (N = 5)</td>
<td>0.14 (&lt;LOQ – 0.15)</td>
<td>2358 (2061 – 2888)</td>
<td>0.47 (0.45 – 0.49)</td>
<td>&lt;LOQ (&lt;LOQ – 0.11)</td>
<td>2.15 (1.97 – 2.44)</td>
<td>7479 (3684 – 4850)</td>
<td>NA</td>
<td>1686 (808 – 1819)</td>
<td>2.53 (1.97 – 2.97)</td>
<td>NA</td>
<td>0.81 (0.65 – 1.07)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Median metal concentrations and ranges (µg/g dw) in invertebrates collected at the different sampling sites in 2012 and 2016. NA = not assessed. Locations are abbreviated according to Fig. 1.
The concentrations of trace elements in the invertebrates from the Morogoro Rivers, were in most cases similar (or within the lower part of the range) to those reported in the Msimbazi River, where concentrations of 1.3 – 17 mg/kg dw, 5.2 – 160 mg/kg dw, 0.6 – 420 mg/kg dw, 6.1 – 4.5 mg/kg dw and 0.1 – 0.3 mg/kg dw were reported for Cr, Cu, Zn, Ni and Cd, respectively (Ak’habuhaya and Lodenius, 1988). On the other hand, the concentrations of Fe, Mn and Al were within the same range or much higher than those reported in the Msimbazi River (78 – 620 mg/kg Fe, 9.9 – 110 mg/kg Mn and 40 – 200 mg/kg Al in the Msimbazi River; Ak’habuhaya and Lodenius, 1988). Compared to the Kenyan part of Lake Victoria, the Cu, Zn and Ni concentrations in invertebrates collected in the present study were within the lower part of the range detected at Lake Victoria (6.45 – 148 mg/kg Cu, 49.7 – 1361 mg/kg Zn and 0.325 – 36.1 mg/kg Ni), and only the Cr concentrations were higher in invertebrates from the Morogoro Rivers (<LOQ – 20.1 mg/kg in the Morogoro Rivers, compared to 0.271 – 5.05 mg/kg in Lake Victoria)(Outa et al., 2020).

The significant differences that were observed in metal concentrations between different invertebrate taxa are likely explained by differences in feeding ecology and trophic level. At Mindu Dam, the concentrations in Palaemonidae (shrimp) were often significantly higher than those in Planorbidae (ramshorn snails) (at M1; p < 0.05) and Odonata species (at M4; p < 0.05). Palaemonidae are omnivorous or even carnivorous species, feeding largely on benthic invertebrates, such as oligochaetes and dipteran larvae (Carnevali et al., 2012), whereas Planorbidae occupy a lower trophic level, feeding primarily on detritus, epiphytic algae and decaying macrophytes (Madsen, 1992). Odonata larvae, on the other hand, are carnivorous (Merrill and Johnson, 1984) and should hence occupy a similar trophic level as Palaemonidae. However, the larval diets of Odonata could change depending on the species, time of the year, and developmental stage of the larvae (Blois, 1985), which might also explain why metal concentrations in Odonata at the Ngerengere River (N2) were higher than those in Palaemonidae (p < 0.05).

Metal concentrations in the fish, collected in 2012, are displayed in Table A6. Significant differences among locations were examined using the whole body metal concentrations of *B. kerstenii* (Fig. 3). Significantly higher concentrations of Al, Cr, Cu and Fe were observed at the Tzugi River (T1) compared to the Ngerengere (N1) and Kikundi (K1) Rivers (p < 0.05). Concentrations of Mn were higher at T1 than at K1 (p = 0.001) and the Na concentrations at K1 were higher than at N1 (p < 0.001). These higher concentrations at downstream sites, are in agreement with the sediment and water concentrations and can possibly be explained by the influence of Morogoro City.
Metal concentrations in the gills, liver and muscle tissues of *O. urolepis* and *C. gariepinus* are displayed in Fig. 4. As only one individual of *A. bengalensis labiata* was caught, this species was excluded from statistical analyses. For Al, Fe and Mn, significantly higher concentrations were observed in the livers of *O. urolepis* than in those of *C. gariepinus* (*p* < 0.05). Regarding the muscle tissue, the Al and Cr concentrations were also significantly higher in *O. urolepis* (*p* < 0.05). Finally, in the gills the Mg concentrations were higher in *C. gariepinus* compared to *O. urolepis* (*p* = 0.003). Differences between the species are likely the result of differences in exposure pathways and dietary sources, which might contain different accumulated concentrations of these trace elements (as has been suggested by numerous studies, e.g. Oberholster et al., 2012; Yousafzai et al., 2017).
Metal concentrations in the different fish tissues of *O. urolepis* (Fig. 4) differed significantly among tissues. For most metals, a general pattern was observed with concentrations in liver ≥ gill > muscle (*p* < 0.05). In *C. gariepinus* (Fig. 4) a similar pattern was observed for most metals (*p* < 0.05). However, the
concentrations of Al, Mg and Mn were significantly higher in the gills, followed by the liver and then the muscle tissue (p < 0.05) and the Na concentrations were significantly higher in the gills compared to the muscle (p < 0.001). With exception of a correlation between gill and muscle Na concentrations (ρ = 1, p = 0.017), no correlations were observed between the metal concentrations in the different tissues of *O. urolepis* (p > 0.05). In *C. gariepinus*, only the Al and Mn concentrations between gills and muscle were correlated (both ρ = 1, p = 0.017).

Although different fish organs accumulated varying quantities of different metals, our result show that the essential metals, such as Cu, Zn and Fe were mainly accumulated in the liver, rather than in the gills or muscle tissue. This is likely linked to the role of the liver in metabolism, as high concentrations of Zn and Cu are often related to metal binding proteins such as metallothioneins (Gorur et al., 2012). Similarly, Fe accumulates mainly in hepatic tissues due to the role of the liver in blood cells and hemoglobin synthesis (Gorur et al., 2012). Both species generally had higher concentrations of Mn, Na and Al in the gills than in the muscle. Due to the very large surface area of gills, facilitating the rapid diffusion of metals, gills act as a main route of metal ion exchange from water (Dhaneesh et al., 2012). Therefore, it is suggested that the accumulated metals in the gills are concentrated from the water. Similar patterns were also reported in other studies on a variety of fish species (El-Moselhy et al., 2014). Although initially we expected more correlations of metals among the different tissues, the lack of such correlations is likely the result of a relatively small dataset. Furthermore, the limited variation in metal concentrations among samples could also explain the absence of such correlations.

Metal concentrations in the different fish tissues (liver, muscle and gill) are compared with literature in Table A7. Concentrations in the muscle and liver from all fish species in the present study were higher than those reported in *Chanos chanos* and *Mugil cephalus* from the Tanzanian coasts (Mwakalapa et al., 2019). Compared to *Lutjanus fulvus*, *Rastrelliger kanagurta* and *Fenneropeanus indicus*, collected at the Tanzanian coasts, the Cu concentrations in the muscle were much lower in the present study, whereas the Fe concentrations were similar (Saria, 2016). Mataba et al. (2016) determined metal concentrations in muscle, liver and gills of *Laboe victorianus* from the Thigithe River. In muscle, the Co and Cr concentrations were lower than those detected in fish from Mindu Dam in the present study, but the Cu and Zn concentrations were much higher in the fish from the Thigithe River. Fish from the Thigithe River also contained higher Zn concentrations in the liver and higher Co concentrations in the gills (Mataba et al., 2016). Similar or slightly higher metal concentrations were reported in muscle tissue of *Siganus sutor*, *Lehrtrinus harak* and *Rastrelliger kanagurta* from Dar es Salaam compared to the fish collected at Mindu.
Dam, whereas the concentrations in the liver of the fish species collected at Dar es Salaam were often much higher than those reported in the present study (Mziray and Kimirei, 2016). The Cu and Zn concentrations in muscle tissue of *Lates niloticus* from Lake Victoria (Machiwa, 2005) were within the same range or higher than those reported for *A. bengalensis labiata*, *C. gariepinus* and *O. urolepis* from Mindu Dam in the present study.

Significant correlations were observed between metal concentrations in the water and sediment for Al, Cr, Fe, Mn and Ni (p < 0.05). Furthermore, the sediment Cr, Fe and Zn concentrations were correlated to those in the invertebrates (p < 0.05) and the concentrations of Cr were correlated between water and invertebrates (p = 0.037). No correlations were observed between the fish and any of the other matrices (p > 0.05).

### 3.2. POPs, including PFAS

Multiple PCBs, HCB, DDXs, PBDEs and PFAS were detected in the muscle tissue of fish collected at Mindu Dam in 2012 (Fig. 5, Table A8). Due to too low recoveries for the PFAS, the water samples in 2012 were excluded from further analyses. In addition, the POP concentrations measured in the individual *A. bengalensis labiata* were also excluded from further statistical analyses. HCB could only be detected in *A. bengalensis labiata* (13 pg/g dw) and in one individual of *O. urolepis* (13 pg/g dw). The ΣPCB, ΣDDX, ΣPBDE, PFOS, PFOA and PFDoDA concentrations did not differ significantly between the fish species (p > 0.05).

The mean HCB and ΣDDX concentrations in the present study were lower than those reported by Mdegela et al. (2009), who reported HCB concentrations of 200 ± 100 pg/g ww and 300 ± 200 pg/g ww, and ΣDDX concentrations of 3.7 ± 1.1 ng/g ww and 10 ± 5.4 ng/g ww in *O. urolepis* and *C. gariepinus*, respectively. Polder et al. (2014) reported HCB concentrations, that were similar to those reported in the present study, in different *Oreochromis* species from Lake Victoria, Lake Tanganyika, Lake Nyasa and Lake Babati (10 – 40 pg/g ww). Compared to this study by Polder et al. (2014), the ΣDDX concentrations in the present study were lower than those in *O. niloticus* from Lake Victoria (190 ng/g ww) and *Oreochromis* species from Lake Nyasa (260 ng/g ww). In the present study, the p,p’-DDE metabolite was the dominant contributor to the ΣDDX concentrations, which is in agreement with previous studies conducted in the aquatic or marine environment of Tanzania and indicates an historical contamination rather than a fresh input of DDT (Mdegela et al., 2009; Polder et al., 2014). Finally, the PCB concentrations were also lower than those detected in *Oreochromis sp.* from Lake Nyasa (20 ng/g ww) (Polder et al., 2014). The HCB, ΣPCB, and ΣDDX
concentrations in the present study were often similar to lower than those reported in other African studies (Govaerts et al., 2018; Verhaert et al., 2017; Wepener et al., 2011).

Figure 5. Mean concentrations and standard deviations (pg/g ww) of POPs in fish muscle tissue collected in 2012. N = 3 for C. gariepinus and O. urolepis, N = 1 for A. bengalensis labiata.

To the best of our knowledge, this is the first study that reports PFAS concentrations in the freshwater environment in Tanzania. Mwakalapa et al. (2018), investigated the presence of PFAS in muscle tissue of farmed and wild marine fish in Tanzania, but found no PFAS concentrations above the detection limit (LOD). Therefore, a comparison was made with other African studies, which were mainly conducted in South Africa. The PFOS concentrations in the present study were comparable to those in the Olifants River Basin (140 – 2700 pg/g ww; Verhaert et al., 2017) and lower than those reported in the Vaal River (1000 – 29000 pg/g ww, Groffen et al., 2018). On the other hand, the PFOA concentrations at Mindu Dam in the present study were higher than those in the Olifants River Basin (<LOQ – 420 pg/g ww; Verhaert et al., 2017) and Vaal River (470 – 1190 pg/g ww Groffen et al., 2018). These results suggest differences in the pollution source between the South African Rivers and Mindu Dam in Tanzania.

Surprisingly, no significant differences in POP concentrations were observed between the different fish species in the present study. Bioaccumulation of some POPs has been reported to be higher in benthic
species compared to benthopelagic and pelagic fish species (Mdegela et al., 2009). As *C. gariepinus* is a benthic species, it was expected that the POP concentrations would have been higher in *C. gariepinus* compared to the benthopelagic *O. urolepis*. The concentrations of OCPs seem to decrease over time, which is most likely the result of the ban of these chemicals in Tanzania, which was a follow-up of the Stockholm Convention on the use of POPs in the late 1990s (Mdegela et al., 2009). In addition, it was already reported that HCB, commonly used as fungicide for seed treatment, was no longer registered for use in Tanzania (Mdegela et al., 2009). Therefore, the detected HCB concentrations are likely the result of historical contamination or they might originate from other sources, including dyes, solvents or plastics.

In 2016, recoveries of PFBS, PFHxS and PFUnDA were too low to quantify these compounds in any of the samples. As PFAS were analyzed in single samples (N = 1 for water, sediment and invertebrates, which were pooled in order to obtain sufficient materials), when statistical results are reported, sampling location and/or species were not taken into account in those statistical analyses.

In water, only PFOA was detected (Table A9), with the highest concentrations in the Ngerengere River, followed by the Kikundi River and Mindu Dam. The PFOA concentrations in the surface water in the present study were comparable to those reported by Mudumbi et al. (2014a) in the Diep River (314 ng/L), Salt River (390 ng/L) and Eerste River (136 ng/L) in South Africa, but were higher than those reported in the Vaal River (0.7 – 4.2 ng/L; Groffen et al., 2018) and Olifants River Basin (<LOQ; Verhaert et al., 2017). Multiple PFAS were detected in the sediment, but only PFBA and PFOA were detected at all locations (Fig. A1). The sediment PFOA concentrations were lower than those reported for the Salt River, Diep River and Eerste River, where, respectively, concentrations up to 187 ng/g, 772 ng/g and 193 ng/g were detected (Mudumbi et al., 2014b).

As different invertebrate species were collected from the sampling locations, an average of the PFAS concentrations (which are reported in Table 2) in all invertebrate samples collected at a certain site was used to test for differences among locations (which was only possible for N2, N3 and T1). We observed no significant difference in PFOA concentrations between the Ngerengere River sites (N2 & N3) and the Tzugi River (T1) (p > 0.05).
Table 2. Concentrations of PFAS (pg/g ww) in invertebrates collected in 2016 at the different sampling sites. Sampling locations are abbreviated according to Fig. 1. Analytes that were <LOQ in all samples were excluded from the Table. All samples (N = 1) contained pools of individuals of the same taxa in order to obtain sufficient materials for extraction.

<table>
<thead>
<tr>
<th>Location</th>
<th>Taxa</th>
<th>PFBA</th>
<th>PFPeA</th>
<th>PFHpA</th>
<th>PFOA</th>
<th>PFDA</th>
<th>PFDoDA</th>
<th>PFOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Palaemonidae</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>71</td>
<td>&lt;LOQ</td>
<td>72</td>
<td>14925</td>
</tr>
<tr>
<td>N2</td>
<td>Gastropoda</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>323</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>N3</td>
<td>Palaemonidae</td>
<td>&lt;LOQ</td>
<td>752</td>
<td>&lt;LOQ</td>
<td>88</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>16905</td>
</tr>
<tr>
<td>N3</td>
<td>Odonata</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>3654</td>
<td>180</td>
<td>&lt;LOQ</td>
<td>204</td>
<td>4133</td>
</tr>
<tr>
<td>N3</td>
<td>Brachyura</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>16683</td>
<td>489</td>
<td>&lt;LOQ</td>
<td>451</td>
<td>320</td>
</tr>
<tr>
<td>N3</td>
<td>Dytiscidae</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>4927</td>
<td>213</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>2965</td>
</tr>
<tr>
<td>N3</td>
<td>Palaemonidae</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>3023</td>
<td>374</td>
<td>&lt;LOQ</td>
<td>160</td>
<td>1119</td>
</tr>
<tr>
<td>K2</td>
<td>Odonata</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>3855</td>
<td>1328</td>
<td>&lt;LOQ</td>
<td>243</td>
<td>642</td>
</tr>
<tr>
<td>K3</td>
<td>Odonata</td>
<td>581</td>
<td>7423</td>
<td>7103</td>
<td>368</td>
<td>&lt;LOQ</td>
<td>78</td>
<td>8448</td>
</tr>
<tr>
<td>T1</td>
<td>Gyrinidae</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>340</td>
<td>&lt;LOQ</td>
<td>102</td>
<td>636</td>
</tr>
<tr>
<td>T1</td>
<td>Chironomidae</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>2822</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
</tbody>
</table>

The PFOS concentrations detected in larvae of Odonata were within the lower half of the range of PFOS concentrations detected in adult Odonata (150 – 16000 pg/g ww) from selected sites in central and northern South Africa (Lesch et al., 2017). Furthermore, the PFOA concentrations in Odonata larvae from these sites in South Africa (<LOQ – 890 pg/g ww; Lesch et al., 2017) were similar to those reported in the Ngerengere River (N3) and Kikundi River (K3), although slightly higher PFOA concentrations were observed in Odonata larvae from K2. Nonetheless, this comparison should be interpreted with caution, as Odonata larvae occupy the aquatic environment whereas adults occupy the terrestrial environment and hence might be exposed to different sources.

The concentrations of PFOA and PFDoDA in Gyrinidae from the Tzugi River were comparable to those reported in Gyrinidae from Thabela Thabeng (300 pg/g ww and 100 pg/g ww, for PFOA and PFDoDA, respectively), a site in the Vaal River, South Africa (Groffen et al., 2018). However, the PFOS concentrations in Gyrinidae from the Vaal River (1990 pg/g ww; Groffen et al., 2018) were three times higher than those in the Tzugi River. The PFOA and PFDoDA concentrations in shrimp (Palaemonidae) from the aquatic environment around Morogoro were also similar to those reported for shrimp (Atyidae) from the Vaal River (200 – 400 pg/g ww and 100 – 200 pg/g ww for PFOA and PFDoDA, respectively) whereas PFOS concentrations in the Vaal River shrimp (5500 – 35500 pg/g ww) were higher (Groffen et al., 2018).

3.3. Risks to human health
The maximum amount of contaminated shrimp and fish that a person of 70 kg can consume per day (q-value), without any health risks is displayed in Table 3 for the different sampling sites. The q-values were calculated based on mean concentrations of the compounds. In case of *A. bengalensis labiata*, only one individual was bought from the fishermen, and therefore the q-values were based on the concentrations detected in that one individual. In general, the q-values for PFAS were lower than for the other pollutants.

In fish from Mindu Dam, the q-values for *A. bengalensis labiata* were lowest for PFOS, followed by PFOA. Although the q-values for the PFAS were slightly higher in the other fish species, they were still lower compared to the metals and other POPs. In shrimp, the lowest q-values were reported for PFOS and Cu at Mindu Dam (M4) and for Cu and Co in the Ngerengere River (N2).

Table 3. Amount of shrimp (at locations M1, M4 and N2) or fish (from Mindu Dam) a person of 70kg can eat a day, presented as a q-value (kg/day), before adverse health effects may occur. Q-values are calculated based on mean concentrations of the compounds. Only one individual of *A. bengalensis* was used in this analysis. NA = not assessed. Values in bold are q-values below 0.1 kg/day and might potentially cause health risks.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRL (ng/kg/day)</th>
<th>Shrimp</th>
<th>q (kg/day)</th>
<th>Fish</th>
<th>Clarias gariepinus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M1</td>
<td>M4</td>
<td>N2</td>
<td><em>Anguilla bengalensis labiata</em></td>
</tr>
<tr>
<td>Al</td>
<td>1,000,000</td>
<td>1.21</td>
<td>0.35</td>
<td>0.11</td>
<td>5.00</td>
</tr>
<tr>
<td>As</td>
<td>5,000</td>
<td>NA</td>
<td>0.65</td>
<td>0.90</td>
<td>NA</td>
</tr>
<tr>
<td>Cd</td>
<td>500</td>
<td>NA</td>
<td>1.17</td>
<td>0.88</td>
<td>NA</td>
</tr>
<tr>
<td>Co</td>
<td>100</td>
<td>&lt;LOQ</td>
<td>0.02</td>
<td>0.01</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Cu</td>
<td>10,000</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.47</td>
</tr>
<tr>
<td>Cr</td>
<td>5,000</td>
<td>3.5</td>
<td>0.29</td>
<td>0.18</td>
<td>3.50</td>
</tr>
<tr>
<td>Zn</td>
<td>300,000</td>
<td>0.66</td>
<td>0.23</td>
<td>0.24</td>
<td>0.50</td>
</tr>
<tr>
<td>PFOA</td>
<td>3</td>
<td>NA</td>
<td>2.39</td>
<td>NA</td>
<td><strong>0.10</strong></td>
</tr>
<tr>
<td>PFOS</td>
<td>2</td>
<td>NA</td>
<td>&lt;0.01</td>
<td>NA</td>
<td><strong>0.06</strong></td>
</tr>
<tr>
<td>PCBs</td>
<td>30</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>16</td>
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<tr>
<td>DDX</td>
<td>500</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>53</td>
</tr>
<tr>
<td>PBDEs</td>
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<td>NA</td>
<td>NA</td>
<td>280</td>
</tr>
<tr>
<td>HCB</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>24348</td>
</tr>
</tbody>
</table>

The average fish consumption in Tanzania was estimated on 5.9 – 9.9 kg/capita/year, which comes down to approximately 0.016 – 0.027 kg/capita/day (Desiere et al., 2018). Mdegela et al. (2009) estimated that the amount of shrimp consumed per day was equal to the amount of fish. Based on the q-values in Table 3, a potential health risk caused by Co, Cu and PFOS is expected at Mindu Dam, through consumption of contaminated shrimp. In addition, the Co and Cu concentrations in shrimp from the Ngerengere River might cause potential health problems. Although no risks for human health are expected through
consumption of contaminated fish at Mindu Dam, there might be a potential health risk caused by PFOS and PFOA in humans that rely heavily on fish consumption, such as local fishermen. Furthermore, the q-values reported in Table 3 were calculated based on muscle tissue, which is consumed in most countries. However, in some countries, fish liver is also consumed (D’Hollander et al., 2010). As livers often have higher concentrations of various pollutants, this might also cause risks to human health.

3.4 Ecological Risks and recommendations

Sediment and water quality control regulations in aquatic ecosystems vary considerably within and between countries and locations. As Tanzania lacks its own quality standards (QS) for metals, the measured concentrations in water and sediment were compared with other guidelines (Tables A4 for water, Table A5 for sediment). We observed an exceedance of the water QS (Table A4) for Al, Cr, Cu and Zn in almost all locations sampled in 2016 and an exceedance of the QS for Mn in 2012, indicating that there might be an ecological risk for the aquatic environment at these sites. This risk might be even more pronounced for Cu and Zn, for which water concentrations sometimes exceed chronic and acute effect values set by South Africa’s target water quality range for aquatic ecosystems (TWQR; DWAF, 1996) and the United States Environmental Protection Agency (USEPA, 2009). Furthermore, the Cr and Zn concentrations in the sediment from the Tzugi River and Ngerengere River also exceed some of the sediment quality standards (Table A5), which further suggests that there might be an ecological risk caused by these metals. The concentrations of PFOS in fish from Mindu Dam, and in Palaemonidae from Mindu Dam and the Ngerengere River exceeded the biota quality standards set by the EU (9.1 µg/kg ww; EU, 2013). Furthermore, the PBDE and HCB concentrations in fish from Mindu Dam also exceeded these EU guidelines (0.0085 µg/kg ww for PBDEs and 10 µg/kg ww for HCB; EU, 2013), indicating that there might be an ecological risk regarding the POP contamination in biota at Mindu Dam.

3.5 Limitations of the study

It should be stated that due to the amount of non-detects, and difficulties and restrictions during our sampling efforts, the datasets used in the statistical analyses were sometimes rather small. Therefore, statistical results should be interpreted with caution. Furthermore, due to the lack of earlier research in this area, we did not know what concentrations of the pollutants to expect. Hence, we did not know whether we could measure these concentrations. Therefore, especially regarding the fish, we only sampled a limited number of individuals to minimize our impact on the fish communities.

4. Conclusion
Metals and POPs have been detected in both the abiotic as well as the biotic compartments of the aquatic environment of Mindu Dam, the Ngerengere River, Kikundi River and Tzugi River. Concentrations of metals and POPs in these compartments were in general comparable to other studies conducted in the Tanzanian or African aquatic environment. Metal concentrations appeared to increase in the downstream sites, which is likely the results of multiple pollution sources along the rivers, including the city of Morogoro. For PFAS, however, we did not observe such decrease, which is most likely the result of the relatively small dataset. The detected concentrations of metals and POPs likely pose an ecological risk to the aquatic environment, as ecological guideline values are exceeded for some metals and POPs. In addition, our results suggest that potential health effects through consumption of contaminated shrimp, and to minor extent fish, are expected, as the daily consumption in Tanzania is similar or higher than the tolerable maximum consumption calculated in the present study. The outcome of this study are preliminary results and could be used in future monitoring studies on metals and POPs in African aquatic ecosystems.

5. Acknowledgements

This work was funded by the Research Foundation Flanders (FWO project G038615N and G018119N). The authors declare no conflicts of interest. We would like to express our gratitude to the Sokoine University of Morogoro, for the possibility to conduct a major part of our research in their laboratory. In addition, we would like to thank Jackson Msalilwa, Arne Iserbyt, Tim Kouba and Tim Lieben for their help collecting the samples. Finally, we wish to thank Wendy D’Hollander, Vera Verhaert, Steven Joosen and Tim Willems for their assistance regarding laboratory work and chemical analyses.

6. References


