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Distribution and bioaccumulation of POPs and mercury in the Ga-Selati River (South Africa) and the rivers Gudbrandsdalslågen and Rena (Norway)

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Abstract

Biomagnification of Hg and persistent organic pollutants (POPs: polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs)) in aquatic food chains can lead to high pollutant concentrations in top predators, including humans. Despite this threat to human health, research concerning bioaccumulation is still underrepresented in the southern hemisphere and in (sub)arctic and (sub)tropical areas, emphasizing the need for research in these locations. In this study, samples of water, sediment and aquatic biota were analyzed to determine concentrations of POPs and total mercury (THg) in the Ga-Selati river (South Africa) and two rivers Rena and Gudbrandsdalslågen in Norway. Trophic magnification factors (TMFs) were determined to evaluate and compare the biomagnification and the threat to human health due to consumption of the fish was assessed.

Concentrations of POPs in sediment and biota samples were generally low except for relatively high concentrations of \sum DDX (dichlorodiphenyltrichloroethane and metabolites) in aquatic biota from the Ga-Selati river (ranging from 1.9 to 133 ng/g ww in invertebrates and 1.9 to 5643 ng/g ww in fish). Dissolved THg concentrations were high in the Ga-Selati river (ranging from 0.009 to 0.036 μ g/l) but THg concentrations in sediment and biota were low in studied rivers compared to other studies. Biomagnification occurred for THg, several DDT-metabolites and PCB compounds, TN and CN. Biomagnification of *p,p'*-DDT and THg differed significantly between the two countries, supporting existing patterns found in literature, although more data is needed to attribute these differences to climatic or other factors. Concentrations in fish from the rivers Ga-Selati and Rena were under the threshold levels reported for THg and POPs, but caution should be taken when consuming Northern pike (*Esox Lucius*) from the subarctic river Gudbrandsdalslågen, to avoid harmful effects due to both elevated THg and PBDE exposure.

Keywords: Persistent Organic Pollutants, Hg, Bioaccumulation, Trophic Magnification Factors, South Africa, Norway

1. Introduction

The release of Persistent Organic Pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and mercury (Hg), into the environment is of great concern due to their toxicity, their ability to accumulate in organisms and their persistence (Jones and de Voogt, 1999; Lavoie et al., 2013). Both POPs and mercury have been shown to biomagnify across the food chain, leading to high concentrations and potential effects in top

51 predators, including humans. (El-Shahawi et al., 2010; Borgå et al., 2012; Lavoie et al., 2013). Despite
52 the urgent need for understanding the impact and global patterns of bioaccumulation in aquatic food
53 webs, the majority of studies on bioaccumulation of POPs and Hg have been conducted in temperate
54 regions, and in the case of POPs predominantly in the northern hemisphere (Lavoie et al., 2013, Walters
55 et al., 2016). This emphasizes the need to investigate bioaccumulation in underrepresented areas, such
56 as countries in the southern hemisphere and (sub)tropical regions. To properly investigate
57 bioaccumulation across different aquatic systems, Trophic Magnification Factors (TMFs) are used.
58 TMFs represent the average prey to predator transfer of pollutants through food webs and have proven
59 to be a reliable and conclusive tool to quantify biomagnification (Walters et al., 2016).

60 The present study investigates bioaccumulation in river ecosystems from two different hemispheres:
61 the Ga-Selati River located in South Africa and the rivers Gudbrandsdalslågen (further on referred to as
62 Laagen) and Rena located in Norway. The Ga-Selati river is heavily impacted by agricultural activities,
63 local communities and a mining activity (King, 2008). Furthermore, although the use of DDT has been
64 banned in most parts of the world (NPIC, 1999; PAN UK, 2008), it is permitted to produce and use DDT
65 in countries where the transmission of malaria and visceral leishmaniasis can be efficiently prevented
66 by the use of DDT, in accordance with WHO (World Health Organization) recommendations and
67 guidelines (van den Berg, 2008). Several African countries, including South Africa, still regularly use
68 DDT. In Norway, the river Laagen flows in the Gudbrandsdalen valley and is influenced by surrounding
69 villages and farms. Few people live in the drainage basin of the Rena river, so the latter is regarded as
70 relatively little impacted by human activities (NINA et al., 2000). Because fish is an important food
71 source for local people, especially around the Ga-Selati river, it is important to monitor the
72 bioaccumulation of pollutants in the fish to assess whether they form a threat for human health.

73 The specific aims of this study are to (1) assess and compare POP and total mercury (THg)
74 concentrations in water, sediment and aquatic biota originating from the Ga-Selati river and the rivers
75 Laagen and Rena (2) examine the degree of biomagnification of the analyzed POPs and THg in the
76 aquatic biota using TMFs, and (3) assess the potential human health risk due to consumption of
77 contaminated fish from this study using Minimum Risk Levels and converting this to a maximum edible
78 amount per day.

80 2. Materials and Methods

82 2.1. Study Area

83 The Ga-Selati River is located in the northeastern province of Limpopo in South Africa and merges
84 with the larger Olifants river at the boundary of the Kruger National Park, near the Phalaborwa mine
85 (Chapman, 2006) (**Figure 1**). Extensive farming takes place along the river and several rural
86 communities, commercial game farms and nature reserves depend on the water flows of the river for
87 irrigation. At the confluence of the Ga-Selati River with the Olifants River, mining activity is very
88 intense with the Palabora Mining Company extracting copper and other by-products, and Foskor, one
89 of the world's largest producers of phosphate and phosphoric acid, as key players (King, 2008). Samples
90 were collected at three locations along the river (**Figure 1**). The first one, Harmonie, is situated in an
91 agricultural area. The second sampling site, Namakgale, is located near the low-income area of
92 Namakgale and the third collection of samples was at Lepelle Bridge (LB), a bridge that is situated in
93 the vicinity of a fertilizer factory and a phosphate mine. The climate of the Ga-Selati River catchment
94 is sub-tropical, with mean temperatures of 25.8°C in the summer and 18.0°C in the winter, and an annual
95 rainfall of 450-600mm (Chapman, 2006).

96 Sampling in Norway was carried out in two of the larger river systems of the country:
97 Gudbrandsdalslågen (Laagen) and Rena. The river Laagen flows through the Gudbrandsdalen valley in
98 the southeast of Norway, and runs about 250 km south through glacially sculptured rural valleys before
99 merging into Lake Mjøsa, the largest lake in Norway (NINA et al., 2000). Several villages and farms
100 are found in the valley. Few people live in the drainage area of Rena, and only minor agricultural areas
101 occur in the vicinity of the river. Both rivers encounter a continental climate in contrast to coastal rivers
102 in south Norway, and are located in an area characterized by a subarctic climate with high precipitation
103 and cold temperatures, with an average annual rainfall of 719mm and mean temperatures of 13.8°C in
104 the summer and -8.1°C in the winter (Yr, 2017).

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2.2. Sample collection

Samples of sediment, water, fish and invertebrates were collected at each location in South Africa between the 17th and 19th of September 2014 and during the first week of November 2014 in Norway.

Water samples were taken in triplicates by manually filling sterile 50 ml polypropylene (PP) tubes at about 10 cm below water surface at all locations. Three sediment samples were collected at each location along the Ga-Selati river and later pooled to obtain one mixed sample per location. Sediment was scooped from the side of the river at Namakgale and Lepelle Bridge and a Van Veen Grab sampler was used at Harmonie to collect the sediment samples onboard a boat. Sediments in Norway were collected with the invertebrate catching net from at least three points down to 1.5 m deep in both rivers. Gomphidae (Odonata) larvae were collected by hand and stored in 5 ml PP tubes at the riverbank of the Ga-Selati river, with an average of 10 individuals per site. In Norway, snails (Lymnaea sp.) were collected by net-sweeps just above the bottom of the river and by manually selecting them among sediments and plants with a metal pincher. Since snails were not found in the Rena River, they were sampled 400 m up the river mouth in lake Løpsjøen, where the river runs through. In South Africa eight fish species were caught: African sharptooth catfish (*Clarias gariepinus*), leaden labeo (*Labeo molybdinus*), plain squeaker (*Synodontis zambeziensis*), redbreast tilapia (*Tilapia rendalli*), Mozambique tilapia (*Tilapia mossambicus*), sawfin suckermouth (*Chiloglanis paratus*), river goby (*Glossogobius callidus*) and threespot barb (*Barbus trimaculatus*). The fish were caught and dissected the same day. The tissues were stored in 50 ml PP tubes and kept with the other samples in a liquid nitrogen tank before being stored in freezers at -20°C in the lab in Belgium. In Norway, five species of fish were collected: Brown trout (*Salmo trutta*), Eurasian ruffe (*Gymnocephalus cernuus*), common minnow (*Phoxinus phoxinus*), alpine bullhead (*Cottus poecilopus*) and the piscivorous Northern pike (*Esox Lucius*). All species were present in the river Laagen, but only the brown trout and the Northern pike were present in the Rena river. All water, sediment and biota samples were stored in a liquid nitrogen tank during field work before being stored in freezers at -20°C in the lab in Belgium.

2.3. POPs

2.3.1. Sample preparation

Samples were extracted following the protocol described in Verhaert et al. (2013). Concerning aquatic biota samples, 29 South African fish and 24 Norwegian fish were used as well as pooled samples of invertebrates for each site in both Norway and South Africa. Accurate amounts of fish (0.4 – 3.4 g wet weight (ww)), invertebrate samples (2.5 - 4.2 g ww) and sediment samples (3.1 – 3.2 g) were extracted using an automated hot Soxhlet extractor as described by Verhaert et al. (2013) (see SII).

2.3.2. Analysis

The following persistent organic pollutants were targeted: PCB congeners (IUPAC numbers 28, 49, 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 151, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 195, 196/203, 199, 206 and 209), dichlorodiphenyltrichloroethane (*p,p'*-DDT and *o,p'*-DDT) and metabolites (*p,p'*-DDD, *p,p'*-DDE, *o,p'*-DDE and *o,p'*-DDD), β - and γ -HCH (hexachloro-cyclohexane) isomers, chlordane metabolites (oxychlordane (OxC), cis-nonachlor (CN) and trans-nonachlor (TN)), hexachloro-benzene (HCB), and PBDEs (BDE 28, 47, 99, 100, 153, 154, and 183). PBDEs, CHLs, and higher PCBs were measured with an Agilent 6890-5973 gas chromatograph coupled with a mass spectrometer system (GC-MS). The GC was equipped with a 30 m x 0.25 mm x 0.25 μ m DB-5ms capillary column (J&W Scientific, Folsom, CA, USA) and the MS was operated in electron capture negative ionisation (ECNI) mode and used in the selected ion-monitoring (SIM) mode. For measuring the levels of lower chlorinated PCBs, DDXs (different DDT compounds), and HCB, an Agilent 6890 GC – 5973 MS system operated in electron ionisation (EI) mode equipped with a 25 m x 0.22 mm x 0.25 μ m HT-8 capillary column was used in the selected ion-monitoring (SIM) mode with 2 ions monitored for each PCB homologue group or individual OCP.

Procedural blanks were included for quality assurance and quality control. The limit of quantification (LOQ) was calculated as three times the standard deviation of the mean of the blank measurements. The analytical procedures were validated through the analysis of certified material SRM 1945 (PCBs,

159 PBDEs and OCPs in whale blubber) for which deviations from certified values were less than 10%. The
160 QC scheme is assessed through participation in the inter-laboratory Comparison Exercise Program for
161 Organic Contaminants in Marine Mammal Tissues, which is organized by the National Institute of
162 Standards and Technology (NIST, USA).

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164 2.4. Mercury

165

166 2.4.1 Sample preparation

167 A 0.5 g wet weight (ww) of sediment and biota samples was accurately weighed using a Mettler
168 AT261 DeltaRange® sensitive balance. The samples were then freeze dried at -55°C for 48 hours to a
169 constant weight using a freeze drier (Heto PowerDry® LL3000), and weighed again to obtain the dry
170 weight (dw). Samples were digested as described by Mataba et al. (2016) (see SI2). After digestion,
171 samples were stored in a freezer (-20°C) until analysis. Reference materials and blanks were handled in
172 a similar manner to ensure quality control. Lyophilized Cod Muscle (BCR 422) from the Institute for
173 Reference Materials and Measurements (IRMM, Geel Belgium), freeze dried blue mussel tissue (no
174 2976) from NIST and channel sediment BCR 320-R were used as reference materials to verify for
175 recoveries. Prior to analysis, all sediment and biota samples were thawed and diluted 2 times with Milli-
176 Q water.

177 To determine the dissolved THg concentrations, 10 ml of each water sample was filtered through a
178 filter with a pore size of 0.2 µm and subsequently acidified with 150 µl HNO₃ (67%). In total, three
179 replicas were prepared per site. Three blanks were used as control, carrying out the same procedure but
180 utilizing MilliQ water. Samples were then stored at -20°C until analysis.

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182 2.4.2. Analysis

183 Analysis of total mercury (THg) was carried out in cold plasma mode (to prevent the Hg to become
184 volatile) by High Resolution Inductively Coupled Mass Spectrometry (HR-ICP-MS) (Thermo scientific
185 Finnigan element 2, Waltham, MA, USA) with a detection limit of 0.001 µg/L. Obtained concentrations
186 agreed well with the certified values, with a mean recovery of 112%.

187

188 2.5. Total Organic Carbon (TOC)

189 Sediment samples were pooled per location and approximately 10 g of sediment of each site was
190 transferred to pre-weighted crucibles and subsequently weighed. The samples were then placed in a
191 muffle oven at 150°C for 24 hours and thereafter allowed to cool down to room temperature. The filled
192 crucibles were then weighed again, placed in the muffle oven and heated up to 550°C in a span of an
193 hour, after which they were kept at this temperature for another 4 hours. After the samples were allowed
194 to cool down to room temperature, they were weighed to obtain the final dry weight. The organic content
195 is inferred through loss of ignition (LOI) from the weight difference between the wet and dry state of
196 the sample as described by Heiri et al., (2001) and the total amount of organic carbon was calculated as
197 described by Nelson and Sommers (1996) and Schumacher (2002) (see SI3).

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199 2.6. Stable Isotopes

200 To assess the isotopic composition, a small amount of each sample was transferred to an Eppendorf
201 tube (2 mL), freeze-dried, and homogenized. The fish and snail samples were encapsulated in pre-
202 weighted 5 x 8 mm Sn capsules and weighted with an accuracy of three decimal places with a Mettler
203 AT261 scale. A similar procedure was used for the sediment and gomphid samples, with the exception
204 of the samples being encapsulated in Ag cups instead of Sn cups, since HCl must be added to samples
205 that contain sediment or invertebrates with an exoskeleton in order to remove traces of non-dietary
206 carbonates (Verhaert et al., 2013). C and N concentrations were determined at the Department of Earth
207 and Environmental Sciences, KU Leuven (Belgium) using a Thermo Flash HT/EA coupled to a Thermo
208 DeltaV Advantage IRMS with a ConFlo IV interface. Isotopic composition is expressed using following
209 formula:

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$$211 \delta^{13}\text{C}, \delta^{15}\text{N} = [(R_{\text{sample}}/ R_{\text{reference}}) - 1] \times 1000$$

212

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

214 Data were calibrated by using a combination of IAEA-C6, IAEA-N1 and acetanilide, which had been
215 calibrated in the lab for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. To relate the pollutant concentration to the trophic level (TL)
216 of the fish, the TL was calculated for all fish and invertebrates using following formula (Borgå et al.,
217 2012):

$$218 \\ 219 \text{TL}_{\text{consumer}} = ((\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{primary consumer}}) / \Delta^{15}\text{N}) + 2$$

220
221 with $\delta^{15}\text{N}_{\text{consumer}} = {}^{15}\text{N}$ concentration of the consumer, $\delta^{15}\text{N}_{\text{primary consumer}} = {}^{15}\text{N}$ concentration of
222 the primary consumer, $\Delta^{15}\text{N}$ = fractionation of ${}^{15}\text{N}$ into predator which has a value of 3‰ because the
223 abundance of ${}^{15}\text{N}$ in tissues of consumers is typically enriched by 3‰ relative to their prey (Pinnegar
224 and Polunin, 1999), and 2 is the trophic level of the primary consumer. The ${}^{15}\text{N}$ concentration of the
225 primary consumer was based on concentrations in *Lymnea* snail samples for Norway and, since snails
226 could not be collected at South African sites and Gomphids cannot be considered as primary consumers,
227 snails (*Tarebia granifera*) collected by Verhaert et al. (2017) from the Olifants River for South Africa.
228 The latter were collected a couple of kilometers downstream of its confluence with the Ga-Selati river
229 and are also present in the Ga-Selati river (Rasifudi et al., 2018).

230 To quantify the Trophic Magnification Factor (TMF), a regression analysis was carried out between
231 the trophic level of a fish/invertebrate and the corresponding concentration of the contaminant. The
232 TMF is calculated as the antilog of the regression slope with base 10 (Borgå et al., 2012):

$$233 \\ 234 \text{Log}[\text{Contaminant}] = a + b \text{ TL} \qquad \text{TMF} = 10^b$$

235

236 2.7. Statistical analysis

237 Statistical analysis was carried out using RStudio (Version 0.98.1103) with a level of statistical
238 significance set at $p < 0.05$. Concentrations below LOQ were given a value of LOQ/2 (Bervoets et al.
239 2005; Custer et al. 2000). The data was tested for normality and homogeneity of variance using a
240 Shapiro-Wilk test and Levene's test respectively, and data were log-transformed since these criteria
241 were not met. Linear regression was used to examine (1) the relation between the concentrations in the
242 TOC-normalized sediment and in lipid-normalized biota (2) the relation between trophic level and
243 corresponding pollutant concentration (see TMF section). To test the relationships between trophic level
244 and concentration of pollutants, as well as to investigate whether this relationship differs between
245 climates, ANCOVA was used to make a regression of the trophic level and the log of the pollutant
246 concentration and then compare the slopes.

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248 2.8. Human health risks

249 The Agency for Toxic Substances and Disease Registry (ATSDR) has developed a list of commonly
250 used hazardous substances and determined significant human exposure levels, which are used to assess
251 whether certain concentrations of pollutants in food items can cause significant acute, subacute, and
252 chronic health effects on humans (ATSDR, 2017). Minimum Risk Levels (MRL) (ng/day) were
253 calculated for a person of 70 kg. Using following formula, the maximum daily intake (g/day) without
254 risk of negative effects due to ingestion of pollutants was calculated for a person of 70 kg, based on the
255 mean concentration of the pollutants found in the muscle tissue of every fish species (ng/g):

$$256 \\ 257 \text{Maximum edible amount} = (\text{MRL for 70 kg person}) / (\text{mean concentration in fish})$$

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259 3. Results and discussion

260

261 3.1. POPs

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263 3.1.1. Sediment

264 TOC values ranged from 0.52 – 2.98 % in South Africa and 2.66 – 10.4 % in Norway and are shown
265 in **Table 1**, together with the mean ΣPCBs , OxC , TN , CN , HCB , ΣDDX , ΣHCHs and ΣPBDEs
266 concentrations measured in the sediment samples.

267 Σ PCBs concentrations in both South Africa and Norway were lower than in other countries in
268 Europe, such as Spain and Belgium (Fernandez, 1999; Van Ael et al., 2012), Asian countries including
269 China, Korea and Vietnam (Koh et al., 2004; Zhang et al., 2004; Minh et al., 2007) and in studies from
270 Egypt and another region in South Africa (El-Kady et al., 2007; Quinn et al., 2009). Concentrations
271 were higher than values from a study in Congo (Verhaert et al., 2013) and comparable to Σ PCBs values
272 in the Olifants River Basin in South Africa (Verhaert et al., 2017). The most frequently occurring PCB
273 congeners were CB110 (18%), CB149 (14%), CB95 (13%) and CB153 (9%) for South Africa and
274 CB149 (14%), CB153 (14%), CB95 (13%) and CB138 (11%) for Norway.

275 Σ PBDEs values were significantly higher in sediment originating from the river Laagen. Values of
276 the present study were however low compared to concentrations reported in sediments from rivers in
277 Belgium, Congo, China and other South African rivers (Hu et al., 2010; Olukunle et al., 2012; Van Ael
278 et al., 2012; Verhaert et al., 2013; Verhaert et al., 2017). The compounds that made up most part of
279 Σ PBDEs were BDE 209 (48%) and BDE 47 (35%) for South Africa, and BDE 209 (52%) and BDE 99
280 (21%) for Norway.

281 All six analyzed DDX metabolites were detected in South African samples, but only *p,p'*-DDE and
282 *p,p'*-DDD were found in sediment from Norway. Σ DDX values were significantly lower in Norwegian
283 samples. Concentrations in South African sediments were also higher compared to rivers in Belgium
284 and Congo, but comparable to concentrations reported in studies in China, Spain, Vietnam and other
285 South African rivers (Fernandez et al., 1999; Zhang et al., 2004; Quinn et al., 2009; Van Ael et al., 2012;
286 Verhaert et al., 2013; Verhaert et al., 2017), with the exception of the very high concentrations at Lepelle
287 Bridge. The high concentrations at Lepelle Bridge are possibly a result of government sponsoring of
288 DDT spraying to kill mosquitoes in the town and lodges nearby, although it is surprising that it is
289 significantly higher than at the Namakgale site, which is closer to said town. Norwegian sediments
290 contained less or similar amounts of DDX compared to previously mentioned studies. The most
291 dominant DDX were *p,p'*-DDT (70%) and *p,p'*-DDE (15%) in South African sediment and *p,p'*-DDE
292 (67%) and *p,p'*-DDD (33%) in Norwegian sediment. DDD and DDE are formed by various chemical
293 breakdowns of DDT, therefore a higher content of DDE and DDD compared to DDT indicates aged
294 DDT that has already been broken down (Aislabie et al., 1997). Only DDE- and DDD-isomers have
295 been found in Norwegian samples, which is not surprising since the use of DDT has been banned from
296 Europe since the 80's (PAN UK, 2008). The same was observed for sediments from the Scheldt river in
297 Belgium and the Netherlands (Van Ael et al., 2012). However, DDT was present in sediment from South
298 Africa and Congo (Verhaert et al., 2013) which suggests DDT is still used in those areas. In countries
299 where the transmission of malaria and visceral leishmaniasis can be efficiently prevented by the use of
300 DDT, it is permitted to produce and use DDT, in accordance with WHO (World Health Organization)
301 recommendations and guidelines (van den Berg, 2008). The results from the present study are consistent
302 with the fact that African countries, as opposed to European countries, still regularly use DDT as a
303 pesticide.

304 HCB concentrations in this study were lower than those reported in sediments from rivers in
305 Belgium, China, Vietnam and other South African rivers (Nakata et al., 2005; Minh et al., 2007; Quinn
306 et al., 2009; Van Ael et al., 2012; Verhaert et al., 2017;) but higher or comparable to concentrations in
307 sediments from rivers located in Spain and Congo (Fernandez, 1999; Verhaert et al., 2013).

308 Concentrations of CHLs and HCHs were low compared to other studies. OxC, TN and CN were not
309 detected in any of the sites, with the exception of Lepelle Bridge (South Africa) where low
310 concentrations of TN and CN were found. CHLs seem to be rarely detected in sediment as they were
311 also under the detection limit in several other studies from Belgium and China (Nakata et al., 2005; Van
312 Ael et al., 2012). Σ HCHs were only detected in South African samples, with γ -HCH as the only
313 compound present. South African HCH concentrations in the present study were lower than
314 concentrations in sediments from rivers in Belgium, Congo, China, Vietnam and other rivers in South
315 Africa (Zhang et al., 2002; Minh et al., 2007; Quinn et al., 2009; Van Ael et al., 2012; Verhaert et al.,
316 2013) but higher than concentrations in sediments from the Olifants river in South Africa (Verhaert et
317 al. 2017). It should be mentioned that α -HCH results were not included in the analysis due to
318 interferences, so we can only say β -HCH was absent but cannot conclude anything concerning α -HCH.
319

320 3.1.2. Invertebrates

321 The lipid content of the invertebrates varied from 1.3% to 1.7% for *Gomphidae* larvae and from 1.3%

322 to 1.6% for *Lymnaea* sp. snails. Concentrations of Σ PCBs, OxC, TN, CN, HCB, Σ DDX, Σ HCHs and
323 Σ PBDEs (ng/g ww) for aquatic invertebrates are given in **Table 2**. PCBs were only found in the snails
324 collected in Norway, and concentrations were low compared to a Belgian study but similar to a study in
325 Congo (Van Ael et al., 2012; Verhaert et al., 2013). CB 153 was the most dominant congener (35%),
326 followed by CB 110 (23%), CB 101 (22%) and CB 138 (20%). Concentrations of OxC, TN, CN, HCB
327 and HCHs were not detected in any of the invertebrates, except for a small amount of TN present in
328 *Gomphidae* larvae at Namakgale. DDX were the most abundant pollutants in South African
329 invertebrates from this study, but were not detected in *Lymnaea* snails in Norway. Σ DDX concentrations
330 in *Gomphidae* larvae from this study very high compared to other invertebrates from Congo, Belgium
331 and another South African river (Van Ael et al., 2012; Verhaert et al., 2013; Verhaert et al., 2017),
332 especially at Namakgale. As stated before, the use of DDT is very common in towns as a way to prevent
333 malaria. The most frequently occurring metabolite was *p,p'*-DDE (73%), followed by *p,p'*-DDT (17%),
334 *p,p'*-DDD (8%) while the remaining congeners contributed less than 1% to the total. Only two PBDE
335 congeners were present in *Lymnaea* snails (BDE 183 (90%) and BDE 47 (10%)), while only BDE99
336 was found in *Gomphidae* larvae.

337

338 3.1.3. Fish

339 Lipid content (%) ranged from 0.6 ± 0.1 % (*Esox lucius*) to 5.5 ± 1.7 % (*Synodontis zambezensis*).
340 An overview of the Σ PCBs, OxC, TN, CN, HCB, Σ DDX, Σ HCHs and Σ PBDEs concentrations (ng/g
341 ww aquatic biota) in the analyzed fish is given in **Table 3**. Relative distribution of indicator PCBs, DDX
342 and PBDE congeners in sediment, invertebrates and fish samples are given in Fig. S1.

343 Dominant congeners in South African samples were CB 153 (18%), CB 74 (16%), CB 138 (13%),
344 CB 118 (11%) and CB 180 (10%). In Norwegian fish, the most dominant congeners were CB 153 (25%),
345 CB 138 (13%), CB 118 (7%) and CB 180 (7%). Σ PCBs concentrations in muscle from South African
346 specimens in this analysis were comparable to studies of relatively pristine rivers in Central Africa and
347 rivers located in Egypt and Central Asia, but were higher in Norwegian specimens from the present
348 study (Kidd et al., 2004; El-Kady et al., 2007; Yang et al., 2010; Verhaert et al., 2013). Concentrations
349 in fish from both South Africa and Norway were much lower compared to a lake located in Burundi and
350 in rivers from more industrialized areas such as in Italy and Belgium (Manirakiza et al., 2002; Miniero
351 et al., 2011; Van Ael et al., 2012).

352 Chlordanes (OxC, TN, CN) were only detected in a few fish from this study, and comparable to
353 concentrations reported in a Belgian study (Van Ael et al. 2012), but lower than in fish from another
354 South African river, the Vaal River, which is heavily influenced by the runoff of surrounding cities,
355 industries and mining activities (Wepener et al., 2011). Detectable concentrations of HCB were only
356 reported in one South African fish sample, while present in all but one of the Norwegian species.
357 Concentrations in Norwegian fish from this study were comparable to those reported in studies on fish
358 from rivers in Belgium and the Tibetan plateau (Yang et al., 2007; Van Ael et al., 2012), but lower than
359 in fish from rivers in Congo (Verhaert et al., 2013) and the heavily polluted Vaal River in South Africa
360 (Wepener et al., 2011).

361 BDE 99 was the only congener present in South African samples from this study. PBDE
362 concentrations were low in South African fish samples compared to lakes in the United States, rivers in
363 Belgium, Congo, Italy, Taiwan and another South African region and especially compared the heavily
364 polluted Lake Mjøsa in Norway (Dodder et al., 2002; Peng et al., 2007; Mariussen et al., 2008; Miniero
365 et al., 2011; Wepener et al., 2011; Van Ael et al., 2012; Verhaert et al., 2013). PBDEs were more
366 abundant in fish from Norway, although they were still generally lower than aforementioned studies.
367 The most dominant congeners were BDE 47 (42%), BDE 99 (35%) and BDE 100 (13%).

368 All six DDT metabolites were detected in fish from this study, except for *o,p'*-DDE being absent in
369 Norwegian fish. Σ DDX concentrations in fish from South African sites were much higher than studies
370 carried out in Belgium and Spain and water bodies from Congo and Chad, but comparable to other
371 African countries such as Burundi and another region in South Africa; concentrations in Norwegian fish
372 were lower than South African fish but still higher than those reported in a study from Belgium and
373 Chad (Kidd et al., 2001; Manirakiza et al., 2002; Bordajandi et al., 2003; Wepener et al., 2011; Van Ael
374 et al., 2012; Verhaert et al., 2013). The most present DDX metabolites in South African fish were *p,p'*-
375 DDE (70%), *p,p'*-DDT (17%) and *o,p'*-DDT (5%), and *p,p'*-DDE (64%), and *p,p'*-DDT (26%) in
376 Norwegian fish. *p,p'*-DDE is very soluble in lipids compared to other congeners which explains its

377 abundance in animal tissue (ATSDR, 2002), as found in other studies (Van Ael et al., 2012; Verhaert et
378 al., 2013).

379 Finally, Σ HCHs concentrations in South African fish from this study were comparable to those
380 reported in studies from Tibet, Congo, Chad and Belgium but lower than other studies from Burundi
381 and the Vaal River in South Africa (Kidd et al., 2001; Manirakiza et al., 2002; Yang et al., 2007;
382 Wepener et al., 2011; Van Ael et al., 2012; Verhaert et al., 2013). Similarly to the sediment samples,
383 only γ -HCH was detected. Σ HCHs concentrations were below the detection limit in Norwegian fish in
384 this study.

385 Relationships between concentrations of POPs in TOC normalized sediment and lipid normalized
386 biota were investigated in South African samples. Concentrations in *Clarias gariepinus* were used as
387 this fish species occurred at each site. No significant relationships could be found between POP
388 concentrations in biota and sediment.

390 3.2. Mercury

391 3.2.1. Surface water

392 Mean dissolved THg concentrations ($\mu\text{g/l}$) (**Table 4**) show no detectable amount of Hg in the water
393 from the Norwegian rivers. Dissolved THg concentrations in the Ga-Selati river however were higher
394 than concentrations reported in studies on the more pristine parts of lake Victoria (Napoleon Gulf,
395 Uganda) and studies from Turkey, Tanzania, Bolivia, Vietnam and Papua New Guinea but still lower
396 than the more polluted areas of the Lake Victoria (Emin Pasha Gulf, Tanzania) (Karadede and Erhan,
397 2000; Maurice-Bourgoin et al., 2000; Bowles et al., 2001; Campbell et al., 2003; Mataba et al., 2016).

399 3.2.2. Sediment

400 Mean concentrations of THg in sediments (**Table 4**) indicate concentrations of THg in both South
401 African and Norwegian sediments lower than those reported for in sediments from the Yangtze river
402 (China), Kwilu river (Congo), Thigithe River (Tanzania) and Lake Victoria (Uganda and Tanzania) but
403 comparable to concentrations in the sediments of the Malagarasi River (Tanzania) (Campbell et al.,
404 2003; Taylor et al., 2005; Yi et al., 2011; Ngelinkoto et al., 2014; Mataba et al., 2016; Chen et al., 2017).
405 Concentrations of THg in sediment from the Norwegian rivers in the present study are higher than
406 dissolved concentrations, which supports the fact that the sediment can act as a reservoir (Foster and
407 Charlesworth, 1996). However, for the South African sites this was not the case. The fact that in general
408 dissolved THg concentrations in the Ga-Selati river were higher in the water compared to sediment is
409 rather surprising since organic matter has been shown to increase the adsorption of metals on sediments
410 (Lin and Chen, 1997). Furthermore, the high pH of water from the Ga-Selati river, ranging from 8.5 to
411 9.8 across all sites, should positively influence the adsorption on sediment because a higher pH leads to
412 a higher binding of Hg to organic material (Haitzer et al., 2003). Dissolved THg concentrations were
413 comparable amongst the three South African sites, but sediment concentration was notably higher at
414 Harmonie, which had the highest TOC value (3%) (**Table 1**). A higher input of pesticides at that site
415 could also play a role, as several commonly used pesticides contain substantial amounts of metals such
416 as Hg (Wuana and Okieimen, 2011).

418 3.2.3. Invertebrates

419 Mean THg concentrations in invertebrates (**Table 4**) are comparable to THg concentrations reported
420 in studies from Tanzania, Central Africa and Vietnam but higher than in invertebrates from a Canadian
421 lake (Kidd et al., 2004; Taylor et al., 2005; Ikemoto et al., 2007; Zhang et al., 2012).

423 3.2.4. Fish

424 Mean THg concentrations in fish muscle tissue in the present study (**Table 4**) were in general slightly
425 lower or comparable to concentrations reported in studies from Congo, Bolivia, Venezuela, Papua New
426 Guinea and Tanzania, but higher than in fish from a lake in Turkey (Maurice-Bourgoin et al., 2000;
427 Karadede and Erhan, 2000; Bowles et al., 2001; Chen et al., 2007; Kwon et al., 2012; Ngelinkoto et al.,
428 2014; Mataba et al., 2016). In general, the uptake of most metals occurs primarily through water and to
429 a lesser extent through food. Hg however occurs primarily as the organic form, methyl mercury (MeHg).
430 Due to the methylation by bacteria, the properties of mercury alter in such a way that it becomes much

431 more lipophilic and more mobile, entering any substrate that contains fat, especially aquatic organisms
432 (Hodson, 1988). As methyl mercury is conserved while energy is lost through trophic transfer, this
433 compound can be highly biomagnified through the food chain resulting in high THg concentrations in
434 top predators (Campbell et al., 2003).

435 Again, relationships between THg concentrations in TOC-normalized sediment and lipid-normalized
436 biota were investigated in South African samples. No significant relationships could be found between
437 THg concentrations in biota and sediment either.

438

439 **3.3. Biomagnification**

440

441 *3.3.1. Trophic web structure*

442 The ranges of nitrogen stable isotopes ratios and trophic levels (Table S1) indicate a variation in
443 trophic level between 1.77 (*Labeo molybdinus*) and 5.34 (*Synodontis zambezensis*). Trophic levels
444 determined in the present study were comparable with those reported on Fishbase (www.fishbase.org)
445 for the majority of fish. Trophic levels of *T.rendalli*, *S.zambesensis*, *C.paratus* and *G.cernuus*, however,
446 were higher compared to levels reported on Fishbase, while the trophic level of *B.trimaculatus* was
447 lower in the present study. The small sample size of aforementioned fish might account for these
448 discrepancies. In general, trophic levels were higher in the subarctic rivers of Norway (Fig. S2).

449

450 *3.3.2. Trophic transfer and magnification*

451 To assess the biomagnification, the relation between the log of the concentration of a compound in
452 biota and the corresponding trophic level was examined. One food web per climate was used, being
453 Lepelle Bridge in South Africa and Laagen in Norway, as they had the highest number of species and
454 samples and to exclude the risk of pooling distinctive food webs together. Significant relations were
455 found between the TL and the log of THg, all DDT-metabolites, several PCB compounds (CB 110, 118,
456 138 and 153), TN and CN concentrations for South African biota, and between the TL and the log of
457 THg, CB153 and 138, TN, CN, *p,p'*-DDE and *p,p'*-DDT for Norwegian biota (**Figure 2**). Calculation
458 of TMFs (**Table 5**) was only done for significant relationships. When biomagnification occurred for a
459 compound in biota from both climates, it was possible to compare both slopes and assess the influence
460 of climate.

461 In general, TMF values for PCBs were comparable to values reported from Congo, China and the
462 U.S. (Zhang et al., 2010; Verhaert et al., 2013; Houde et al., 2017). TMFs of DDT compounds were
463 found to be high in the South African food web compared to food webs from Norway (present study),
464 Congo, China and the U.S. (Zhang et al., 2010; Verhaert et al., 2013; Houde et al., 2017). The initial
465 concentrations of DDT compounds, which are usually higher in African countries, should not play a
466 role in calculating the TMFs, as TMFs are not related to exposure concentrations at the base of the food
467 webs (Borgå et al., 2012). It has been previously reported that the biomagnification of POPs, with the
468 exception of DDT-compounds, didn't follow a clear relationship with trophic level and that
469 consequently $\delta^{15}\text{N}$ wasn't effective to predict the accumulation of other POPs than DDT (Campbell et
470 al., 2000; Guo et al., 2008; Deribe et al., 2011). In this study, only *p,p'*-DDT bioaccumulated differently
471 in the different climates ($p = 0.03$). A global analysis of studies on trophic magnification of POPs
472 suggests that latitude doesn't influence biomagnification in food webs, and that differences in TMF
473 values likely result from differences in food web composition rather than temperature dependent
474 processes such as growth and physiological changes (Walters et al., 2016). However, major data gaps
475 still exist as a result of overrepresentation of studies mainly conducted in temperate climates of the
476 northern hemisphere, which emphasizes the need to investigate biomagnification in the southern
477 hemisphere and other types of climates (Walters et al., 2016).

478 In the current study, the TMF value for THg was significantly higher in Norway than in South Africa
479 ($p=0.0005$). TMF values from studies in lakes located in Uganda and Burkina Faso and a river in
480 Vietnam were in general comparable to the TMF value in the South African river from the present study
481 (Ikemoto et al., 2007; Ouédraogo et al., 2015; Poste et al., 2015). In contrast to POPs, it has been shown
482 that the global biomagnification of mercury is positively related to latitude. Indeed, research shows that
483 Trophic Magnification Slopes are significantly higher in polar and temperate regions as opposed to
484 tropical ones (Lavoie et al., 2013). Although there is not enough data in this study to attribute the

485 differences in biomagnification of THg to climatic or other factors, values from this study do follow the
486 pattern found in literature. Several factors related to climatic conditions would explain this lower
487 biomagnification of Hg in temperate or arctic food webs. The subtropical climate of the Ga-Selati river
488 experiences higher temperatures throughout the year, resulting in a high primary productivity, which
489 stimulates growth rates in the fish. This can lead to growth biodilution of Hg, as the amount of Hg per
490 unit of body mass decreases with growth. Fish from the Norwegian rivers however have growth rates
491 limited by the low temperatures and have to starve for long periods of the year. Shorter life spans of fish
492 living in tropical conditions and a higher species diversity in tropical food webs can also play a role in
493 reducing the trophic transfer of Hg (Lavoie et al., 2013; Poste et al., 2015). However, a few of the
494 aforementioned African lakes had a TMF value comparable or even higher than the one reported for
495 Norwegian rivers in the present study (Ouédraogo et al., 2015), depicting trophic status as a more
496 influencing factor rather than latitude. Indeed, the lakes with highest TMF values were described as
497 eutrophic and mesotrophic compared to the other hypereutrophic lakes. The fact that higher TMF values
498 can also be found in more tropical systems shows that latitude is just one of many factors that influence
499 the biomagnification of Hg, and that trophic status also plays a key role in the biomagnification of Hg
500 in an aquatic food web. Indeed, it has been shown that in African lakes trophic status has a negative
501 relationship with Hg TMF and thus appears to be an important driver of TMFs (Poste et al., 2015).
502 Further research needs to be carried out to better understand the determining factors influencing
503 bioaccumulation of Hg.

504

505 **3.4. Implications for human health**

506 The mean concentrations of POPs and THg in fish captured in the Ga-Selati River and the Norwegian
507 rivers have been compared with the corresponding Minimum Risk Levels for oral intake (ATSDR,
508 2017). This information has been translated into the maximal amount of fish a human with the average
509 weight of 70kg can consume before experiencing negative effects due to exposure to excessive
510 concentrations of pollutants. Taking into account that, according to the FAO, the South African
511 population consumes an average of 21g of fish a day (FAO, 2010), it is unlikely that the recommended
512 limits to avoid adverse health effects is exceeded when consuming fish from this study. The average
513 fish consumption for the Norwegian population is 52 g of fish a day, as reported by the Norwegian
514 Scientific Committee for Food Safety (VKM report 2014). Considering this, caution should be taken
515 when consuming Northern pike (*Esox lucius*) from the river Laagen to avoid harmful effects due to an
516 elevated THg and PBDE exposure (Table 6). A detailed overview of all maximal amounts per fish
517 species can be found in Table S2 for the Ga-Selati river and Table S3 for the rivers Laagen and Rena.

518

519 **4. Conclusion**

520 Overall, concentrations of POPs and mercury in water, sediment and aquatic biota were relatively low
521 in the studied rivers. However, caution should be taken when consuming Northern pike from the river
522 Laagen as PBDEs and THg concentrations exceeded the Minimum Risk Levels.

523 Biomagnification was detected for THg, all DDT-metabolites, several PCB compounds (CB 110, 118,
524 138 and 153), TN and CN in the South African river system while THg, CB153 and 138, TN, CN, *p,p'*-
525 DDE and *p,p'*-DDT were found to be biomagnified in the Norwegian river system. The biomagnification
526 of THg and *p,p'*-DDT differed significantly between both river systems, THg being more biomagnified
527 in the Norwegian river and *p,p'*-DDT being more biomagnified in the South African river. Although
528 this study doesn't have enough data to attribute these differences to climatic or other factors,
529 biomagnification of THg has been shown in various studies to be higher in polar and temperate regions
530 as well as being influenced by trophic status of the aquatic system. Biomagnification of POPs, however,
531 does not seem to be significantly influenced by climate. Differences in biomagnification of POPs might
532 be more influenced by the composition of the food web rather than by differences in temperature. Further
533 research in underrepresented areas is essential to investigate global patterns and to identify the factors
534 influencing bioaccumulation of POPs and Hg.

535

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537

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544

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768 **Tables and Figures**

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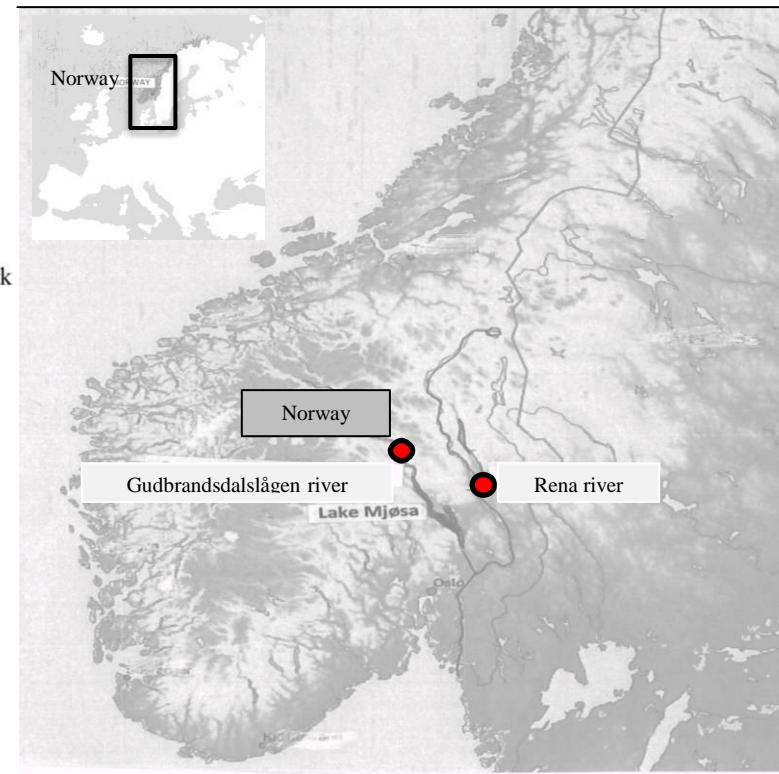
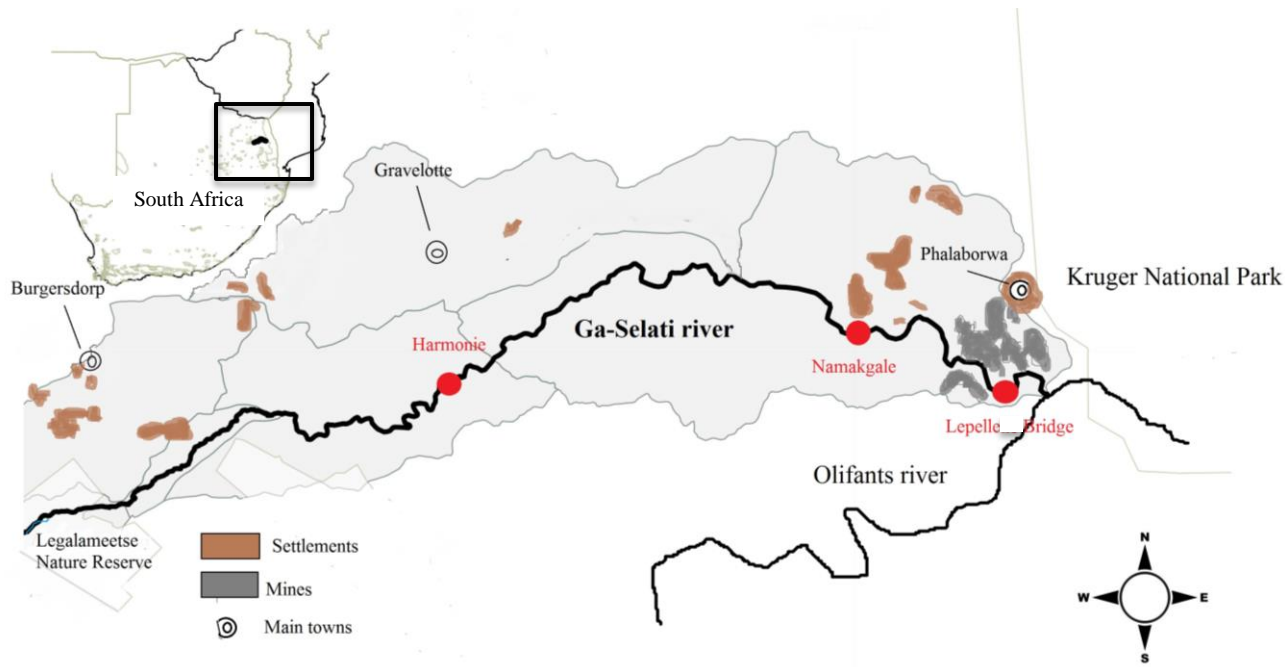


Figure 1. Map of the Ga-Selati catchment, South Africa, and the rivers Laagen and Rena, Norway, with sampling sites indicated in red.

Table 1. Values of total organic carbon (TOC%) and mean sediment concentrations (ng/g) of Σ PCBs, OxC, TN, CN, HCB, Σ DDX, Σ HCHs and Σ PBDEs for sites in South Africa and Norway in this analysis compared to other studies.

	TOC (%)	Σ PCBs	OxC	TN	CN	HCB	Σ DDX	Σ HCHs	Σ PBDEs
This study									
South Africa									
Harmonie	2.98	0.16	<LOQ	<LOQ	<LOQ	0.0059	0.17	0.0044	0.077
Namakgale	2.09	0.25	<LOQ	<LOQ	<LOQ	0.0057	1.5	0.0056	0.049
Lepelle Bridge	0.52	0.22	<LOQ	0.0062	0.0026	0.0062	80	0.0064	0.042
Norway									
Laagen	2.66	0.21	<LOQ	<LOQ	<LOQ	0.0072	0.051	<LOQ	0.406
Rena	10.36	0.09	<LOQ	<LOQ	<LOQ	0.0046	0.019	<LOQ	0.033
Congo River, Congo ^a		0.08	-	-	-	<LOQ	0.12	0.036	0.23
Scheldt estuary, Belgium ^b		6.43	<LOQ	<LOQ	<LOQ	0.01	0.03	0.02	6.9
Tonghui River, China ^c		3.3±2.6	-	-	-	-	1.1±1.1	0.17±0.10	-
Ebro River, Spain ^d		14±7.6	-	-	-	0.007±0.009	3.1±3.1	-	-
Hyeongsan River, Korea ^e		62	-	-	-	-	-	-	-
Vaal River, South Africa ^f		3.02*	-	-	-	0.12	1.65	0.52	-
Nile River, Egypt ^g		0.22±0.04	-	-	-	-	-	-	-
Lake Tai, China ^h		<LOQ	<LOQ	0.03±0.01	0.03±0.02	0.08±0.05	0.72±0.18	0.43±0.11	-
Fuhe River, China ⁱ		-	-	-	-	-	-	-	2.3
Jukskei River, South Africa ^j		-	-	-	-	-	-	-	3.4
Mekong River, Vietnam ^k		0.89	-	-	-	0.016	6.5	0.1	-
Olifants River Basin, South Africa ^l		0.16	-	-	-	0.031	0.64	<LOQ	1.5

<LOQ = below Limit of Quantitation, * Σ PCBs (7 indicator PCBs)

^a Verhaert et al., 2013, ^b Van Ael et al., 2012, ^c Zhang et al., 2004, ^d Fernandez, 1999, ^e Koh et al., 2004, ^f Quinn et al., 2009, ^g El-Kady et al., 2007, ^h Nakata et al., 2005, ⁱ Hu et al., 2010, ^j Olukunle et al., 2012, ^k Minh et al., 2007, ^l Verhaert et al., 2017

Table 2. Σ PCBs, OxC, TN, CN, HCB, Σ DDX, Σ HCHs and Σ PBDEs concentrations (ng/g ww invertebrate) in invertebrates from this study compared to other studies.

	Lipid %	Σ PCBs	OxC	TN	CN	HCB	Σ DDX	Σ HCHs	Σ PBDEs
This study									
South Africa (<i>Gomphidae</i> sp.)									
Harmonie	1.7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3.9	<LOQ	0.51
Namakgale	1.3	<LOQ	<LOQ	0.13	<LOQ	<LOQ	133	<LOQ	<LOQ
Lepelle Bridge	1.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.9	<LOQ	<LOQ
Norway (<i>Lymnaea</i> sp.)									
Laagen	1.6	2.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.33
Rena	1.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.43
Congo River Basin, Congo (<i>Pila</i> sp.) ^a	1.2	2.1	-	-	-	<LOQ	0.14	0.060	0.040
Scheldt estuary, Belgium (Polychaeta) ^b	1.3	53	<LOQ	0.03	<LOQ	0.14	0.55	0.06	1.2
Olifants River Basin, South Africa (<i>Gomphidae</i>) ^c	0.82	<LOQ	-	-	-	<LOQ	0.33	<LOQ	<LOQ

<LOQ = below Limit of Quantitation

^a Verhaert et al., 2013, ^b Van Ael et al., 2012, ^c Verhaert et al., 2017

Table 3 . Means \pm SD of Σ PCBs, OxC, TN, CN, HCB, Σ DDX, Σ HCHs and Σ PBDEs concentrations (ng/g ww) in fish from this study compared to other studies.

	Lipid %	Σ PCBs	OxC	TN	CN	HCB	Σ DDX	Σ HCHs	Σ PBDEs
This study									
South Africa									
Harmonic									
<i>Labeo molybdinus</i>	2.5	1.8	<LOQ	<LOQ	<LOQ	<LOQ	21	<LOQ	<LOQ
<i>Tilapia mossambicus</i>	0.97	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.4 \pm 0.43	<LOQ	<LOQ
<i>Clarias gariepinus</i>	1.2	1.9 \pm 0.40	<LOQ	<LOQ	<LOQ	<LOQ	64 \pm 74	<LOQ	<LOQ
Namakgale									
<i>Labeo molybdinus</i>	3.6	2.6	<LOQ	3.7	1.3	0.13	5643	2.7	<LOQ
<i>Clarias gariepinus</i>	0.57	<LOQ	0.16 \pm 0.16	0.76 \pm 1.0	0.27 \pm 0.31	<LOQ	361 \pm 275	0.48 \pm 0.091	0.29 \pm 0.058
Lepelle Bridge									
<i>Labeo molybdinus</i>	3.0	1.7 \pm 0.030	<LOQ	<LOQ	<LOQ	<LOQ	60 \pm 55	<LOQ	0.27 \pm 0.04
<i>Tilapia rendalli</i>	1.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	16 \pm 4.4	<LOQ	<LOQ
<i>Clarias gariepinus</i>	1.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	21 \pm 23	<LOQ	<LOQ
<i>Synodontis zambezensis</i>	5.5	2.7 \pm 0.45	<LOQ	0.22 \pm 0.08	0.17 \pm 0.04	<LOQ	410 \pm 141	0.17 \pm 0.07	0.25 \pm 0.08
Norway									
Laagen									
<i>Gymnocyphus cernuus</i>	1.7	2.5 \pm 1.3	<LOQ	<LOQ	<LOQ	<LOQ	1.2 \pm 0.45	<LOQ	0.58 \pm 0.34
<i>Phoxinus phoxinus</i>	1.4	3.1 \pm 0.23	<LOQ	<LOQ	<LOQ	<LOQ	2.2 \pm 0.53	<LOQ	0.47 \pm 0.22
<i>Salmo trutta</i>	1.5	4.6 \pm 1.7	<LOQ	0.11 \pm 0.05	<LOQ	0.15 \pm 0.03	2.8 \pm 1.1	<LOQ	1.0 \pm 0.30
<i>Esox lucius</i>	0.65	9.5 \pm 7.7	<LOQ	0.069 \pm 0.032	0.065 \pm 0.026	<LOQ	4.5 \pm 2.8	<LOQ	11 \pm 14
<i>Cottus poecilopus</i>	1.5	7.1 \pm 5.5	<LOQ	0.11 \pm 0.10	0.077 \pm 0.046	0.12 \pm 0.13	2.0 \pm 1.6	<LOQ	2.0 \pm 1.6
Rena									
<i>Salmo trutta</i>	1.9	1.7 \pm 0.01	<LOQ	<LOQ	<LOQ	0.13 \pm 0.031	0.85 \pm 0.016	<LOQ	0.19 \pm 0.017
<i>Esox lucius</i>	0.57	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.83 \pm 0.019	<LOQ	0.27 \pm 0.14
Congo river, Congo (<i>Synodontis alberti</i>) ^a		1.5	-	-	-	0.078	0.090	0.16	1.2
Olifants River Basin, South Africa (<i>Clarias gariepinus</i>) ^b		0.14 \pm 0.12	-	-	-	<LOQ	8.0 \pm 11	<LOQ	<LOQ
Nile River, Egypt (<i>Oreochromis niloticus</i>) ^c		0.00082 \pm 0.00011	-	-	-	-	-	-	-
Lakes of the Tibetan Plateau ^d		0.32	-	-	-	0.21	4.0	0.55	-
Lake Chad, Chad (<i>Synodontis schall</i>) ^e		2.6	-	-	-	-	0.79	0.69	-
Tiber River, Italy (<i>Leuciscus cephalus</i>) ^f		99	-	-	-	-	-	-	13
Scheldt estuary, Belgium (<i>Platichthys flesus</i>) ^g		105	0.00	0.01	0.00	0.16	0.56	0.06	1.2
Lake Tanganyika, Burundi (<i>Stolothrissa tanganikae</i>) ^h		64 \pm 15	-	-	-	6.5 \pm 1.2	124 \pm 19	55 \pm 12	-
Vaal River, South Africa (<i>Labeo capensis</i>) ⁱ		371 \pm 113	<0.5	5.8 \pm 1.8	2.6 \pm 0.6	257 \pm 204	161 \pm 47	10 \pm 0.9	5.9 \pm 1.0
Hadley Lake, US (<i>Pomoxis annularis</i> , <i>Lepomis macrochirus</i>) ^j		-	-	-	-	-	-	-	65 \pm 8.0
Lan-Yang River, Taiwan ^k		-	-	-	-	-	-	-	25 \pm 11
Erh-Jen estuary, Taiwan ^k		-	-	-	-	-	-	-	281 \pm 210
Lake Mjøsa, Norway (<i>Salmo trutta</i>) ^l		-	-	-	-	-	-	-	342 \pm 312
River Turia, Spain (<i>Salmo trutta</i>) ^m		6.9	-	-	-	-	4.3	-	-

<LOQ = below Limit of Quantification

^a Verhaert et al., 2013, ^b Verhaert et al., 2017, ^c El-Kady et al., 2007, ^d Yang et al., 2010, ^e Kidd et al., 2004, ^f Miniero et al., 2011, ^g Van Ael et al., 2012; ^h Manirakiza et al., 2002; ⁱ Wepener et al., 2011

^j Dodder et al., 2002; ^k Peng et al., 2007; ^l Mariussen et al., 2008; ^m Bordajandi et al., 2003

Table 4. Mean concentration (\pm SD) of THg in water samples ($\mu\text{g/l}$) (n=3), sediments ($\mu\text{g/g dw}$) (n=2) and biota ($\mu\text{g/g ww}$ biota) from the Ga-Selati river (South-Africa) (n=5) and the rivers Laagen (n=3) and Rena (n=3) (Norway) compared to other studies.

	Water	Sediment	Invertebrates	Fish	Water	Sediment	Invertebrates	Fish				
This study												
South Africa					Norway							
Namakgale	0.03 \pm 0.01	0.02	<i>Gomphidae</i> sp. 0.03 \pm 0.008	<i>Clarias gariepinus</i> 0.11 \pm 0.02 <i>Labeo molybdinus</i> 0.03 <i>Chiloglanis paratus</i> 0.30 <i>Glossogobius callidus</i> 0.06 \pm 0.02 <i>Clarias gariepinus</i> 0.04 \pm 0.05 <i>Labeo molybdinus</i> 0.01 \pm 0.003 <i>Synodontis zambeziensis</i> 0.11 \pm 0.03 <i>Tilapia rendalli</i> 0.02 \pm 0.001 <i>Clarias gariepinus</i> 0.25 \pm 0.14 <i>Labeo molybdinus</i> 0.03 <i>Tilapia mossambicus</i> 0.02 \pm 0.004 <i>Barbus trimaculatus</i> 0.09 \pm 0.008	Rena	<LOQ	0.02	<i>Lymnaea</i> sp. 0.03 \pm 0.003 <i>Salmo trutta</i> 0.06 \pm 0.01 <i>Esox lucius</i> 0.13 \pm 0.07 Laagen	<LOQ	0.07	0.03 \pm 0.004	<i>Gymnocapalus cernuus</i> 0.24 \pm 0.07 <i>Phoxinus phoxinus</i> 0.09 \pm 0.006 <i>Salmo trutta</i> 0.05 \pm 0.005 <i>Esox lucius</i> 0.55 \pm 0.17 <i>Cottus poecilopus</i> 0.11 \pm 0.03
LB	0.02 \pm 0.005	0.002	0.01 \pm 0.005									
Harmonie	0.02 \pm 0.006	0.001	0.007 \pm 0.004									
Lake Victoria ^a												
Napoleon Gulf, Uganda	0.0037 \pm 0.0011	0.18	-	-								
Emin Pasha Gulf, Tanzania	0.88 \pm 0.76	0.59	-	-								
Atatürk Dam lake, Turkey(<i>Capoetta trutta</i>) ^b	<LOQ	<LOQ	-	<LOQ								
Thigithe River, Tanzania (<i>Labeo victorianus</i>) ^c	<LOQ	0.077 \pm 0.15	-	0.05-0.90								
Madeira River, Bolivia (<i>Pseudoplastystoma tigrinum</i>) ^d	0.0077 \pm 0.0013	-	-	0.99								
Mekong River, Vietnam (<i>Macrobranchium equidens</i>) ^e	<LOQ	-	0.08 \pm 0.03*	-								
Lake Murray, Papua New Guinea (<i>Arius berneyi</i>) ^f	0.0014 \pm 0.0012	0.11 \pm 0.06	-	0.23 \pm 0.17								
Yangtze River, China ^g	-	0.19 \pm 0.21	-	-								
Nikonga River, Tanzania (Gastropoda sp.) ^h	-	0.02	0.040	-								
Canon river, Taiwan ⁱ	-	3.41 \pm 2.95	-	-								
Kwilu River, Congo (<i>Clarias pachynema</i>) ^j	-	0.22 \pm 0.32	-	0.44 \pm 0.49								
Lake Chad, Central Africa (<i>Etheria elliptica</i>) ^k	-	-	0.019 \pm 0.009	-								
Lake Ontario, Canada (<i>Echinogammarus fasciatus</i>) ^l	-	-	0.0087 \pm 0.0025	-								
Río Las Marías, Venezuela (<i>Astyanax integer</i>) ^m	-	-	-	0.37 \pm 0.22								

<LOQ = below Limit of Quantitation

^a Campbell et al., 2003, ^b Karadede and Erhan, 2000, ^c Mataba et al., 2016, ^d Maurice-Bourgoin et al., 2000, ^e Ikemoto et al., 2007, ^f Bowles et al., 2001, ^g Yi et al., 2011, ^h Taylor et al., 2005, ⁱ Chen et al., 2007, ^j Ngelinkoto et al., 2014, ^k Kidd et al., 2004, ^l Zhang et al., 2012, ^m Kwon et al., 2012

* Concentration in dry weight, compared to a mean THg concentration of 0.10 \pm 0.080 and 0.16 \pm 0.053 $\mu\text{g/g dw}$ in samples from South Africa and Norway respectively.

Table 5. TMFs of significant relationships between the log of compound concentration and trophic level in aquatic biota from the present study, compared to other studies. (*) depicts a significant difference between climates.

	South Africa	Norway	Congo ^a	China ^b	U.S. ^c	Uganda ^d	Burkina Faso ^e	Vietnam ^f
THg	2.3*	5.4*	-	-	-	1.9-5.6	2.9-6.5	1.3
CB153	3.7	4.0	2.5	3.6	3.4	-	-	-
CB138	3.1	3.9	2.6	2.3	3.3	-	-	-
CB 118	2.8	-	-	3.6	-	-	-	-
CB 110	1.9	-	2.4	1.8	-	-	-	-
TN	3.1	2.6	-	-	3.6	-	-	-
CN	2.6	2.5	-	-	-	-	-	-
<i>o,p'</i> -DDE	24	-	-	-	-	-	-	-
<i>p,p'</i> -DDE	6.2	4.1	-	1.4	4.0	-	-	-
<i>o,p'</i> -DDD	9.1	-	-	-	-	-	-	-
<i>p,p'</i> -DDD	8.8	-	-	1.7	-	-	-	-
<i>o,p'</i> -DDT	10.3	-	-	-	-	-	-	-
<i>p,p'</i> -DDT	14*	4.2*	1.7	1.1	-	-	-	-

^a Verhaert et al., 2013, ^b Zhang et al., 2010, ^c Houde et al., 2017, ^d Poste et al., 2015, ^e Ouédraogo et al., 2015, ^f Ikemoto et al., 2007

Table 6. Minimal Risk Levels for *p,p'*-DDT, Σ PBDEs (lower brominated), Σ PCBs, γ -HCH, CHLs and THg (ATSDR 2017), the corresponding mean concentrations in *Esox lucius* river Laagen (Norway), and the maximum amount of fish recommended to avoid risk of adverse non-cancer health effects due to the ingestion of the pollutants. (*) Depicts an amount exceeded by the average daily fish consumption of the Norwegian population.

	<i>p,p'</i> -DDT	Σ PBDEs (lower brominated)	Σ PCBs	γ -HCH	CHLs	THg
Minimal Risk Level (ng/kg body weight/day)	500	3	30	10	600	230
MRL (ng/day) for a 70kg person	35,000	210	2100	700	42,000	16,100
Mean concentration in <i>Esox lucius</i> (ng/g ww)	0.78	8.1	7.1	<LOQ	0.14	550
Maximum edible amount of <i>Esox lucius</i> for a person of 70kg (g)	45,000	30*	300	-	300	30*

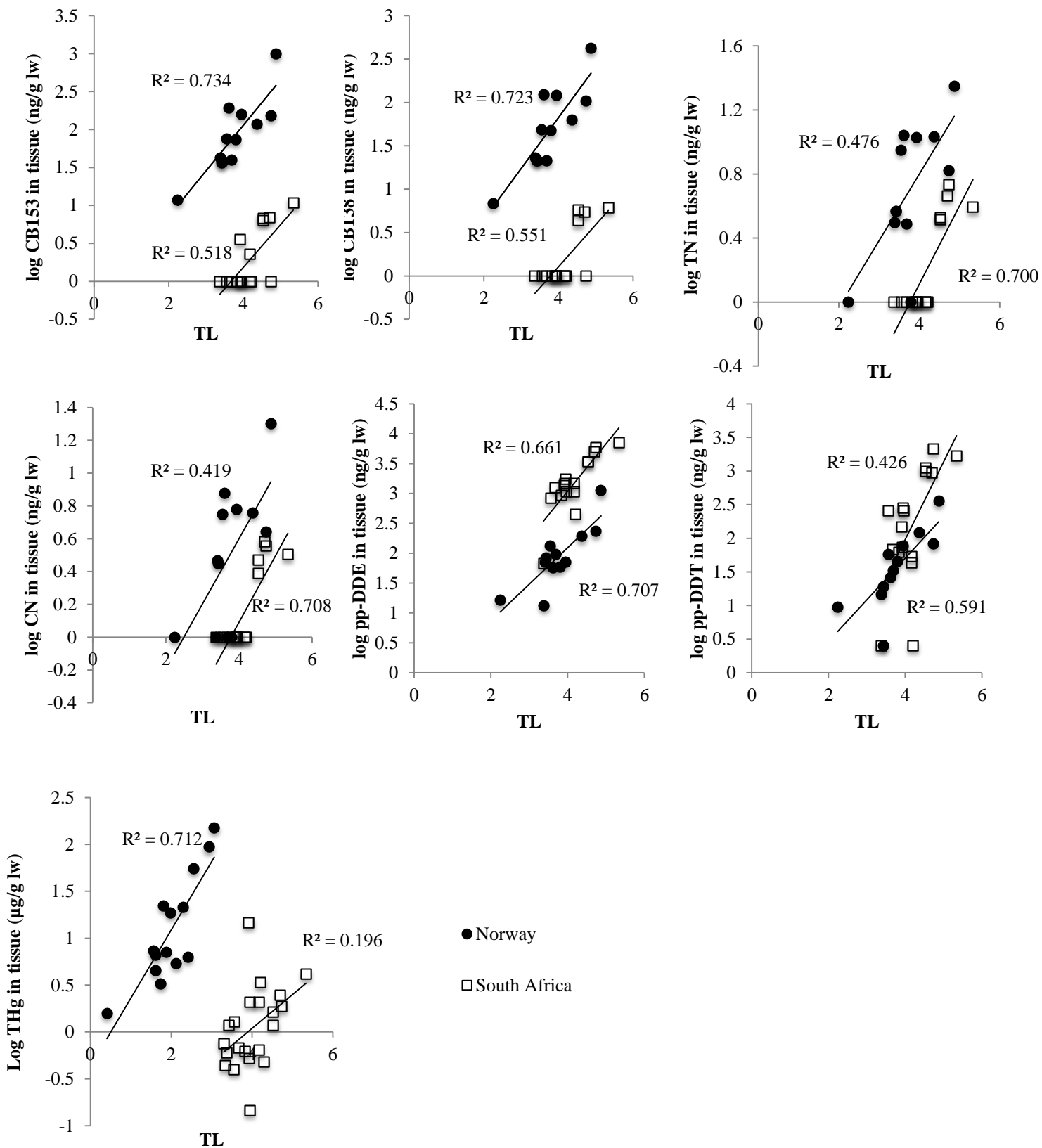


Figure 2. Trophic transfer for CB153, CB138, TN, CN, *p,p'*-DDE, *p,p'*-DDT and THg in aquatic biota from South Africa and Norway