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Accumulation of poly- and perfluoro-alkylated substances (PFASs) and mercury in fish tissue from Lake Tana, Ethiopia

Evaluation of human exposure due to increased fish consumption

Margareta Sjöholm

ABSTRACT

Accumulation of poly- and perfluoroalkylated substances (PFASs) and mercury in fish tissue from Lake Tana, Ethiopia – evaluation of human exposure due to increased fish consumption.

Margareta Sjöholm

Both poly- and perfluoroalkylated substances (PFASs) and mercury (Hg) are persistent bioaccumulative, and toxic substances (PBTs) of great concern due to their health effects on humans. These pollutants are ubiquitously occurring in the global aquatic environment and dietary intake of fish is the major exposure pathway for humans. PFASs and Hg are widely studied in the temperate zones, but little is known from the tropical aquatic systems in Africa. Lake Tana, Ethiopia, is of high ecological value and predicted to increase its fish production and export during following years, but knowledge of human health effects due to bioaccumulated pollutants loading from this lake is lacking. The objective of this study was therefore to assess Hg and PFAS concentrations in fish tissue at Lake Tana, Ethiopia, and evaluate their spatial distribution, species-specific uptake and the human health risk with increased fish consumption.

During October 2014, a total of 97 fish specimens from five species (*Labeobarbus megastoma, Labeobarbus gorguari, Labeobarbus intermedius, Oreochromis niloticus* and *Clarias gariepinus*) were collected from seven sites in Lake Tana. The fish was dissected in Bahir Dar, where muscle samples were taken from the dorsal line, and later analyzed for Hg and PFASs at the Swedish University of Agricultural Sciences (SLU), Uppsala. To determine differences and correlations between sites and species as well as for Hg and PFASs, statistical analyses were conducted and to determine the health risks in increased fish consumption a hazard ratio (HR) was calculated for both substances.

The results showed several similarities between Hg and PFASs, including higher concentrations in piscivorous fish species (*L. megastoma* and *L. gorguari*) than non-piscivorous (*L. intermedius*, *O. niloticus* and *C. gariepinus*) and also spatial distribution similarities. Hg concentrations ranged from 0-639 ng g^{-1} wet weight (ww) (mean = 135 ng g^{-1} ww; median = 52.4 ng g^{-1} ww) for all species. Seven PFASs were detected (PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS, PFOS), and the ∑PFAS concentrations ranged from non-detected to 5.78 ng g^{-1} ww (mean = 1.15 ng g^{-1} ww; median = 0.479 ng g^{-1} ww). PFDA was found at all sites and species, compared to PFOS, which only was found in elevated levels in piscivorous species. The positive correlation between Hg and PFOS imply that these substances have similar accumulation patterns. The HRs showed that the consumption of fish contaminated with PFAS and Hg will not cause any harmful effects for the Ethiopian population. However, varied fish consumption is of importance though since several individuals from the piscivorous species contained Hg concentrations exceeding the WHO marketing limit of 500 ng g^{-1} ww.

Keywords: mercury, PFAS, fish, bioaccumulation, human exposure, Lake Tana, Ethiopia

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REFERAT

Ackumulation av poly- och perfluoralkylerade ämnen (PFASer) och kvicksilver i fisk från Lake Tana, Etiopien – utvärdering av mänsklig exponering med anledning av ökad fiskkonsumtion.

Margareta Sjöholm

Både poly- och perfluoralkylerade ämnen (PFASer) och kvicksilver (Hg) är persistenta, bioackumulerande och toxiska (PBT) ämnen som kan utgöra stor hälsorisk för människor. PFASer och Hg förekommer globalt i den akvatiska miljön och den mest betydande källan för mänsklig exponering av dessa ämnen är fiskkonsumtion. Studier av PFASer och Hg är vanligt förekommande i de tempererade zonerna, men väldigt lite är känt från de tropiska akvatiska systemen i Afrika. Lake Tana, Etiopiens största sjö, är av stort ekologiskt värde och fiskproduktion och export från sjön förutspås öka under kommande år. Däremot saknas kunskap om hur denna föroreningsbelastning med ökat fiskintag kommer påverka befolkningen. Syftet med denna studie var därför att jämföra Hg- och PFAS-koncentrationer mellan områden och arter, utvärdera ackumuleringsmönster och bedöma hälsoriskerna med ökad fiskkonsumtion i landet.

Under oktober 2014 samlades totalt 97 individer in från fem arter (*Labeobarbus megastoma, Labeobarbus gorguari, Labeobarbus intermedius, Oreochromis niloticus* och *Clarias gariepinus*) och från sju olika platser i Lake Tana. Dissektionen utfördes i Bahir Dar (där muskelprover togs från dorsala rygglinjen) och sedan fördes proverna till Sveriges lantbruksuniversitet (SLU) för analys. För att bestämma skillnader och korrelationer mellan områden och arter, samt mellan Hg och olika PFASer, utfördes statistiska analyser och för att utvärdera hälsorisken av en ökad fiskkonsumtion beräknades riskfaktorer för båda ämnena.

Resultaten påvisade flertalet likheter mellan Hg och PFASer, bland annat högre koncentrationer i piskivora fiskarter (*L. megastoma* and *L. gorguari*) än icke-piskivora (*L. intermedius*, *O. niloticus* and *C. gariepinus*) och även likheter i koncentrationer mellan provområdena. Hg-koncentrationerna varierade mellan 0-639 ng g^{-1} våtvikt (vv) (medel = 135 ng g^{-1} vv; median = 52,4 ng g^{-1} vv) för alla arter. Sju PFASer detekterades i analysen (PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS, PFOS), där ∑PFAS koncentrationerna varierade mellan icke-detekterbara till 5,78 ng g^{-1} vv (medel = 1,15 ng g^{-1} vv; median = 0,479 ng g^{-1} vv). PFDA förekom i alla arter och områden, medan PFOS bara fanns i förhöjda värden i piskivora arter. Den funna positiva korrelationen mellan PFOS och Hg antyder att dessa ämnen har liknande ackumulationsmönster. De beräknade riskfaktorerna visade att en fiskkonsumtionsökning inte skulle utgöra en risk för den etiopiska befolkningen med avseende på Hg- och PFAS-halter. En varierad fiskkost är dock av stor vikt eftersom flertalet individer från de piskivora arterna innehöll högre Hg-koncentrationer än den av WHO rekommenderade gränsen på 0,5 μg g^{-1} vv.

Nyckelord: kvicksilver, PFAS, fisk, bioackumulation, mänsklig exponering, Lake Tana, Etiopien

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PREFACE

This Master Thesis covers 30 credits and represents the last part of the Master Programme in Environmental and Water Engineering at Uppsala University. The fieldwork was conducted together with an Ethiopian PhD student in Ethiopia during October 2014. The laboratory work and writing were done in Sweden at The Swedish University of Agricultural Sciences (SLU) between November 2014 and February 2015. The subject examiner has been Kevin Bishop, professor at the Department of Earth Sciences, Program for Air, Water and Landscape Sciences at Uppsala University. The supervisor has been Staffan Åkerblom researcher at SLU in Uppsala at the Department of Aquatic Sciences and Assessment.

This thesis is the product of many months of hard work both abroad and in a laboratory in Sweden. None of this would have been possible without the help from many talented and dedicated people, whom I would like to thank below. First of all I would like to thank Habiba Gashaw, PhD student at Addis Ababa University, who let me be a part of her field work, took care of me and taught me how to dance Ethiopian traditional dances. I also want to send my appreciations to the researchers and workers at the Fish and Research Center in Bahir Dar and especially to Benyam Hailu who helped us more than required and always was there for us if we had questions or requests. I also want to thank Lutz Ahrens researcher at SLU, Uppsala at the department of Aquatic Sciences and Assessment, Organic environmental chemistry and ecotoxicology for his expertise in PFASs, for teaching me the laboratory work and his patience before all my questions.

My supervisor Staffan Åkerblom deserves the greatest ovations, since he came down to Ethiopia to help and support me in the field work, always answered my emails and questions as soon as he could, contributed with many valuable advices and comments related to both the report writing as well as the laboratory work.

Lastly I also want to thank my Johan for all help and support during this period.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

Ackumulation av poly- och perfluoralkylerade ämnen (PFASer) och kvicksilver i fisk från Lake Tana, Etiopien – utvärdering av mänskliga exponeringsnivåer med anledning av ökad fiskkonsumtion.

Margareta Sjöholm

I många av jordens vattendrag, sjöar och hav finns idag spår av miljögifter. Dessa gifter tas upp av alla vattenlevande organismer och halterna ökar ju större och äldre organismerna blir. Vissa miljögifter är svårnedbrytbara i kroppen och koncentrationen ökar därför från bytesdjur till rovdjur. Man säger då att miljögifterna bioackumuleras upp i näringskedjan och i sjöar är det större rovdjursfiskar, som har de högsta nivåerna av skadliga och giftiga ämnen. För människor är det genom luft, vatten och föda vi intar farliga ämnen. I allra störst utsträckning är det när vi ofta äter fisk innehållandes höga halter som vi kan bli allvarligt sjuka.

Två bioackumulerande och farliga miljögifter är poly- och perfluoralkylerade substanser (PFASer) och kvicksilver (Hg). Dessa ämnen har hittats i höga nivåer i vatten, fisk och människa på många platser i världen och de har studerats flitigt globalt. Det finns dock kunskapsluckor för dessa föroreningar i många vattensystem, och framförallt i Etiopien, där inga tidigare studier har gjorts för PFASer och Hg i fisk från Lake Tana.

Lake Tana är Etiopiens största sjö, lokaliserad i nordvästra Etiopien på cirka 1800 m höjd och är ursprunget till Blå Nilen. Sjön är av stor betydelse som vattentillgång och fiskevatten, samt för biologisk mångfald, kultur och turism. Även om sjön är den största i landet (jämförbar med svenska Vänern) och har den största teoretiska fiskproduktionen, har det huvudsakliga fisket ägt rum i den södra delen av landet, i mindre sjöar närmare huvudstaden Addis Abeba. De sjöarna börjar dock bli utfiskade och lösningen är därför att öka produktionen från Lake Tana. Fiskproduktionen beräknas öka med 44 % under kommande år, men hur denna ökning kommer påverka befolkningen vad gäller upptag av bioackumulerande och farliga ämnen såsom PFAS och Hg är ännu inte känt.

Kvicksilver och PFASer används dagligen i människans vardag. Kvicksilver används i lågenergilampor, amalgam, termometrar, elektriska apparater och vid silver- och guldtillverkning. PFASer används i alla sammanhang där material är olje- och vattenresistenta, t.ex. GORE-tex kläder, teflon och textilier, men används även som brandbekämpningsmedel. Från produktionsstart till avfall sprids dessa föroreningar till närmiljön och då främst till luft och vatten. Även om PFASer är ämnen som tillverkats på mänsklig väg och kvicksilver är ett grundämne finns likheter mellan dessa ämnen. De är båda svårnedbrytbara och blir kvar länge i miljön, samt att de kan färdas långväga i atmosfären och hamna på platser långt från direkta utsläpp. De är båda även bioackumulerande och giftiga. PFASer tros vara cancerframkallande och hormonstörande och kvicksilverförgiftning är skadligt för nerv-, immun- och matsmältningssystem samt för lungor, njurar, hud och ögon. Det är cirka åtta av 1000 barn från fiskarbefolkningar i världen som visar upp symtom orsakade av konsumtion av fisk innehållandes höga värden av kvicksilver.

Den etiopiska befolkningen är för närvarande ett av de länder som äter minst fisk i världen med ett medel på endast 200 g/person/år. Men i de delar av landet där fiske är vanligt uppgår konsumtionen till cirka 10 kg/person/år. En ökad fiskkonsumtion skulle dock kunna påverka befolkningen negativt med avseende på Hg- och PFAS-halter. Den här rapporten har därför fokuserat på att undersöka hur höga halter olika arter innehåller samt om det finns någon skillnad i halter i olika områden runt sjön. De fem undersökta arterna ingår i olika delar i näringskedjan; två växtätare (botten av näringskedjan), en blandätare och två fiskätare (överst i näringskedjan). Det har även undersökts hur väl dessa arter bioackumulerar PFAS och Hg, alltså om det finns något samband mellan uppmätt halt och längd på fiskarna.

Resultaten visade att det fanns tydliga samband mellan Hg-koncentrationer och längd för fiskätarna, inte lika tydliga för blandätaren och inga alls för växtätarna. Detta betyder att Hg bioackumuleras upp i näringskedjan i Lake Tana och att de högsta koncentrationerna förekommer i fiskätarna som är högst upp i näringskedjan. Av alla PFASer var det endast en (PFOS, perfluoroktansulfonsyra) som visade upp samma bioackumulationsmönster som Hg. Överlag upptäcktes lägre koncentrationer av Hg och PFASer än i t.ex. Europa och Nordamerika, men i ungefär samma intervall som i andra studerade akvatiska system i Afrika. Det fanns däremot vissa individer som översteg de rekommenderade gränserna för Hg i fisk.

Det visade sig dock att PFAS- och Hg-koncentrationerna i fiskarna tillsammans med den nuvarande låga fiskkonsumtionen i Etiopien inte utgjorde någon hälsofara för den etiopiska befolkningen. Inte heller en uppskattad framtida ökning av fiskkonsumtion på 44 % utgjorde någon fara. Något som dock är viktigt både i det här fallet och för fiskkonsumtion globalt, är att ha en artvarierad kost, då hälsorisken ökar om man äter mycket fisk från toppen av näringskedjan.

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1 INTRODUCTION

Poly- and perfluoroalkyl substances (PFASs) and mercury (Hg) are persistent, bioaccumulative and toxic (PBT) substances of concern for environmental and human health (Labadie & Chevreuil 2011; UNEP 2013). An important exposure pathway for human intake of these substances is fish of both freshwater and marine origin (Dórea 2008). Hg in fish has been studied globally, mostly in the temperate zones where the potential ecological effects and human exposure have been of concern (Johansson et al. 2004; Goulet et al. 2007), but also in South America (Mol et al. 2001), Asia (Jin et al. 2006), and Africa (Hanna et al. 2015). Even though Africa is the second highest Hg emitter after Asia (Pacyna et al. 2006), this is not reflected in the Hg levels in fish, which generally show lower concentrations than elsewhere globally (Black et al. 2011). PFASs have also been widely investigated in fish in Europe (Berger et al. 2009; Labadie & Chevreuil 2011), South America (Quinete et al. 2009) and Asia (Yang et al. 2012), while there is a lack of knowledge of PFASs in aquatic ecosystems in Africa. Bioaccumulation of Hg has been studied for several fresh water bodies in East Africa, however Lake Tana, the largest lake in Ethiopia and origin of the Blue Nile, is of high interest for further investigation of substances with PBT characteristics (Desta et al. 2007; Poste et al. 2015). Fish production and export is predicted to increase from Lake Tana (Gordon et al. 2007), but to what extent this increased production will affect people around the lake, with respect to pollutants loading, is still unclear.

1.1 LAKE TANA, ETHIOPIA

Lake Tana, located in the Amhara region in Northwestern Ethiopia (Fig. 1), is of high ecological value and important as water source and food supply, for hydropower and tourism for the approximate 807,000 residents living around the lake (Ligdi et al., 2010). The largest city in the region is Bahir Dar, a fast growing city located in the southern gulf of the lake (Gordon et al. 2007). Lake Tana has a high biodiversity with a diverse fish fauna of at least 29 different species from which 20 are endemic (Mohammed et al. 2011). The most common fish genus is Labeobarbus (Cyprinidae family) with 23 species from which 15 are endemic (Mohammed et al. 2011). The Lake also hosts the African sharptooth catfish (*Clarias gariepinus*, Clariidae family) and the Nile tilapia (*Oreochromis niloticus*, Cichlidae family) in big numbers (de Graaf 2003). The fish production in the lake is estimated to 13,000 tons per annum, but the latest landings data from 1996 estimated a total catch of only 1,000 ton (Berhanu et al. 2001). The reasons for the low production may be artisanal fishing technique[1] (Fig. 2), environmental[2] or the exceptionally low fish consumption in the country[3] estimated to only 200 g capita^{-1} year^{-1} (Berhanu et al. 2001; Gordon et al. 2007). The fish consumption in Addis Ababa is higher than the national average with around 900 g capita^{-1} year^{-1}, and near production areas the per capita consumption is 8.5 kg year^{-1} (Breuil1995). Fishery is considered to be a potentially increasing industry to get protein to

[1] There are few motorized boats and most fishermen use reed (papyrus) boats, *Tanqwas*. Difficulties in obtaining decent fishing gear forces use of artisanal material such as gill nets, cast nets baskets, traps, and line fishing (Gordon et al. 2007).

[2] Polluted shorelines force the fishermen to travel further out from shore to catch a substantial amount of fish (Berhanu et al. 2001).

[3] The Ethiopian population is historically and culturally meat eating, but during fasting months and two days a week meat is not allowed (Gordon et al. 2007).

a growing population around the lake, but also to the capital Addis Ababa and other urban areas, which have mainly been supplied from the southern Rift Valley lakes, which are now heavily overexploited (Gordon et al. 2007).

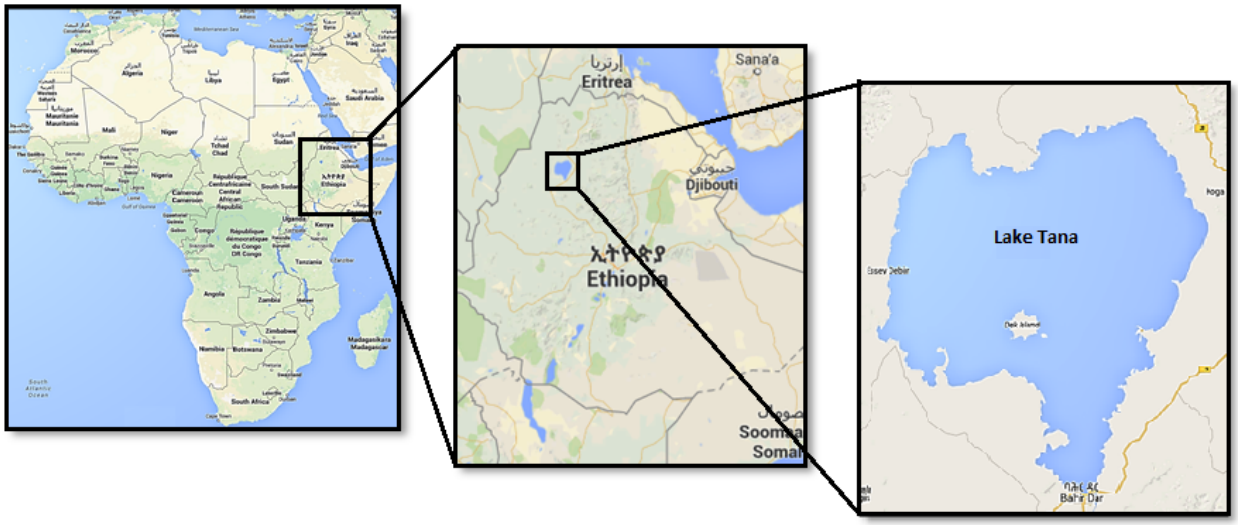

Figure 1 Location of Lake Tana, Ethiopia, Africa (Google maps, 2015).

Figure 2 Fishermen of Lake Tana in Tanqwas, traditional reed boats made of papyrus (Sjöholm 2014).

Lake Tana faces several environmental threats like untreated wastewater, deforestation, erosion and reduction in water level that impacts the chemistry and biology of the lake (Dejen 2003). Since the commercial fish in the rift valley lakes show high Hg concentrations compared to other East African water bodies (Black et al. 2011) there exist valid reasons to investigate the loading of PBTs in Lake Tana.

1.2 MERCURY

Hg emissions in the environment from natural sources [4] are comparable to those from anthropogenic sources[5] (Pacyna et al. 2006). Hg is mainly emitted into the atmosphere, where it can be carried over great distances and deposited in soil, water and vegetation far from its emission origin (Pacyna et al. 2006). Via anaerobic organisms inorganic Hg transforms into the organic compound methylmercury (MeHg), which absorbs and accumulates in the tissue of aquatic biota more efficiently than inorganic Hg (Mahaffey 1999). The health effects of Hg contamination are mainly neurological but may also cause damages on lungs, heart, liver, kidneys and skin (WHO 2013). To protect humans there are several health advisory limits where the European Union and the WHO have two thresholds. The estimated marketing limit is 0.5 μg Hg per gram fish muscle in wet weight (ww) and to protect vulnerable groups[6] a limit is set to 0.2 μg Hg per gram fish muscle ww (WHO 2013).

Comparing mean Hg concentrations in piscivorous freshwater fish around the world, the highest concentrations of the temperate zones were found in Swedish lakes (0.90 μg g^{-1} ww) followed by Northeast North America lakes (0.60 μg g^{-1} ww), Northwestern Canada lakes (0.40 μg g^{-1} ww) and US lakes/reservoirs (0.35 μg g^{-1}g ww) (Black et al. 2011). In the tropical zones, Rio Negra, Brazil, had higher concentrations (0.60 μg g^{-1} ww) than any African water body (0.10 μg g^{-1} ww). Black et al. (2011) also compared lakes in Eastern Africa and found that lake Tanganyika, Tanzania and Lake Awassa, Ethiopia had the highest concentrations with around 0.20 μg g^{-1} ww, while others had less than 0.10 μg g^{-1} ww. In Lake Awassa a study was made for the omnivorous African Big Barb (*L. intermedius*), where concentrations ranged from 0.01 to 0.94 μg g^{-1} ww with a mean of 0.285 μg g^{-1} ww (Desta et al. 2006). Studies on fish species *O. niloticus* (non-piscivorous) and *C. gariepinus* (omnivorous) in Lake Ziway, Ethiopia, showed mean concentrations of 0.011 and 0.033 μg g^{-1} ww respectively (Tadiso et al. 2011). These species are the most common commercial fish species in Lake Tana, where Hg concentrations in fish are yet to be evaluated.

1.3 PFASs

Most PFASs are man-made chemicals used in numerous commercial products such as paint, lubricants, paper, textiles, water and stain proofing agents (Berger et al. 2009; Ahrens & Bundschuh 2014). PFASs can be divided into three classes: perfluoroalkyl substances (PerFASs), polyfluoralkyl substances (PolyFASs) and fluorinated polymers (Ahrens & Bundschuh 2014). Food and drinking water, as well as breathing indoor air, are sources of PFAS exposure for humans, but the main exposure pathway is a fish and seafood diet (Borg & Håkansson 2012). PFASs are emitted continuously from production to disposal and around 95 % are released into the aquatic environment and 5 % into the atmosphere (Ahrens & Bundschuh 2014). PFASs can be transported long distances in both air and water and the long-chained perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs), belonging to PerFASs, are of particular concern (Labadie & Chevreuil 2011; Ahrens & Bundschuh 2014).

[4] Volcanoes, weathering of rocks, emissions from aquatic and terrestrial ecosystems etc. (Pacyna et al. 2006).

[5] Human activities such as artisanal small-scale gold mining, coal-fired power stations, cement production, dental amalgam and other wastes from consumer products in landfills (Pacyna et al. 2006).

[6] The mercury health effects affect pregnant women, infants and children the most and are therefore called the vulnerable groups (WHO 2013).

These groups are also suggested to have similar transport and bioaccumulation patterns as Hg (Ahrens et al. 2010).

PFASs in fish have not been as widely studied as for Hg, and most publications focus on perfluorooctane sulfonate (PFOS), which was banned from production in 2009, but is still found in animals and the environment worldwide (Baduel et al. 2014; Ahrens & Bundschuh 2014). Liver samples of Tilapia and Japanese perch from Taiwan had mean PFOS concentrations of 310 and 260 ng g^{-1} ww respectively (Tseng et al. 2006), but in India fish liver samples ranged only from 0.248 to 27.9 ng g^{-1} (Yeung et al. 2009). However, the concentration level depends on the emission sources and fish species and another study examined the influence of atmospheric deposition on the PFAS concentrations in fish from high mountain lakes in France (Ahrens et al. 2010). They found mean PFOS concentrations of 3.61-4.24 ng g^{-1} ww and comparison of PFASs with mercury concentrations showed a positive correlation between total Hg and ∑PFCAs[7] (Ahrens et al. 2010).

1.4 ACCUMULATION OF POLLUTANTS IN AQUATIC FOOD CHAINS

An aquatic organism's intake of pollutants can either be directly from the water or through diet (Deribe et al. 2011). However, even though many pollutants are efficiently accumulated in the bottom of the aquatic food chain (microorganisms, plankton and algae), the efficiency in transfer to the next trophic level is determined by the loading of pollutants in the water, how efficiently the pollutant is bioaccumulated in the organisms and length of the aquatic food chain (Morel et al. 1998; McLeod et al. 2014). At the top of the food chain (mostly piscivorous fish) there are also individual differences in concentrations, which depend on growth rate, dietary shifts, sex, habitats and seasonal weight losses (McLeod et al. 2014). The bioaccumulation of persistent organic pollutants (POPs) is slightly different than for metals. POPs are lipophilic and accumulate therefore favorably in lipids and are greater in fish with high lipid content (Sharma et al. 2009; Deribe et al. 2011). This is also true for MeHg in mammals and birds, but differs in fish, which seems to have a selectivity of the intestine wall regarding MeHg absorption and MeHg is therefore more concentrated in the muscle tissue (Morel et al. 1998). One aspect regarding Hg in fish, compared with other trace metals, is 'growth enrichment' which means that larger fish contain higher concentrations of pollutants and mercury is thus generally accumulated in fish in proportion to its size (Wang 2012). Another aspect is 'growth dilution', where fast growing fish species have lower Hg concentrations than slow growing ones (Wang 2012).

Lake Tana's food chain is ranging from two to five trophic levels and typical pathways may look like this (Wondie et al. 2012):

- Phytoplankton < herbivorous zooplankton < small Labeobarbus species < piscivorous Labeobarbus species
- Phytoplankton < herbivorous zooplankton < zooplanktivore Labeobarbus species < African catfish
- Phytoplankton < herbivorous species < African catfish

[7] The sum of all substances in the perfluoroalkyl carboxylates group (Ahrens et al. 2010).

1.5 OBJECTIVES

Fish and seafood is an important exposure pathway of PFASs and Hg in humans. There is thus a need to evaluate the contamination of fish to estimate the impact of fish consumption on human health. This is especially important for the aquatic environment in developing countries such as around Lake Tana in Ethiopia. There is very little information available for Lake Tana on the PFAS and Hg contamination in fish, but also a hope that the Ethiopian population will consume more of the lake's fish production in the future. The main objectives of this study were therefore to:

- investigate the spatial distribution of Hg and PFAS concentrations in fish tissue from Lake Tana
- evaluate the Hg and PFAS concentrations between different fish species
- compare Hg and PFAS concentrations and accumulation patterns
- assess the human health risk with increased fish consumption.

2 MATERIALS AND METHODS

2.1 SAMPLING SITES

The sampling was performed in the period between 10/10-2014 and 25/10-2014 with support from the Fish and Research Center in Bahir Dar. The study site, Lake Tana, is located in the Amhara region in northwestern Ethiopia at around 1,800 meters above sea level. The climate in the region is "tropical highland monsoon" with two rainy seasons, one major between June and September and one minor between April and March. Annual mean rainfall in the Lake Tana catchment area (15,054 km^2) is around 1,300 mm. The lake itself has a shifting total area of 3,000-3,600 km^2, with a mean depth of 8 m (max 14 m). It is approximately 84 km long, 66 km wide and has a volume of 28 000 km^3. Some 61 rivers and streams feed the lake. Of these, four are perennial and contribute with more than 95 % of the inflow. The only natural outflow is the Abbay River (Blue Nile River) in the southeastern part of the Bahir Dar Gulf (Ligdi et al. 2010).

Due to the large lake size, the sampling sites were chosen to cover diverse environments like river inflows and outflow, wastewater outlets, agricultural and forested areas etc. Five spots were located in the southern part of the lake, the Bahir Dar gulf, and two in the northern part, close to the city of Gorgora (Fig. 3). In the south there were two wastewater outlets chosen, one from Bahir Dar prison (P) and one from Bahir Dar hospital (H). The areas surrounding both these wastewater outlets were around 4 m deep and characterized by much organic material such as macrophytes and papyrus (Table 1). Samples were taken near the Cherechera hydro electrical dam (C), which is located at the only lake outflow to the Blue Nile River. The fourth site was the Yegashu river inlet in the northeast part of the bay, with very shallow waters (depth 2 m) and surrounding agricultural land (Y). The fifth site in the south was the large forested Zegi peninsular (Z), where a wood industry was situated. In the northern part of the lake one site was located outside the city of Gorgora (G) and the other north of the city, close to the Dirma River inlet (D).

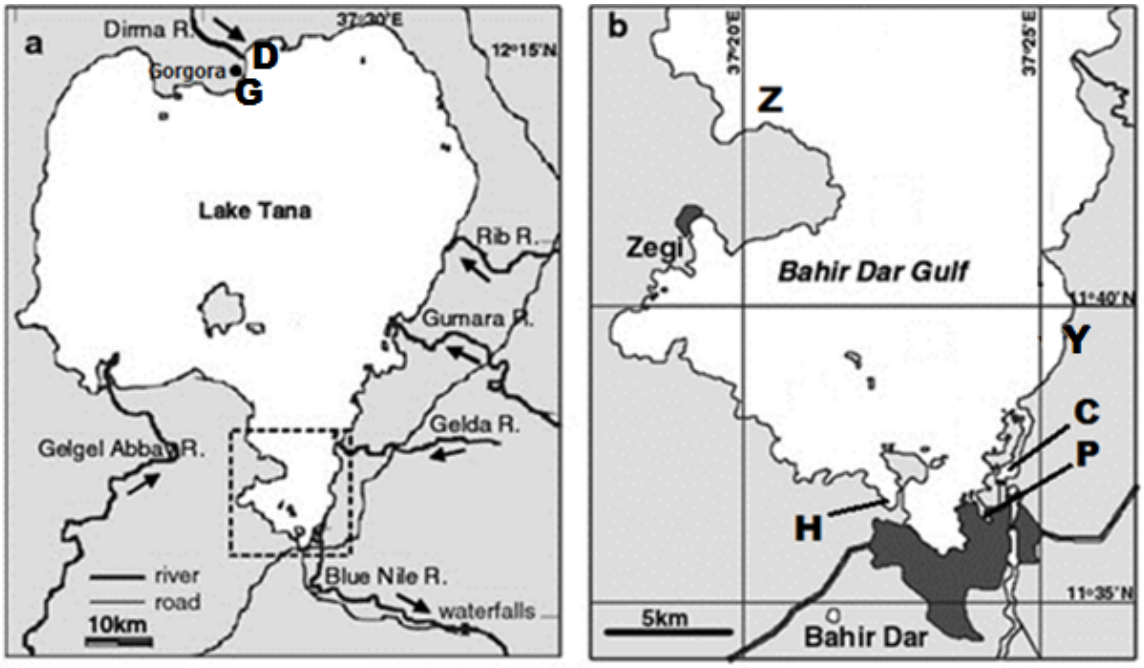

Figure 3 Sampling sites around Lake Tana (Modified after Dejen et al. 2009). With permission.
Cherechera (**C**). Outflow from Lake Tana to the Blue Nile River.
Dirma (**D**). Dirma River inlet close to the city Gorgora, located in northern Lake Tana.
Gorgora (**G**). Location outside city of Gorgora, located in northern Lake Tana.
Bahir Dar Hospital (**H**). Wastewater outlet from the Bahir Dar Hospital.
Bahir Dar Prison (**P**). Wastewater outlet from Bahir Dar prison and from the entire town.
Yegashu (**Y**). Yegashu river inlet. Agricultural land
Zegi Peninsular (**Z**). Forested peninsular with a wood industry.

Table 1 Geographic condition of the sampling sites (C, D, G, H, P, Y, Z) at Lake Tana

Site	GPS coordinates[a]		Elevation (MASL)[b]	Depth (m)	Sampling date
	North	East			
C	11° 37’ 17.1”	37° 24’ 34.1”	1792	4.0	11 10 2014
D	12° 15’ 41.4”	37° 18’ 49.7”	1788	5.5	24 10 2014
G	12° 15’ 54.8”	37° 17’ 30.4”	1776	5.0	25 10 2014
H	11° 36’ 52.0”	37° 22’ 21.8”	1793	4.0	14 10 2014
P	11° 36’ 23.8”	37° 24’ 02.9”	1795	4.1	11 10 2014
Y	11° 37’ 42.3”	37° 25’ 26.9”	1794	2.2	13 10 2014
Z	11° 43’ 08.6”	37° 20’ 09.6”	1792	6.0	14 10 2014

[a] WGS84 coordinate system was used.
[b] MASL = Meters above sea level.

2.2 SAMPLING

Five species were used in this study; *Labeobarbus megastoma* (*L. megastoma*), *Labeobarbus intermedius (L. intermedius*), *Labeobarbus gorguari* (*L. gorguari*), *Clarias gariepinus* aka African catfish (*C. gariepinus*) and *Oreochromis niloticus* aka Nile Tilapia (*O. niloticus*) (Fig. 4). The three Labeobarbus species were chosen due to their habitats and occurrence in the lake as well as their varying feeding habits, where *L. megastoma* and *L. gorguari* are piscivorous and *L. intermedius* is omnivorous (Table 2). The herbivorous *O. niloticus* is the most important fish in Ethiopia and stands for 60 % of the commercial fishery in the country as well as 30 % of the fishery in Lake Tana (Tadesse 2011). The omnivorous *C. gariepinus* is also an important commercial fish as it is fast growing and thus a large protein source (Desta et al. 2007).

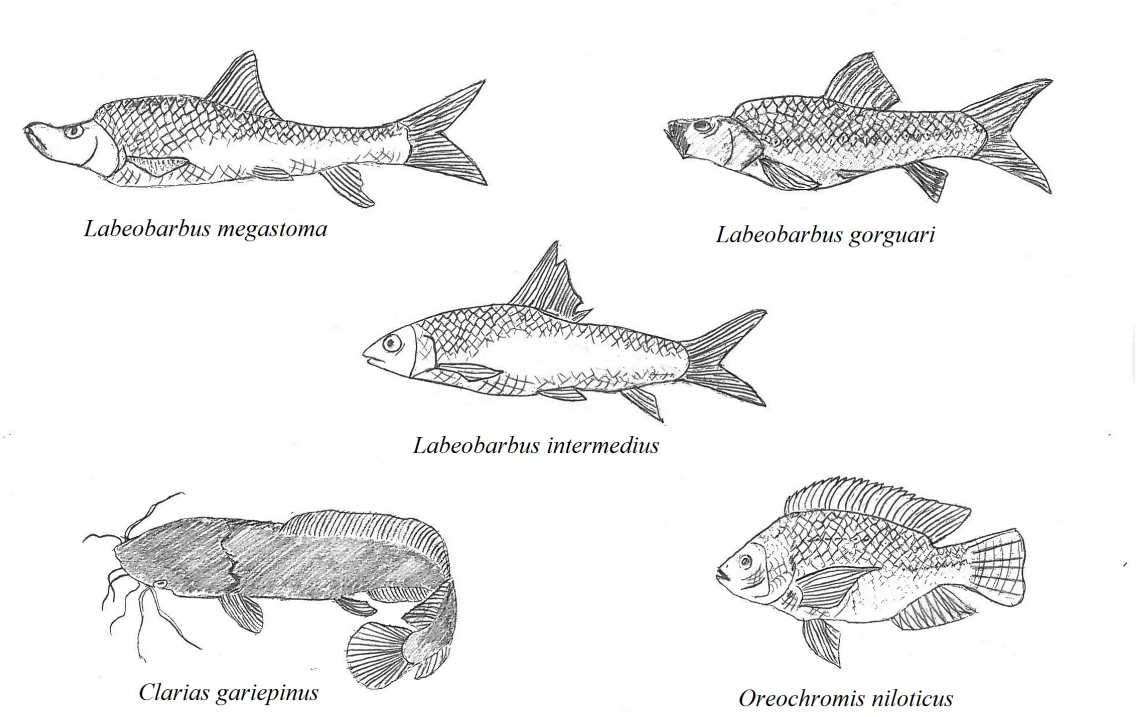

Figure 4 Sampled fish species, *Labeobarbus megastoma, Labeobarbus intermedius, Labeobarbus gorguari, Clarias gariepinus* and *Oreochromis niloticus* from Lake Tana (Sjöholm 2015).

Table 2 Habitat and diet of the five chosen fish species from Lake Tana

Specie	Habitat	Diet	
Labeobarbus megastoma [b] (82 cm) [a]	Endemic Lake Tana Littoral (~35 %) Sub littoral (~20 %) Off shore (~45 %)	Specimen <20 cm Fish 20 % (<10cm) Fish 65 %, (10-20 cm) Insects and insect larvae (remaining %)	Specimen >20 cm Fish (85 %) Insects (2 %) Detritus (7 %) Macrophytes (6 %)
Labeobarbus intermedius[c] (48.9 cm)	Widely spread in Ethiopia Littoral (~60 %) Sub littoral (~25 %) Off shore (~15 %)	Specimen <20 cm Insects (15 %) Detritus (35 %) Phytoplankton (50 %)	Specimen >20 cm Insects (10 %) Detritus (55 %) Phytoplankton (5 %) Zooplankton (15 %) Other (15 %)
Labeobarbus gorguari[b] (53 cm)	Endemic Lake Tana Littoral (~75 %) Sub littoral (~15 %) Off shore (~10 %)	Specimen <20 cm Fish 20 % (<10 cm) Fish 50 % (10-20 cm) Insects and insect larvae (~50 %)	Specimen >20 cm Fish (80 %) Detritus (15 %) Macrophytes (5 %)
Clarias gariepinus[d] (170 cm)	Widely spread in Ethiopia Smaller species in littoral zones Larger species in open water	Specimen <40 cm Fish (15 %) Insects (25 %) Detritus (30 %) Macrophytes (15 %) Zooplankton (15 %)	Specimen >40 cm Fish (37 %) Insects (15 %) Detritus (15 %) Macrophytes (3 %) Zooplankton (30 %)
Oreochromis niloticus[e] (60 cm)	Widely spread in Ethiopia Most in shallow littoral zones	Specimen <10 cm Detritus (10 %) Zooplankton (20 %) Algae (40 %) Insects (30 %)	Specimen >10 cm Detritus (20 %) Zooplankton (20 %) Algae (20 %) Insects (40 %)

[a] Maximum recorded length (Froese & Pauly 2015)
[b] (de Graaf 2003; Getahun & Dejen 2012)
[c] (Dadebo et al. 2013)
[d] (Dadebo 2000; Desta et al. 2007)
[e] (Tadesse 2011)

The fish were collected from professional fishermen around the area of the sampling spots (C, D, G, H, P, Y, Z). If the number of fish caught *in situ* was not sufficient, fishermen were hired to put out nets and bring the catch to the Fish and Research Center. After delivery the fish were weighed and measured (standard length) and directly dissected where a muscle sample was taken above the dorsal line, in between the dorsal and adipose fin. The samples were then carefully wrapped into aluminum foil and packed into a zip lock plastic bag together with an identification card. These samples were then frozen to -20 ºC awaiting transportation to Sweden.

During the fish-sampling period in Lake Tana a total of 97 fish specimens were collected with a species composition that differed between sampling sites (Table 3). Of all species collected *O. niloticus* was the only species found at all sites followed by *C. gariepinus* that was found at all but one site. The most diverse fish species composition was found at sites Z and H where all species were collected. Site Z also had most specimens in total followed by Y > C > H > D > G and P. At site P only 2 species were found (*C. gariepinus* and *O. niloticus*).

Table 3 Fish species and total number of fish specimens caught at seven sampling sites (C, D, G, H, P, Y, Z) in Lake Tana[a]

Site	*L. megastoma*	*L. intermedius*	*L. gorguari*	*C. gariepinus*	*O. niloticus*	Total
C	8	N/A	3	N/A	3	14
D	N/A	5	N/A	4	3	12
G	4	5	N/A	1	2	12
H	2	1	1	5	5	14
P	N/A	N/A	N/A	4	3	7
Y	3	5	N/A	7	2	17
Z	6	4	2	5	4	21
Total	23	20	6	26	22	**97**

[a] N/A = Not available. For details of the sampling sites see Fig. 3.

The average weight and standard length showed that *C. gariepinus* was on average the largest and heaviest fish, followed by *L. megastoma* (Table 4). *L. intermedius* and *L. gorguari* were almost equal in both length and weight and the smallest fish on average was *O. niloticus*. A complete description of sites and fish species weights, and lengths can be seen in Appendix 1.

Table 4 Average weight and standard length (mean ± SD) for fish species in Lake Tana

Species	Amount (*n*)	Average weight (g)	Standard length (cm)
L. megastoma	23	348 ± 73.7	28.8 ± 2.33
L. intermedius	20	187 ± 55.1	22.0 ± 1.88
L. gorguari	6	193 ± 39.6	22.0 ± 1.75
C. gariepinus	26	369 ± 101	33.1 ± 3.52
O. niloticus	22	161 ± 25.7	17.5 ± 1.14

2.3 LABORATORY ANALYSES

2.3.1 Mercury

All 97 collected fish specimen were used for Hg analysis. The analysis was made with a Perkin Elmer SMS 100 Solid Mercury analyzer, which uses thermal decomposition, gold amalgamation and cold vapor atomic absorption spectrometry for the mercury measurements. The analysis was always begun with two blank samples and two calibration standards to make sure the machine was functioning correctly. Fish samples analyzed were weighed in at around 10 mg dry weight and every fifth sample was duplicated to validate that the range between the same samples wasn't greater than ±10 %. The data output from the analysis was in ng absolute Hg or in ng g^{-1} dry weight Hg. The wet content of the individual fish muscles was determined by weighing fish samples before and after drying (freeze drying for ~48 hours). Fish Hg concentration was reported based on wet weight after normalization to wet content.

2.3.2 PFASs

For the PFAS analysis only 30 fish specimens were chosen among the 97 specimens collected in total from Lake Tana (Table 5). Two to three *O. niloticus* were chosen from all the sites giving a total of 19 specimens to investigate the spatial distribution of the PFAS contamination at Lake Tana. In addition, from site Z, two to three different species were chosen with a total of 14 samples to investigate interspecies differences in the PFAS contamination.

Table 5 Fish species used for PFAS analysis from sampling sites (C, D, G, H, P, Y, Z) in Lake Tana[a]

Sites	*L. megastoma*	*L. intermedius*	*L. gorguari*	*C. gariepinus*	*O. niloticus*	Total
C	-	N/A	-	N/A	3	3
D	N/A	-	N/A	-	3	3
G	-	-	N/A	-	2	2
H	-	-	-	-	3	3
P	N/A	N/A	N/A	-	3	3
Y	-	-	N/A	-	2	2
Z	3	3	2	3	3	14
Total	3	3	2	3	19	**30**

[a] N/A = Not available. For details of the sampling sites see Fig. 3.

In total 26 PFASs were included for analysis: four perfluoroalkane sulfonates (PFSAs) (PFBS, PFHxS, PFOS, PFDS), 13 perfluoroalkyl carboxylates (PFCAs) (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFHxDA, PFOcDA), three perfluorooctane sulfonamides (FOSAs) (FOSA, N-MeFOSA N-EtFOSA), two perfluorooctane sulfonamidoethanols (FOSEs) (N-MeFOSE, N-EtFOSE), three perfluorooctane sulfon-amidoacetic acids (FOSAAs) (FOSAA, MeFOSAA, N-EtFOSAA) and one fluorotelomer carboxylate (6:2 FTSA) (Table 6). In addition, 16 internal standards were used which were spiked before extraction (i.e. $^{13}C_8$-FOSA, d3-N-MeFOSAA, d5-N-EtFOSAA, d3-N-MeFOSA, d5-N-EtFOSA, d7-N-MeFOSE, d9-N-EtFOSE, $^{13}C_4$ PFBA, $^{13}C_2$ PFHxA, $^{13}C_4$ PFOA, $^{13}C_5$

PFNA, $^{13}C_2$ PFDA, $^{13}C_2$ PFUnDA, $^{13}C_2$ PFDoDA, $^{18}O_2$ PFHxS, $^{13}C_4$ PFOS) and 1 Injection standard (InjS) was used ($^{13}C_4$ PFOA).

Table 6 The 26 targeted PFAS analytes with acronyms

PFASs	Acronym	Name
perfluoroalkane sulfonates (PFSAs)	PFBS	perfluorobutane sulfonate
	PFHxS	perfluorohexane sulfonate
	PFOS	perfluorooctane sulfonate
	PFDS	perfluorodecane sulfonate
perfluoroalkyl carboxylates (PFCAs)	PFBA	perfluorobutanoate
	PFPeA	perfluoropentanoate
	PFHxA	perfluorohexanoate
	PFHpA	perfluoroheptanoate
	PFOA	perfluorooctanoate
	PFNA	perfluorononanoate
	PFDA	perfluorodecanoate
	PFUnDA	perfluoroundecanoate
	PFDoDA	perfluorododecanoate
	PFTriDA	perfluorotridecanoate
	PFTeDA	perfluorotetradecanoate
	PFHxDA	perfluorohexadecanoate
	PFOcDA	perfluorooctadecanoate
perfluorooctane sulfonamides	FOSA	perfluorooctanesulfonamide
	N-MeFOSA	*N*-methylperfluorooctansulfonamide
	N-EtFOSA	*N*-ethylperfluorooctanesulfonamide
perfluorooctane sulfonamidoethanols	N-MeFOSE	*N*-methylperfluorooctanesulfonamido-ethanol
	N-EtFOSE	*N*-ethylperfluorooctanesulfonamido-ethanol
perfluorooctane sulfonamidoacetic acids	FOSAA	perfluorooctanesulfonamidoacetic acid
	N-MeFOSAA	*N*-methylperfluorooctanesulfonamidoacetic acid
	N-EtFOSAA	*N*-ethylperfluorooctanesulfonamidoacetic acid
x:2 fluorotelomer carboxylates	6:2 FTSA	6:2 fluorotelomer sulfonate

Extraction and analysis of PFAS content were done according to Ahrens et al. (2009). Before extraction all materials were rinsed with tap water, ethanol, deionized water, dish washed at 70 ºC, burnt at 400 ºC and rinsed with methanol before usage in order to reduce blank contamination. An aliquot of fish muscle (2 ± 0.2 g) were homogenized using Ultra-Turrax with a stainless steel probe in a 50 mL PP-tube. For extraction, the samples were spiked with 100 μL of IS-standard mix (20 pg/μL) and 8 mL methanol was added. After wrist-action shaking for 30 min at 200 rpm and centrifuging for 10 min at 3000 rpm the supernatant was decanted into a new 50 mL PP-tube. The extraction was repeated with 4 mL of methanol, and after shaking and centrifugation the supernatant was combined with the first fraction. The sample was then concentrated to 1 mL under N_2. For the clean-up, the 1 mL sample extract was transferred into a 1.7 mL PP-tube containing 25 mg ENVI-Carb and 50 μL glacial acetic acid. The 1.7 mL PP-tube

was vortexed for 30 seconds and then centrifuged for 15 min at 4,000 rpm. Lastly, 0.5 mL of the supernatant solution was transferred into an autoinjector brown glass vial (Eppendorf) and spiked with 10 μL of InjS (200 pg/L). The extraction also included five laboratory blanks, which were prepared in the same way as the fish samples but without the fish muscle tissue. The PFAS analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Ahrens et al. 2009).

2.4 DATA AND STATISTICAL ANALYSES

The concentration values did not follow a normal distribution hence normalization to logarithmic values was performed before statistical analysis. The statistical tool *Analysis of variance* (ANOVA) (EXCEL 2013) was used to determine if the mean values of different groups had significant variations. Values were first normalized and the significance level was 5 %, thus $p < 0.05$ significance, $p > 0.05$ no significance. The F-value is the test statistic in ANOVA and is the ratio between the variations among groups and the variation within groups. A high F-value indicates that the variation among groups is actually caused by one of the given variables rather than chance. All results of the ANOVA analyses can be found in Appendix 2.

2.4.1 Mercury

Mean and standard deviation (SD) values were compiled as well as box-whiskers diagrams to understand the distribution between species and sites as well as the skewness of data. A regression analysis with normalized values was made with all species to see the differences between species and possible accumulation patterns. The regression equation ($y = rx + m$), coefficient of determination (R^2) and significance level ($p = 0.05$) were included in the charts. The regression coefficient (r) is the slope of the regression line and explains the rate of change of the function and m is the functions intersection of the y-axis. To examine how the accumulation varies between sites *L. megastoma* was chosen for this purpose, since it showed the highest significance, and dependence values were found in all sites except D and P.

2.4.2 PFASs

After analysis only the detected PFASs (PFNA, PFDA, PFUnDA, PFDoDA, PFTeDA, PFBS, PFOS) of the 26 targeted were included in further data processing. All data for PFAS analysis is compiled in Appendix 3. To be able to conduct a statistical analysis of the analyzed fish samples half of the detection limit was used for PFASs not detected in the fish species (zero-values). Mean and standard values were compiled for the individual detected PFASs as well as for the total sum of all PFASs (∑PFASs). To evaluate the composition profile in the different fish species and within sites a relative abundance chart where the PFASs percentage values were displayed. A cluster analysis was made to discover similarities between fish species and between sites with respect to the PFAS composition profile.

2.4.3 Comparison between PFAS and Mercury concentrations

To compare PFAS and Hg concentrations with each other, the same specimens were selected from the Hg analyzed species as the ones chosen for PFAS analysis. A Pearson correlation matrix was made for individual PFASs and ∑PFCAs, ∑PFSAs, ∑PFASs, and Hg. The Pearson correlation coefficient (R) measures the dependence between two variables and gives a value between -1 and +1, where ±0.5-1 is high correlation, ±0.3-0.5 is medium correlation and ±0.1-0.3 is low correlation.

2.5 HUMAN HEALTH ASSESSMENT

To evaluate the uptake of PFASs and Hg by humans with varied fish species consumption from Lake Tana, a hazard ratio was calculated. First an average daily intake (ADI) [μg g^{-1} d^{-1}] was estimated according to the equation

$$ADI = substance\ concentration \cdot fish\ consumption \quad (1)$$

where the substance concentration can be either for PFAS or Hg in ng g^{-1}, and the fish consumption in g $kgbw^{-1}$ d^{-1} (Assuming body weight of 60 kg). Then the hazard ratio could be calculated with the equation

$$Hazard\ ratio\ (HR) = {}^{ADI}/_{Reference\ Dose\ (RfD)} \quad (2)$$

where a HR value greater than 1 suggests that the average exposure level exceeds the benchmark concentration. RfD can be either for PFAS or Hg in μg g^{-1} d^{-1}.

These calculations were done for two scenarios, one contemporary and one prospective with a 44 % increased fish consumption in the country (Gordon et al. 2007). Also different country averages were included with contemporary consumptions of 0.67 g d^{-1} (national average), 2.47 g d^{-1} (Addis Ababa average) and 27.4 g d^{-1} (production area average) (Breuil 1995). There are no RfDs for other PFASs than PFOS, hence only PFOS was included and an assumption was made that Hg = MeHg. The environmental working group (EWG) in the United States has a PFOS RfD of 25 ng kg bw^{-1} d^{-1} and The German Federal institute for Risk Assessment (BfR) has a PFOS RfD of 100 ng kg bw^{-1} d^{-1} (Tao et al. 2008). The European Food Safety Authority (EFSA) has set a RfD of 185 ng kg bw^{-1} d^{-1} (EFSA 2012) and WHO has a MeHg RfD of 229 ng kg bw^{-1} d^{-1} (FAO 2011). For the substance concentrations a Hg mean of 137 ng g^{-1} was used (all specimens included) and for PFOS the mean was 0.137 ng g^{-1} (30 species included).

3 RESULTS

3.1 MERCURY CONCENTRATIONS

Mean Hg concentration within fish species were highest in *L. megastoma* followed by *L. gorguari* > *L. intermedius* > *C. gariepinus* and *O. niloticus* and with significant differences between fish species (ANOVA, F = 123, $p < 0.0001$) (Table 7). Mean Hg concentration at sampling sites were highest at site C followed by Z > G > Y > H > D and P (ANOVA, F = 3.42, $p < 0.004$). The distribution and skewness of Hg concentrations can be seen in Figure 5.

Table 7 Hg concentrations (mean ± SD ng g^{-1} ww) for individual fish species at sampling sites (C, D, G, H, P, Y, Z) in Lake Tana[a]

Site	*L. megastoma*	*L. intermedius*	*L. gorguari*	*C. gariepinus*	*O. niloticus*	Mean
C	404 ± 66.3	N/A	249 ± 26.2	N/A	4.77 ± 1.51	313 ± 106
D	N/A[a]	83.8 ± 10.9	N/A	63.1 ± 12.9	18.2 ± 5.27	60.5 ± 16.8
G	360 ± 57.9	58.5 ± 8.57	N/A	52.4	3.58 ± 1.30	149 ± 84.3
H	159 ± 6.82	25.7	603	15.8 ± 4.81	5.24 ± 1.75	75.1 ± 80.6
P	N/A	N/A	N/A	31.6 ± 8.22	3.76 ± 0.86	19.7 ± 9.46
Y	293 ± 40.4	31.2 ± 6.27	N/A	64.5 ± 25.4	0.99 ± 0.07	87.2 ± 54.6
Z	438 ± 69.3	50.0 ± 6.86	195 ± 8.92	61.2 ± 8.77	3.38 ± 1.75	177 ± 97.9
Mean	386 ± 71.9	54.4 ± 12.8	290 ± 79.6	48.8 ± 17.3	5.86 ± 3.26	

[a] N/A = Not available. For details of the sampling sites see Fig. 3.

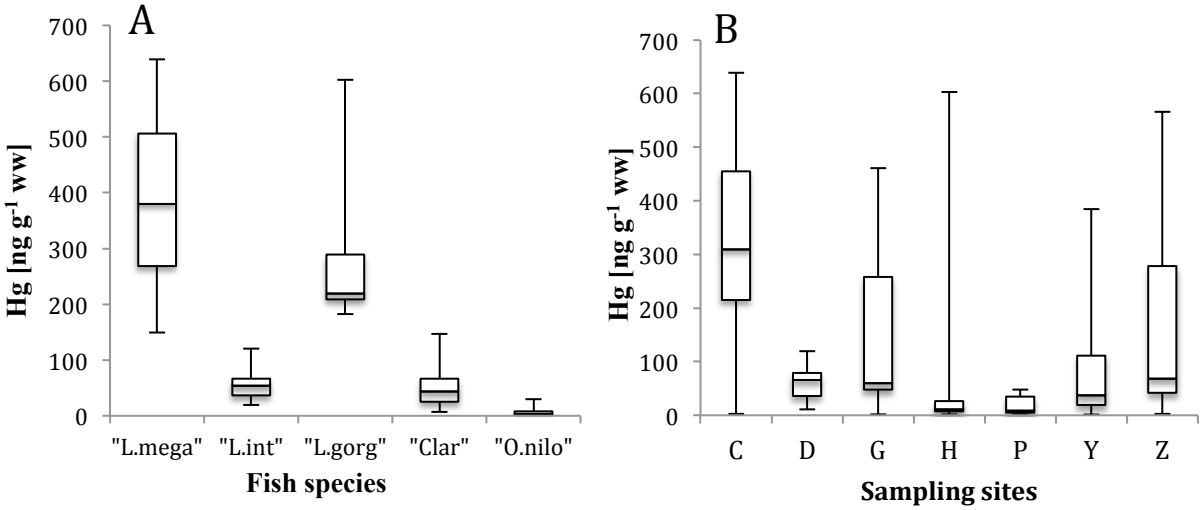

Figure 5 Box and whisker plots of Hg concentration for A) individual fish species and B) between sampling sites at Lake Tana. The box-whiskers indicate the maximum (upper error bar), 3rd-quartile (upper box), median (horizontal line), 1st-quartile and minimum values.

Regression analysis between fish size and Hg-concentrations showed that two species had a significant dependence, which indicate that accumulation of Hg is related to fish size (Fig. 6). The Hg accumulation rate, estimated from regression coefficient (r), was higher for *L. megastoma* (r = 1.9, R^2 = 0.60, $p < 0.001$) than *C. gariepinus* (r = 1.7 R^2 = 0.17, p = 0.0053). Insignificant dependences between fish length and Hg concentrations were given for *L. gorguari* (r = 2.1, R^2 = 0.58, p = 0.13), *O. niloticus* (r = 1.2, R^2 = 0.001, p = 0.90) and *L. intermedius,* which also was the only specie with a negative regression slope (r = -1.4, R^2 = 0.17, p = 0.18).

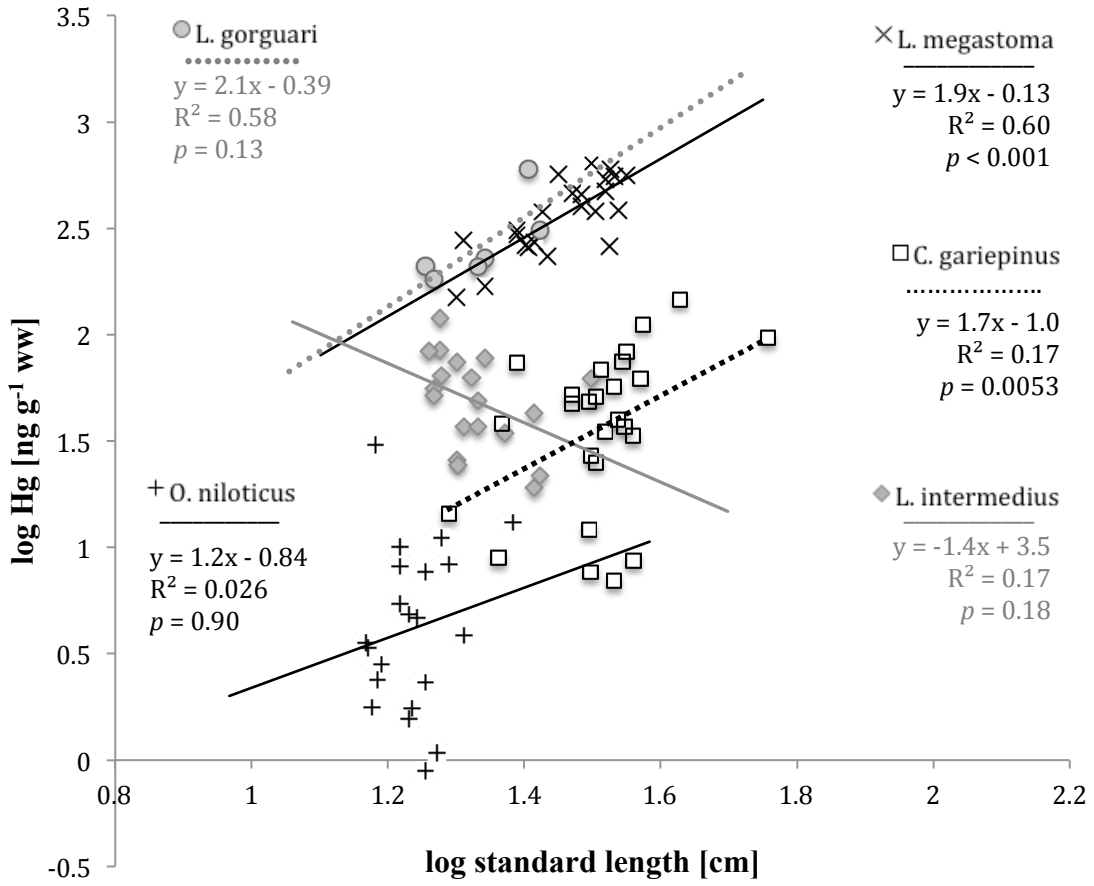

Figure 6 Regression chart of standard length (cm) against Hg concentration (ng g^{-1} ww) made for the fish species *L. megastoma*, *L. intermedius*, *L. gorguari*, *C. gariepinus* and *O. niloticus,* from Lake Tana.

The Hg accumulation rate for *L. megastoma,* which was sampled at five sites, varied significantly (ANOVA, F = 5.13, p = 0.006) (Fig. 7). Strongest dependence and highest Hg bioaccumulation rate were both found at site G (r = 3.3, R^2 = 0.97, p = 0.014). Sites Z and C also had significant but weaker dependences than site G (R^2 = 0.67, p = 0.049 and R^2 = 0.69, p = 0.024) and lower accumulation rates (r = 1.8 and r = 1.4). Site Y and H showed insignificant dependences.

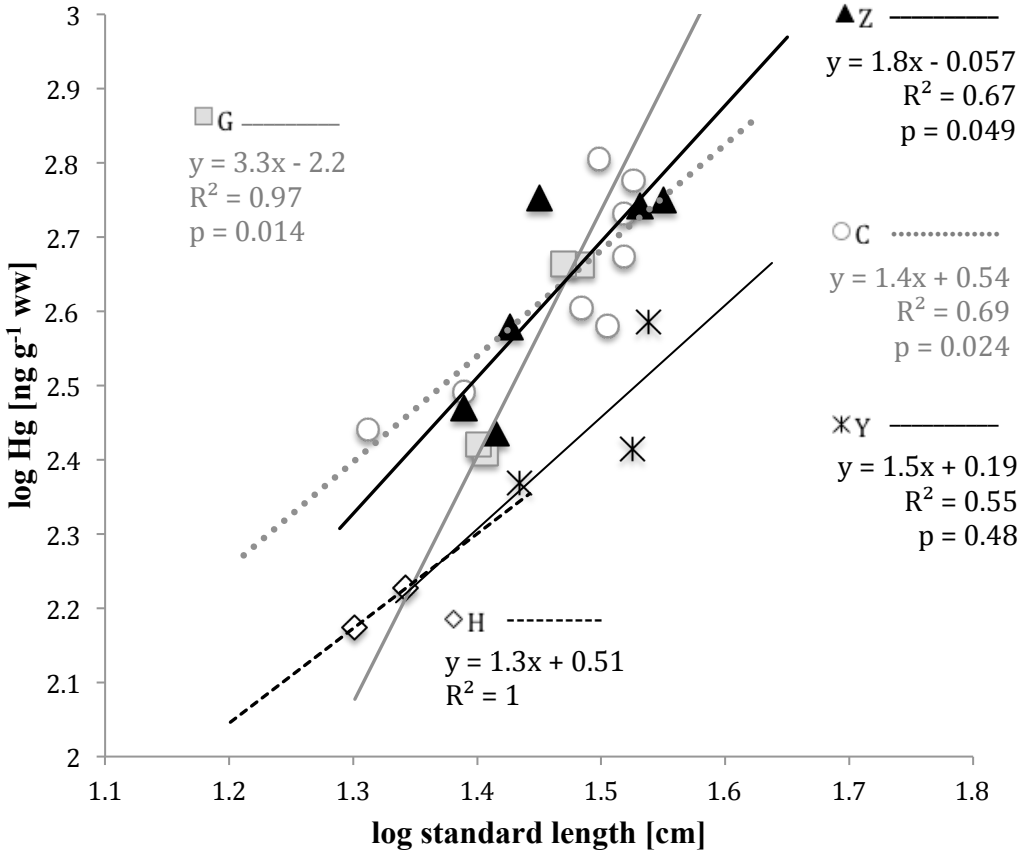

Figure 7 Regression chart for the fish species *L. megastoma* for standard length (cm) against Hg concentration (ng g^{-1} ww) from sampling sites (C, G, H, Y, Z) at Lake Tana.

3.2 PFAS CONCENTRATIONS

Between the five analyzed fish species, PFCA and PFSA concentrations differed significantly (ANOVA, F = 6.4, p = 0.018). PFDA was the PFCA that showed high concentrations for all species, with highest for *L. gorguari* (0.93 ± 0.33 ng g^{-1}) and lowest for *L. megastoma* (0.32 ± 0.076 ng g^{-1}) (Table 8). PFTeDA was not detected at site Z. Three species (*L. intermedius, C. garipenius, O. niloticus*) showed higher values of PFBS than of PFOS whilst *L. megastoma and L. gorguari* had higher PFOS concentrations. *L. gorguari* had the highest ∑PFAS concentration, followed by *L. megastoma* > *C. gariepinus* > *L. intermedius* and *O. niloticus*.

Table 8 PFCA, PFSA and ∑PFAS concentrations (mean ± SD ng g^{-1} ww) for all species from sampling site Z at Lake Tana[a]

Species	PFCAs					PFSAs		∑PFASs
	PFNA	PFDA	PFUnDA	PFDoDA	PFTeDA	PFBS	PFOS	
L. megastoma	<0.025	0.32 ± 0.076	0.41 ± 0.051	0.18 ± 0.030	<0.01	<0.025	0.77 ± 0.037	1.7 ± 0.19
L. intermedius	0.28 ± 0.19	0.57 ± 0.27	0.11 ± 0.096	<0.01	<0.01	0.21 ± 0.13	0.046 ± 0.029	1.2 ± 0.48
L. gorguari	0.16 ± 0.10	0.93 ± 0.33	0.31 ± 0.22	<0.01	<0.01	0.16 ± 0.11	0.58 ±0.017	2.1 ± 0.34
C. gariepinus	0.064 ± 0.044	0.53 ± 0.22	0.11 ± 0.089	0.51 ± 0.43	<0.01	0.50 ± 0.32	<0.025	1.7 ± 0.84
O. niloticus	0.120 ± 0.095	0.42 ± 0.19	<0.005	0.16 ± 0.13	<0.01	0.22 ± 0.15	0.045 ± 0.019	0.97 ± 0.40

[a] <x, below limit of detection.

The relative abundance between individual PFASs at the different species showed that PFDA was the major compound in the species *L. intermedius*, *L. gorguari* and *O. niloticus* with 45 %, 43 % and 40 % of the ∑PFASs, respectively (Fig. 8). *L. megastoma* and *L. gorguari* had higher percentages of PFOS (45 % and 28 %) and PFUnDA (25 % and 15 %) than the other three species. *C. gariepinus* had almost equal shares of PFDA, PFDoDA and PFBS. The cluster analysis gave close similarities between *L. intermedius* and *O. niloticus* and also for the two piscivores *L. megastoma* and *L. gorguari* (Fig. 9). *C. gariepinus* is more similar to the non-piscivores than the piscivores.

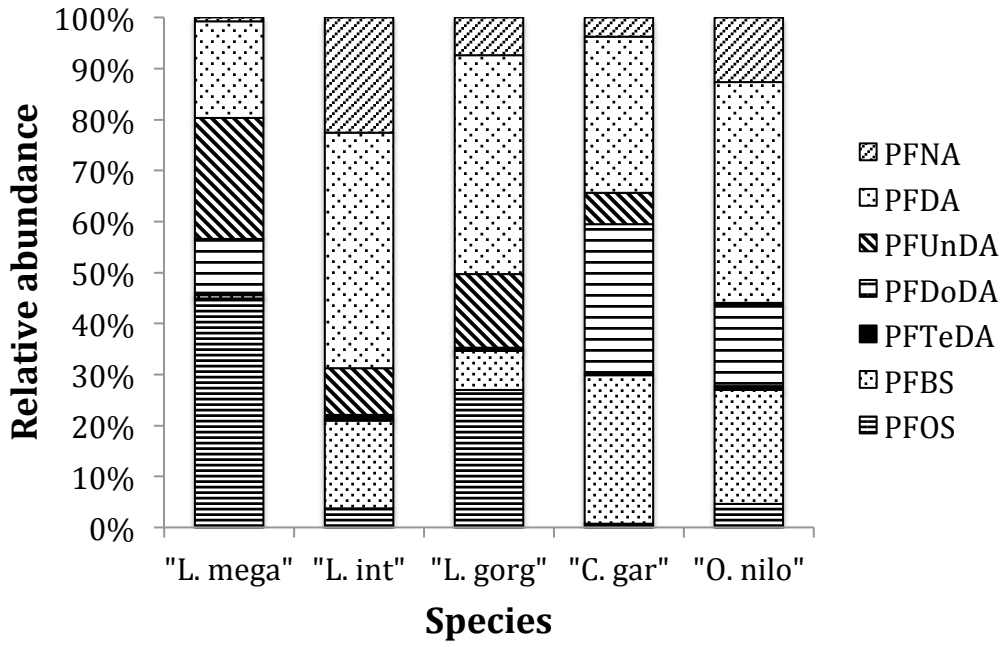

Figure 8 Relative abundance of PFASs in five sampled fish species (*L. megastoma*, *L. intermedius*, *L. gorguari*, *C. gariepinus*, *O. niloticus*) at Lake Tana.

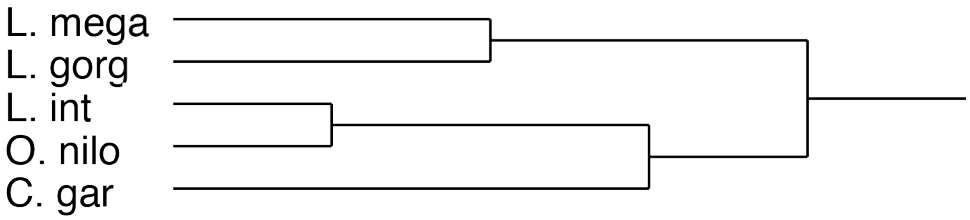

Figure 9 Cluster diagram of the PFAS distribution in five sampled fish species (*L. megastoma*, *L. intermedius*, *L. gorguari*, *C. gariepinus*, *O. niloticus*) at Lake Tana.

There was significantly higher PFCA concentrations compared to PFSA for *O. niloticus* at the seven sampling sites (ANOVA, F = 21, $p < 0.0001$). For the PFCAs, PFUnDA had the highest concentration (1.3 ± 0.70 ng g^{-1}) at site Y (Table 9). Overall PFDA had higher values (> 0.10 ng g^{-1}) in most sites compared to other PFCAs. For the two PFSAs, PFBS was generally found at higher concentrations (on average, 0.11 ± 0.12 ng g^{-1}) compared to PFOS (0.025 ± 0.012 ng g^{-1}). Site Y had the highest concentrations of PFCAs (0.37 ± 0.35 ng g^{-1}) followed by C (0.34 ± 0.44 ng g^{-1}) and Z (0.14 ± 0.12 ng g^{-1}). Highest concentrations of PFSAs were found at site C (0.18 ± 0.18 ng g^{-1}), followed by Z (0.13 ± 0.098 ng g^{-1}). The sites D, G, H and P showed in general low values for both PFCAs and PFSAs. The ∑PFASs gave the highest concentration in site C (2.1 ± 1.6 ng g^{-1}) followed by Y (1.9 ± 0.99 ng g^{-1}) and Z (0.97 ± 0.40 ng g^{-1}). Site D, G, H and P had the lowest concentrations.

Table 9 Individual PFCA and PFSA concentrations and ∑PFAS concentrations (mean ± SD ng g^{-1} ww) for *O. niloticus* at sampling sites (C, D, G, H, P, Y, Z) at Lake Tana [a]

Sites	PFCAs					PFSAs		∑PFASs
	PFNA	PFDA	PFUnDA	PFDoDA	PFTeDA	PFBS	PFOS	
C	0.47 ± 0.39	1.2 ± 0.93	<0.005	0.017 ± 0.0087	0.0093 ± 0.0024	0.34 ± 0.29	0.025 ± 0.0011	2.1 ± 1.6
D	<0.025	0.16 ± 0.058	0.012 ± 0.0080	0.011 ± 0.0038	<0.01	0.055 ± 0.022	0.038 ± 0.022	0.29 ± 0.047
G	<0.025	0.19 ± 0.019	0.037 ± 0.024	0.038 ± 0.022	<0.01	0.12 ± 0.031	<0.025	0.42 ± 0.048
H	<0.025	<0.05	0.040 ± 0.011	0.017 ± 0.0087	0.077 ± 0.0057	<0.025	0.020 ± 0.0069	0.20 ± 0.016
P	<0.025	0.089 ± 0.038	0.029 ± 0.016	0.017 ± 0.0088	0.097 ± 0.022	<0.025	<0.025	0.27 ± 0.047
Y	0.027 ± 0.010	0.51 ± 0.28	1.3 ± 0.70	<0.01	<0.01	<0.025	<0.025	1.9 ± 0.99
Z	0.12 ± 0.095	0.42 ± 0.19	<0.005	0.16 ± 0.13	<0.01	0.22 ± 0.15	0.045 ± 0.019	0.97 ± 0.40

[a] <x, below limit of detection. For details of the sampling sites see Fig. 3.

The relative abundance between all PFASs in *O. niloticus* at the different sites showed that PFDA was the dominant compound with 58 %, 55 %, 48 % and 41 % of the ∑PFASs at the sites C, D, G and Z, respectively (Fig. 10). Site Y contained 70 % of PFUnDA and 25 % of PFDA. At sites H and P, PFTeDA was the prominent compound but barely seen at any other site. Cluster analysis between the sites showed that there were close similarities between sites H, P and D, G (Fig. 11). Sites C and Z also showed similarities, but differed more than the sites close to cities.

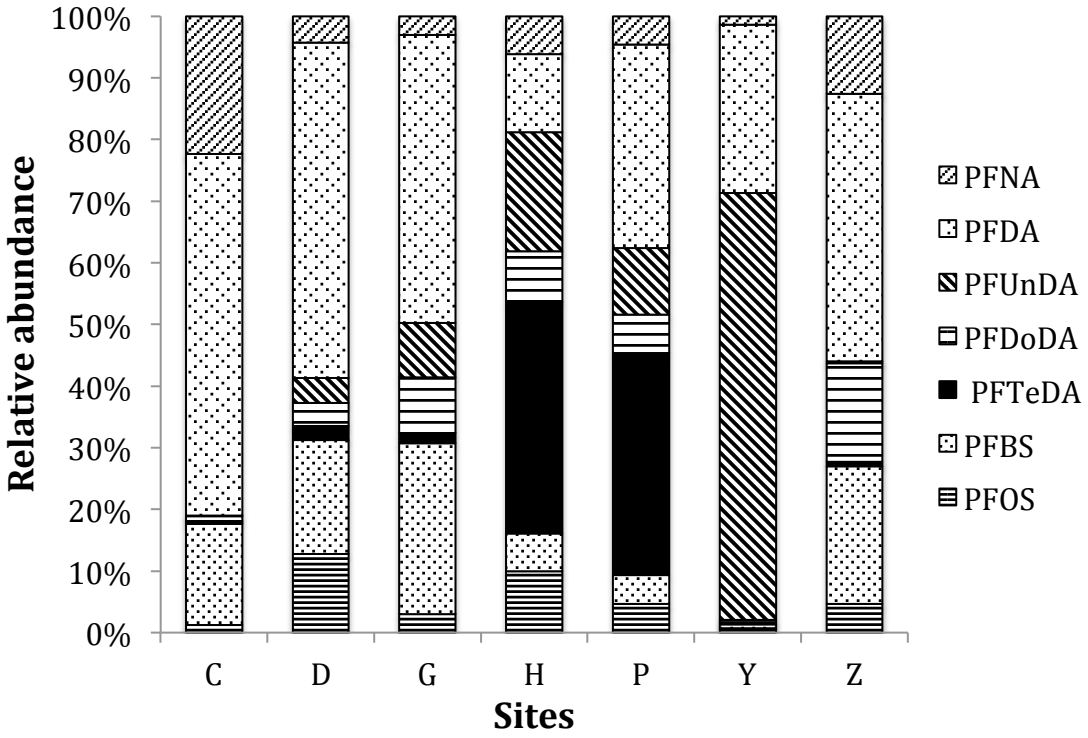

Figure 10 Relative abundance of PFASs in *O. niloticus* within sites (C, D, G, H, P, Y, Z) at Lake Tana. For details of the sampling sites see Fig. 3.

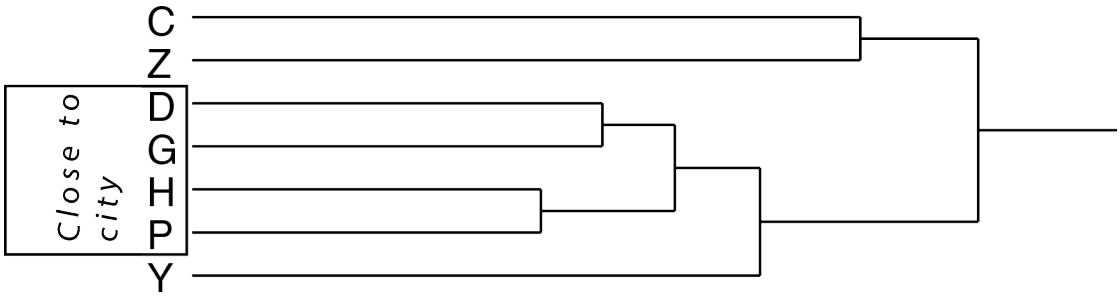

Figure 11 Cluster diagram of the PFAS distribution in *O. niloticus* between sites (C, D, G, H, P, Y, Z) at Lake Tana. For details of the sampling sites see Fig. 3.

3.3 COMPARISON BETWEEN PFAS AND MERCURY CONCENTRATIONS

The correlation matrix (Table 10) between Hg and PFASs showed that Hg and PFOS had a strong correlation (R = 0.94) and neither of them correlated with any other compound. For PFCAs, only PFNA showed a correlation with PFDA (R = 0.91). For the PFSAs, PFBS was correlated with three (R = 0.71 for PFNA, R = 0.71 for PFDA, R = 0.60 for PFDoDA) of the five PFCAs. ΣPFCAs showed a high correlation with PFNA (R = 0.78 and PFDA (R = 0.90) and also with PFBS (R = 0.71). ∑PFSAs was correlated (R = 0.50 - 0.75) with all compounds except PFUnDA and PFTeDA, which don't correlate with any group or compound.

Table 10 Pearson correlation coefficients (R) for individual PFASs and ∑PFCAs, ∑PFSAs, ∑PFASs, and Hg[a]

	PFCAs					PFSAs		ΣPFCA	ΣPFSA	ΣPFAS	Hg
	PFNA	**PFDA**	**PFUnDA**	**PFDoDA**	**PFTeDA**	**PFBS**	**PFOS**				
PFNA	1										
PFDA	0.91	1									
PFUnDA	-0.13	0.058	1								
PFDoDA	-0.013	0.044	-0.069	1							
PFTeDA	-0.17	-0.27	-0.17	-0.14	1						
PFBS	0.71	0.71	-0.20	0.60	-0.23	1					
PFOS	-0.093	0.043	0.20	0.052	-0.23	-0.16	1				
ΣPFCA	0.78	0.90	0.39	0.26	-0.29	0.71	0.090	1			
ΣPFSA	0.52	0.62	-0.028	0.53	-0.35	0.71	0.58	0.65	1		
ΣPFAS	0.77	0.89	0.31	0.36	-0.33	0.76	0.23	0.98	0.79	1	
Hg	-0.091	0.031	0.19	0.11	-0.23	-0.12	0.94	0.096	0.57	0.23	1

[a] Black color indicates coefficients between 0.75-1 and gray color coefficients between 0.5-0.75.

In Figure 12, PFOS and Hg concentrations were correlated for all 30 analyzed specimens and normalized in logarithmic scale. The regression analysis showed significance, but without a strong dependence ($R^2 = 0.55$, $p < 0.0001$) between PFOS and Hg when all specimens were included. A significant and strong dependence ($R^2 = 0.93$, $p < 0.0001$) was whereas found when excluding samples (19 out of 30) which were below the detection limit for PFOS (Fig. 13).

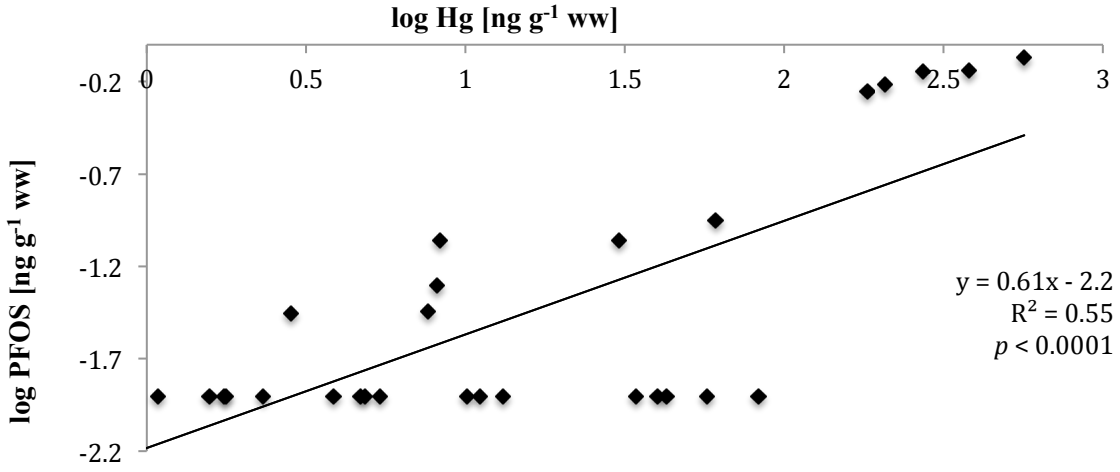

Figure 12 Regression chart for 30 fish specimen with PFOS (ng g^{-1} ww) against Hg concentration (ng g^{-1} ww) from sampling sites (C, D, G, H, P, Y, Z) at Lake Tana. Note: PFOS concentrations were below method detection limit (MDL) in 19 of 30 samples and the values were replaced by 0.0125 ng g^{-1} ww (log value = -1.90 ng g^{-1} ww).

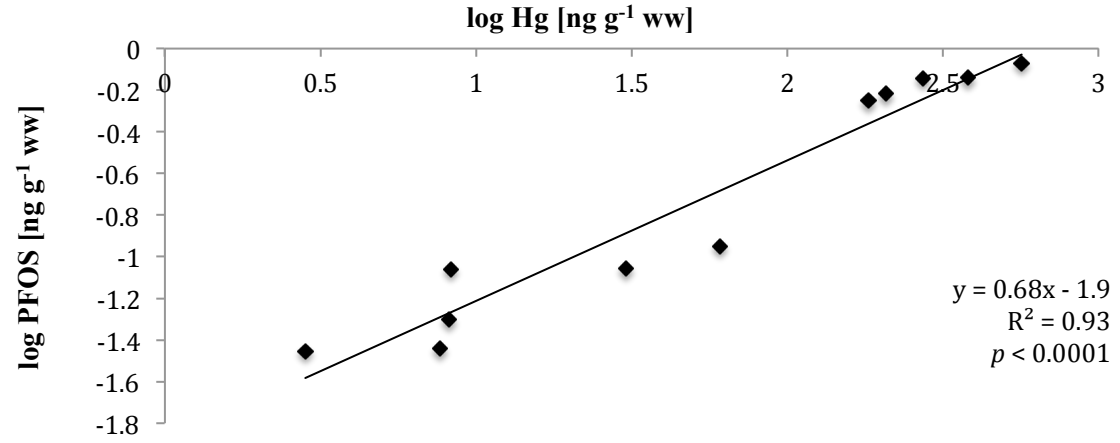

Figure 13 Regression chart for PFOS (ng g^{-1} ww) against Hg concentration (ng g^{-1} ww) from sampling sites (C, D, G, H, P, Y, Z) at Lake Tana excluding PFOS concentrations below MDL (n = 19).

Comparison of ∑PFAS, PFOS and Hg showed that neither ∑PFASs (ANOVA, F = 1, $p = 0.47$) nor PFOS (ANOVA, F = 0.74, $p = 0.63$) had any significant variability between sites. Hg on the other hand varied between sites in the group of fish specimen used for analysis of both Hg and PFAS (ANOVA, F = 6.88, $p = 0.003$). The PFAS concentration between the fish species did not vary significantly (ANOVA, F = 0.70, $p = 0.61$), while significant variation between fish species was found both for PFOS (ANOVA, F = 17, $p < 0.001$) and Hg (ANOVA, F = 40, $p < 0.0001$).

3.4 HUMAN HEALTH ASSESSMENT

For both Hg and PFOS, neither the areas with different country fish consumption averages nor the two scenarios (prospective and 44 % increased consumption) had a calculated hazard ratio (HR) over one (Table 11). The largest ratio (0.49) was found for the EFSA Hg reference dose in the production areas together with the increased consumption scenario. All PFOS HR-values laid several magnitudes below the ratio of one.

Table 11 PFOS and Hg hazard ratios (HR) for three average fish consumptions and two scenarios; a contemporary and a prospective with 44 % increased fish consumption. Two Reference Doses (RfD) were used for each pollutant

		National		Addis Ababa		Production areas	
		Today	44 % Incr.	Today	44 % Incr.	Today	44 % Incr.
PFOS	EWG[a]	$6.1 \cdot 10^{-5}$	$8.8 \cdot 10^{-5}$	$2.3 \cdot 10^{-4}$	$3.3 \cdot 10^{-4}$	$2.5 \cdot 10^{-3}$	$3.6 \cdot 10^{-3}$
	BfR[b]	$1.5 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$	$5.6 \cdot 10^{-5}$	$8.1 \cdot 10^{-5}$	$6.3 \cdot 10^{-4}$	$9.0 \cdot 10^{-4}$
Hg	EFSA[c]	$8.3 \cdot 10^{-3}$	0.012	0.031	0.044	0.34	0.49
	WHO[d]	$6.7 \cdot 10^{-3}$	$9.6 \cdot 10^{-3}$	0.025	0.035	0.23	0.34

[a] Environmental working group (EWG) in the US. PFOS RfD = 25 ng kg bw^{-1} d^{-1}
[b] The German Federal Institute for Risk Assessment (BfR) PFOS Rfd = 100 ng kg bw^{-1} d^{-1}
[c] European Food Safety Authority (EFSA) MeHg RfD = 185 ng kg bw^{-1} d^{-1}
[d] World Health Organization (WHO) MeHg RfD = 229 ng kg bw^{-1} d^{-1}

4 DISCUSSION

Highest Hg and PFAS concentrations were both found in the piscivorous fish species (*L. megastoma* and *L. gorguari*) and the highest mean concentrations were found at four sites (C, G, Y, Z) (Table 7, Fig. 5). The PFAS composition profile differed between sites and species, where groupings were found for the piscivores and the non-piscivores and for the city-close sites (Fig. 9 and 11). For the length-Hg relations, Hg accumulation patterns were found in two species, *L. megastoma* and *C. gariepinus* (Fig. 6). A central finding was the positive correlation between PFOS and Hg, which implies that these pollutants have similar accumulation patterns (Table 10). Even though the mean Hg concentrations for the piscivorous species exceeded WHOs lower guideline of 0.2 μg g^{-1} ww, a varied fish species consumption from Lake Tana seems not to be a problem for the Ethiopian population, with respect to PFOS and Hg content, and also regarding the predicted increase in fish consumption of 44 % (Table 11).

4.1 MERCURY CONCENTRATIONS

4.1.1 Species specific Hg concentrations

The two endemic piscivorous species *L. megastoma* and *L. gorguari* had on average higher Hg concentrations (386 ± 71.9 ng g^{-1} and 290 ± 79.6 ng g^{-1}, respectively) (Table 7) than the guideline value of 0.2 μg g^{-1} ww, which is recommended by WHO to protect vulnerable groups to Hg toxicity (Section 1.2). In addition, seven samples (six *L. megastoma* and one *L. gorguari*) also exceeded the higher guideline values of 0.5 μg g^{-1} ww (Appendix 1). That these species had higher concentrations than the other three was expected since these species are strictly piscivorous at the highest trophic level. Since these species are endemic no studies for comparison exist from other lakes in Ethiopia or Africa. The third Labeobarbus specie *L. intermedius* had much lower mean Hg concentrations (54.4 ± 12.8 ng g^{-1} ww) (Table 7), compared with a study made for the same specie in Lake Awassa, Ethiopia, (285 ng g^{-1} ww, Desta et. al 2006), but similar to levels found studies from Lake Koka (39 ± 0.3 ng g^{-1} ww) and higher than for Lake Ziway (13 ± 0.2 ng g^{-1} ww). The probable explanations for the concentration differences are dietary and ecological factors, where studies of big barbs show that *L. intermedius* tend to change diets depending on food availability and can be both strict herbivorous and omnivorous (Desta et al. 2006).

The Hg concentrations for *C. gariepinus* in this study (48.8 ± 17.3 ng g^{-1} ww) (Table 7) seem to be similar as reported for other Ethiopian lakes, Lake Awassa (43.6 ± 24.1 ng g^{-1} ww) (Desta et al. 2007) and Lake Ziway (non detected to 160 ng g^{-1} ww) (Deribe et al. 2014). The herbivorous *O. niloticus* has shown in average very low Hg values (~10 ng g^{-1} ww) in Ethiopian lakes (Tadiso et al. 2011) and in other Africa lakes (Campbell et al. 2003), which coincide with the results in this study (5.86 ± 3.26) (Table 7).

The fish species had much lower Hg mean concentrations comparing with piscivores and non-piscivores in many other countries globally (Black et al. 2011), and the theory about the 'African mercury anomaly' seem to also be true for the fish species in Lake Tana. This theory states that fish from African freshwater systems has much lower Hg concentrations in general than in other ecosystems worldwide.

Patterns in Hg accumulation showed strong dependence to fish size in *L. megastoma* ($p < 0.001$) and *C. gariepinus* ($p < 0.01$) (Fig. 6). *L. gorguari* also gave a high dependence, but due to few

collected specimens this dependence was not significant (p = 0.13). However with a higher number of collected specimens, size dependence would most likely have been found since *L. gorguari* and *L. megastoma* have similar diets (de Graaf 2003). *C. gariepinus* has shown different accumulation patterns in studies around Ethiopia, for instance in Lake Awassa, *C. gariepinus* showed no dependence between length and Hg concentrations (R^2 =0.043, p = 0.127) (Desta et al. 2007) but significant dependence (R^2 =0.406, p = 0.001) in Lake Ziway (Tadiso et al. 2011). Since *C. gariepinus* is a fast growing omnivorous fish, with piscivority increasing with size, these patterns may be explained with 'growth dilution' (Section 4.1.1) of Hg in this specie. *O. niloticus* and *L. intermedius* showed no significant accumulation patterns and this is found also in other African lakes (Campbell et al. 2003; Tadiso et al. 2011).

It would have been interesting to sample both smaller and larger fish from Lake Tana and see if there might occur any change in dependence and also analyze the age of the specimens to have more substantial facts for discussion.

4.1.2 Spatial distribution of Hg

The mean Hg concentrations between sites in Table 7 together with the spatial distribution box-whisker plot in Fig. 5B showed large differences between the five studied sites. Site C, close to the Cherechera hydroelectric dam, showed highest mean value (313 ng g^{-1} ww) and around two times greater than site Z, forested Zegi peninsular and site G, outside the city of Gorgora in the north. Sites Y (the agricultural area), H (Bahir Dar Hospital waste water outlet), and D (Dirma river outlet outside of Gorgora) were in the same concentration range (60-90 ng g^{-1} ww) while site P (Bahir Dar Prison waste water outlet) had the lowest concentration (19.7 ng g^{-1} ww). The box-whiskers plot shows though that the data is not normally distributed with large skewness for almost all sites.

The differences in Hg concentrations at the sites can mainly be explained by the fish specie distribution, which is not obvious when observing Table 7 or Fig. 5B. That site C had such high concentration was because eleven of the 14 collected fishes were piscivorous species, *L. megastoma* and *L. gorguari*. Also sites Z and G had a high ratio of the two piscivorous species. One reason for the low concentrations in the city-close sites (H, P, D) is that the piscivorous specie *L. megastoma* is scarcely found in littoral zones since it preys in more open water (Table 2). *L. gorguari* is commonly found in littoral zones, but found in few numbers in this study and therefore the most common species found were *L. intermedius*, *C. gariepinus* and *O. niloticus*, which contains low Hg concentrations. The concentrations at the different sites are therefore highly dependable of the fish species composition. With a larger and more similar fish catch quantity at each site it would have been possible to make proper and scientific conclusions about the site-specific differences. Differences in weight, length and age should also be considered.

L. megastoma showed significant differences between sites and significant dependences between Hg concentrations and size for three (C, G, Z) of five sites (Fig. 7). Between these three sites the slopes seem to differ, but this was not statistical tested. Site G gave the steepest slope (r = 3.3) and may therefore have a higher accumulation rate and hence also higher Hg loading than site Z (r = 1.8) and C (r = 1.4). The highest mean concentrations at sites though were in the magnitude C > Z > G, which is the reversed for the accumulation patterns for *L. megastoma*, but

accumulation of Hg depends on many factors for both locations and individuals and many fish samples would have been needed to make a statistical significant comparison.

4.2 PFAS CONCENTRATIONS

4.2.1 Species specific PFAS concentrations

The mean ∑PFAS concentrations of the five evaluated species at site Z were quite similar, but with slightly elevated concentrations for the piscivores *L. gorguari* (2.1 ng g^{-1} ww) and *L. megastoma* (1.7 ng g^{-1} ww) compared to the herbivorous *O. niloticus* (0.97 ng g^{-1} ww) (Table 8). These results can be compared with another African study for PFOS in *O. niloticus*, where mean values between five sites in Lake Victoria ranged from 1.23 ± 0.19 to 4.89 ± 2.11 ng g^{-1} ww (Orata et al. 2008). Other studies of PFASs in African freshwater fish were not found, and most global studies focus on PFOS, but in Asia ∑PFAS concentrations in freshwater fish were analyzed (Murakami et al. 2011). Vietnam (0.05-0.3 ng g^{-1} ww), Thailand (mean = 0.05 ng g^{-1} ww), India and Malaysia (0.05-0.2 ng g^{-1} ww), are comparable to the mean ∑PFASs in this study (1.15 ng g^{-1} ww) but are much lower compared to Japan (5.1-22 ng g^{-1} ww) (Murakami et al. 2011). None of these concentrations are especially high and are more related to background concentrations originated from atmospheric deposition, while in areas, where PFASs are widely distributed, concentrations in certain fish species might range up to 551 ng g^{-1} ww (Nordic pike, *Esox lucius*) (Kallenborn et al. 2004).

The composition profile (Fig. 8) showed that PFDA was present in all species independent of trophic level, e.g. with low percentages in *L. megastoma* and high percentages in *L. gorguari*. PFBS was found in low percentages (~0-5 %) in piscivores but higher (~20-30 %) in non-piscivores. The opposite was discovered for PFOS and PFUnDA, which were found in higher percentages in piscivores (~25-45 % and ~15-25 %, respectively) compared to non-piscivores (~0-5 % and ~0-10 %, respectively). Interestingly, *C. gariepinus* had high percentages of PFBS (~30 %), PFDoDA (~30 %) and PFDA (~30 %), while *L. megastoma* had high percentages of PFOS (~45 %) and PFUnDA (~25 %). Overall short-chain PFASs were found in herbivores and omnivores and longer-chain PFASs in piscivores. The dominant PFASs in all species were PFCAs with between 55-80 %, while PFSAs were just found between 20-45 %.

The cluster diagram of the PFAS distribution in the species showed that *L. intermedius* and *O. niloticus* had the closest relationship between their PFAS composition (Fig. 9). The two piscivores *L. megastoma* and *L. gorguari* also had a close relation but a bit further apart and *C. gariepinus* were more similar to the non-piscivores than the piscivores. This may imply that the compound composition may depend on the trophic position of the species.

4.2.2 Spatial distribution of PFASs

The highest ∑PFAS concentrations were found at the Cherechera dam (site C = 2.1 ng g^{-1} ww), Yeagashu River (site Y = 1.9 ng g^{-1} ww) and Zegi peninsular (site Z = 0.97 ng g^{-1} ww) (Table 9). The city close sites (H and P) showed low levels (∑PFAS = 0.2 and 0.27 ng g^{-1} ww) as they did for Hg. Interestingly, *O. niloticus* showed as high ∑PFAS concentrations at sites C and Y (2.1 and 1.7 ng g^{-1} ww, respectively, Table 9) as the piscivores *L. gorguari* and *L. megastoma* (2.1 and 1.7 ng g^{-1} ww, respectively) at site Z (Table 8). This might mean that the ∑PFAS concentration does not depend on trophic levels, since piscivores and non-piscivores showed the same concentration values, or that the pollutant loading in site C and Y was higher than in Z and

that piscivores at these would also show higher concentrations. Even though only one species (*O. niloticus*) was chosen to compare the concentration between sites, the same argumentation as for Hg can be made. Two to three individuals from each site are not an adequate amount of samples to make scientific conclusions.

The composition profile (Fig. 10) shows that PFDA is found at all sites, but differs considerable at three sites. Site H and P were the only sites containing PFTeDA to around 30 % although this compound was barely seen at the other sites. These sites H and P were both near wastewater outlets from Bahir Dar and a possibility is therefore that this is a point source for that compound. At site Y, PFUnDA was predominant with 70 %, which is close to the agricultural lands.

The cluster diagram (Fig. 11) gives a clear view that the city close sites H and P as well as D and G from the north part of the lake, has close relationships. Site C and Z have a weak relationship, and are far from similar to the other sites. Site Y is closer to the city-close sites than sites C and Z.

4.3 COMPARISON BETWEEN PFAS AND MERCURY CONCENTRATIONS

The strongest correlation found between different PFASs and Hg was that of PFOS and Hg ($r = 0.94$) (Table 10). No correlation was seen for any other PFASs with either Hg or PFOS. However, PFBS, which serves as replacement compound for PFOS since it got banned from production, showed correlation with several PFCAs (PFNA, PFDA, PFDoDA).

The regression analysis between PFOS and Hg showed a significant, but weak dependence when all species were included (Fig. 12). 19 of the samples were under detection limit for PFOS, why the figure displays many samples in a straight line with identical PFOS concentration but different Hg concentrations. Removing the samples without detected PFOS concentration gave a significant and high dependence between PFOS and Hg. (Fig. 13), which implies that PFOS and Hg might have the same accumulation patterns. However, PFCAs and Hg has been studied in high mountain lakes in France Ahrens et al. (2010), where correlation was found between PFCAs and Hg, which is the opposite of what was found in this study.

4.4 HUMAN HEALTH ASSESSMENT

The Hazard ratios (Table 11) for PFOS were magnitudes below 1, indicating that the current PFOS concentrations in fish are not harmful for the people. Also Hg showed values below 1 although several factors higher than for PFOS. Neither people with fish consumption according to the national or Addis Ababa average seem to be at any contamination risk even after 44 % increased fish consumption. The production areas might be in risk of Hg contamination though if the fish consumption exceeds 44 %, when HR for the EFSA reference dose for Hg after 44 % fish consumption increase lies at 0.49.

Overall it seems that a various fish consumption is of no risk regarding either PFOS or Hg for the Ethiopian population even after a 44 % fish consumption increase in the country.

5 CONCLUSIONS

When changing any consumption patterns, attention to the consequences regarding the uptake of Hg and PFASs needs to be emphasized. The Ethiopians have had very low fish consumption for centuries and a drastic increase might affect the pollutant loading in the population. Mercury and PFASs are ubiquitous in the environment with high concentrations in fish in the temperate zones (Ahrens et al. 2010; Black et al. 2011), where intake of fish may cause adverse health effects for humans. However, the concentrations of PFAS and Hg in Lake Tana fish were of no harm to the Ethiopian population, even with a 44 % increased fish consumption. However, the importance of a varied fish species diet should be stressed though, since individuals from the piscivorous species contain levels that might be harmful when ingested in high quantities.

Even though PFASs are man-made chemicals and mercury is a natural element, they seem to have similar spatial and trophic level distributions regarding pollutant loading. The positive correlation between PFOS and mercury also show that these substances have similar accumulation patterns in fish muscle, where higher concentrations were found in larger species in higher trophic levels. The overall PFAS and Hg concentrations in fish from lake Tana were comparable with fish from African lakes (Orata et al. 2008; Black et al. 2011), other tropic regions (Black et al. 2011; Murakami et al. 2011) but lower than reported from temperate zones (Black et al. 2011; Labadie & Chevreuil 2011).

It is important to gain a better understanding of the spatial distribution of pollutants in African water systems like Lake Tana since only very few studies were performed in this region. However, further research is required regarding tropic aquatic systems and bioaccumulation of toxic bioaccumulative pollutants to provide adequate information about the risks with high fish consumption in Africa.

6 REFERENCES

Ahrens, L., Siebert, U. and Ebinghaus, R. (2009) Total body burden and tissue distribution of polyfluorinated compounds in harbor seals (Phoca vitulina) from the German Bight. *Marine Pollution Bulletin*. 58, 520–525.

Ahrens, L., Marusczak, N., Rubarth, J., Dommergue, A., Nedjai, R., Ferrari, C. and Ebinghaus, R. (2010). Distribution of perfluoroalkyl compounds and mercury in fish liver from high-mountain lakes in France originating from atmospheric deposition. *Environmental Chemistry.* 7, 422-428.

Ahrens, L. and Bundschuh, M. (2014). Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: a review. *Environmental Toxicology and Chemistry*. 33, (9), 1921-1929.

Baduel, C., Lai, F. Y., Townsend, K . and Mueller, J. F. (2014). Size and age-concentration relationships for perfluoroalkyl substances in stingray livers from eastern Australia. *Science of Total Environment.* 496, 523-530.

Berger, U., Glynn, A., Holmström, K., Berglund, M., Halldin Ankarberg, E. and Törnkvist, A. (2009). Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden – Analysis of edible fish from Lake Vättern and the Baltic Sea. *Chemosphere.* 76, 799-804

Berhanu, T., Lee, R., and Girma, Z. (2001). Development Initiatives and Challenges for Sustainable Resource Management and Livelihood in the Lake Tana Region of Northern Ethiopia. *Proceedings of the Wetland Awareness Creation and Activity Identification Workshop in Amhara National Regional State*, January 23rd 2001 Bahar Dar, Ethiopia.

Black, F. J., Bokhutlo, T., Somoxa, A., Maethamako, M., Modisaemang, O., Kemosedile, T., Cobb-Adams, C., Mosepele, K. and Chimbari, M. (2011). The tropical African mercury anomaly: lower than expected mercury concentrations in fish and human hair. *Science of The Total Environment.* 409, (10), 1967-1975.

Borg, D. and Håkansson, H. (2012). Evironmental and health risk assessment of perflouroalkylated and polyflouroalkylated substances (PFASs) in Sweden. Swedish Environmental Protection Agency. Report 6513 September 2012.

Breuil, C. (1995). Review of the fisheries and aquaculture sector: Ethiopia. *FAO Fisheries Circular*. 890.

Campbell, L., Osano, O., Hecky, R. and Dixon, D. (2003). Mercury in fish from three rift valley lakes (Turkana, Naivasha and Baringo), Kenya, East Africa. Environmental Pollution. 125, (2), 281-286

Dadebo, E. (2000) Reproductive biology and feeding habits of the catfish Clarias Gariepinus (Burchell) (Pisces:Clariidae) in Lake Awassa, Ethiopia. *SINET: Ethiopian Journal of Science.* 23, (2), 231-246.

Dadebo, E., Tesfahun, A. and Teklegiorgis, Y. (2013). Food and feeding habits of the African big barb Labeobarbus intermedius. *Agricultural Research and Development.* 3, (4), 049-058.

de Graaf, M. (2003). Lake Tana's piscivorous Barbus (Cyprinidae, Ethiopia): Ecology • Evolution • Exploitation. Doctoral thesis, Experimental Zoology Group, Wageningen University.

Dejen, E. (2003). Lake Tana biodiversity potentials and threats. ARARI, Bahir Dar, Ethiopia.

Dejen, E., Vijverberg, J., Nagelkerke, L. and Sibbing, F. (2009). Growth, biomass, and production of two small barbs (*Barbus Humilis* and *B. tanapelagius*, Cyprinidae) and their role in the food web of Lake Tana (Ethiopia). *Hydrobiologia*. 636, 89-100.

Deribe, E., Rosseland, B. O., Borgstrom, R., Salbu, B., Gebremariam, Z., Dadebo, E., Norli, H. R. and Eklo, O. M. (2011). Bioaccumulation of persistent organic pollutants (POPs) in fish species from Lake Koka, Ethiopia: The influence of lipid content and trophic position. *Science of Total Environment*. 410-411, 136-145.

Deribe, E., Masresha, A. E., Gade, P. A., Berger, S., Rosseland, B. O., Borgstrom. R., Dadebo, E., Gebremariam, Z., Eklo, O. M., Skipperud, L. and Salbu, R. (2014). Bioaccumulation of mercury in fish species from the Ethiopian rift valley lakes. *International Journal of Environmental Protection*. 4, (1), 15-22.

Desta, Z., Borgstrom, R., Rosseland, B. O. and Gebre-Mariam, Z. (2006). Major differences in mercury concentrations of the African big barb, *Barbus intermedius* (R.) due to shifts in trophic position. *Ecology of freshwater fish*. 15, 532-543.

Desta, Z., Bergstrom, R., Rosseland, B. O. and Dadebo, E. (2007). Lower than expected mercury concentrations in piscivorous African sharptooth catfish *Clarias gariepinus* (Burchell). *Science of Total Environment*. 376, 134-142.

Dórea, JG. (2008). Persistent, bioaccumulative and toxic substances in fish: human health considerations. *Science of the Total Environment.* 400, (1-3), 93-114.

EFSA (2012). Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal*. 10 (12), 2985-3226

FAO (2011). Joint FAO/WHO food standards programme codex committee on contaminants in foods. Fifth session, The Hague, The Netherlands, 21-25 March 2011.

Froese, R. and Pauly, D. (2015-02-12). Editors. 2014. FishBase. World Wide Web electronic publication. www.fishbase.org, version (11/2014).

Getahun, A. and Dejen, E. (2012). Fishes of Lake Tana: A Guide Book

Gordon, A., Demissie, T. and Tadesse, M. (2007). Marketing systems for fish from Lake Tana, Ethiopia: Opportunities for improved marketing and livelihoods. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 2. ILRI (International Livestock Research Institute), Nairobi, Kenya.

Goulet, R. R., Lalonde J. D., Chapleau, F., Findlay, S. C. and Lean, D. R. S. (2007) Temporal trends and spatial variability of mercury in four fish species in the Ontario segment of the St. Lawrence river, Canada. *Archives of Environmental Contamination and Toxicology*. 54, 716-729.

Hanna, D., Solomon, C., Poste, A., Buck, D. and Chapman, L. (2015). A review of mercury concentrations in freshwater fishes of Africa: Patterns and predictors. *Environmental Toxicology and Chemistry*. 34, (2), 215-223.

Jin, L., Liang, L., Jiang, G. and Xu, Y. (2006). Methylmercury, total mercury and total selenium in four common freshwater fish species from Ya-Er Lake, China. *Environmental Geochemistry and Health*. 28, (5), 401-407.

Johansson, C., Sällsten, G., Schütz, A., Sjörs, A. and Barregård, L. (2004). Hair mercury levels versus freshwater fish consumption in household members of Swedish angling societies. *Environmental Research.* 96, (3), 257-263.

Kallenborn, R., Berger, U. and Ärnberg, U. (2004). Perfluorinated alkylated substances (PFAS) in the Nordic environment. TemaNord 2004:552. Nordic Council of Ministers, Copenhagen 2005. ISBN 92-893-1051-0.

Labadie, P. and Chevreuil, M. (2011). Partitioning behavior of perfluorinated alkyl contaminants between water, sediment and fish in the Orge River (nearby Paris, France). *Environmental Pollution*. 159, 391-397.

Ligdi, E.E., El Kahloun, M. and Meire, P. (2010). Ecohydrological status of Lake Tana = a shallow highland lake in the Blue Nile (Abbay) basin in Ethiopia: Review. *International journal of Ecohydrology & Hydrobiology.* 10, (2-4), 109-122.

Mahaffey, K. R. (1999). Methylmercury: a new look at the risks. *Public Health Reports.* 114, (5), 396-399, 402-413.

McLeod, A., Paterson, G., Drouillard, K. G., and Haffner, G. D. (2014). Ecological factors contributing to variability of persistent organic pollutant bioaccumulation within forage fish communities of the Detroit River, Ontario, Canada. *Environmental Toxicology*. 33, (8), 1825-1831.

Mohammed, B., de Graaf, M., Nagelkerke, L., Anteneh, W. and Mingist, M. (2011). Lake Tana's (Ethiopia) endemic Labeobarbus spp. Flock: An uncertain future threatened by exploitation, land use and water resources developments. In: *Impacts of climate change and population on tropical aquatic resources*, proceedings of the Third International Conference of the Ethiopian Fisheries and Aquatic Sciences Association (EFASA), editors: Brook Lemma and Abebe Getahun. AAU Printing Press, Addis Ababa. pp. 301-313

Mol, J., Ramlal, J., Lietar, C. and Verloo, M. (2001). Mercury contamination in freshwater, estuarine and marine fishes in relation to small-scale gold mining in Suriname, South America. *Environmental Reserch*. 86, (2), 183-197.

Morel, F., Kraepiel, A. and Amyot, M. (1998). The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics*. 29, 543-566

Murakami, M., Adachi, N., Saha, M., Morita, C. and Takada, H. (2011). Levels, temporal trends, and tissue distribution of perfluorinated surfactants in freshwater fish from Asian countries. Arch Environ Contam Toxicol. 61, 631-641.

Orata, F., Quinete, N., Maes, A., Werres, F. and Wilken, R. D. (2008). Perfluorooctanoic acid and perflurooctane sulfonate in Nile Perch and Tilapia from gulf of Lake Victoria. African Journal of Pure and Applied Chemistry. 2, (8), 075-079.

Pacyna, E. G.; Pacyna, J. M.; Steenhuisen, F.; Wilson, S. (2006). Global anthropogenic mercury emission inventory for 2000. *Atmospheric Environment.* 40, (22), 4048-4063.

Poste, A., Muir, D., Guildford, S. and Hecky, R. (2015). Bioaccumulation and biomagnification of mercury in African lakes: the importance of trophic status. *Science of Total Environment.* 15, (506-507), 126-136.

Quinete, N., Wu, Q., Zhang, T., Yun S. H., Moreira, I. and Kannan, K. (2009). Specific profiles of perfluorinated compounds in surface and drinking waters and accumulation in mussels, fish, and dolphins from southeastern Brazil. *Chemosphere*. 77, 863-869.

Sharma, C. M., Rosseland, B. O., Almvik, M. and Eklo O. M. (2009). Bioaccumulation of organochloride pollutants in the fish community in Lake Årungen, Norway. *Environmental Pollution.* 157 (8-9), 2452-2458.

Tadesse, Z. (2011). Diel feeding rhythm, ingestion rate and diet composition of *Oreochromis niloticus* L. in Lake Tana, Ethiopia. In: *Impacts of climate change and population on tropical aquatic resources*, proceedings of the Third International Conference of the Ethiopian Fisheries and Aquatic Sciences Association (EFASA), editors: Brook Lemma and Abebe Getahun. AAU Printing Press, Addis Ababa. pp. 70-78.

Tadiso, T. M., Borgstrom, R. and Rosseland, B. O. (2011). Mercury concentrations are low in commercial fish species of Lake Ziway, Ethiopia, but stable isotope data indicated biomagnification. *Ecotoxicology and Environmental Safety*. 74, (4), 953-959.

Tao, L., Kunisue, T., Libelo, E. L., Tanabe, S. and Kannan, K. (2008). Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States. *Environmental Science and Technology*. 42, (22), 8597-8602.

Tseng, C, Liu, L, Chen, C. and Ding, W. (2006). Analysis of perfluorooctanesulfonate and related fluorochemicals in water and biologic tissue samples by liquid chromatography-ion trap mass spectrometry. *Journal of Cromatography A*. 1105, (1-2), 119-126.

UNEP (2013). Global mercury assessment 2013: Sources, emissions, releases and environmental transport. UNEP Chemicals Branch, Geneva, Switzerland.

Yang, L., Tian, S., Zhu, L. and Zhang, Y. (2012). Accumulation and distribution of perfloroalkyl acids in seafood products from Bohai Bay, China. *Environmental Toxicology and Chemistry*. 31, (9), 1972-1979.

Yeung, L., Yamashita, N., Taniyasu, S., Lam, P., Sinha, R., Borole, D. and Kannan, K. (2009). A survey of perfluorinated compounds in surface water and biota including dolphins from the Ganges River and in other waterbodies in India. *Chemosphere.* 76, (1), 55-62.

Wang, W. (2012). Biodynamic understanding of mercury accumulation in marine and freshwater fish. *Advances in Environmental Research*. 1, (1), 15-35.

WHO (2013). Mercury and health. Fact sheet no. 361. Updated September 2013. http://www.who.int/mediacentre/factsheets/fs361/en/

Wondie, A., Mengistou, S. and Fetahi, T. (2012). Trophic interactions in Lake Tana, a large turbid highland lake in Ethiopia. IN: Jórdan, F., Jorgensen, S.E. (Eds), *Models of the Ecological Hierarchy: From Molecules to the Ecosphere*. Elsevier. 14, 217-235

APPENDIX 1: FISH SAMPLE INFORMATION AND HG ANALYSIS

Sampling dates, sampling sites, sex, total weight and standard length for the sampled species in Lake Tana together with analyzed Hg in ng g^{-1} dry weight and wet weight

Date	Site	Specie name	Code	Total weight (g)	Standard length (cm)	Sex	Hg (ng g^{-1}) dry weight	Hg (ng g^{-1}) wet weight
111014	H	C. gar	1	364	34.0	m	37.39	6.930
111014	H	C. gar	2	284	31.3	m	65.09	12.12
111014	H	C. gar	3	304	32.0	f	139.3	25.05
111014	H	C. gar	4	283	31.4	f	43.11	7.660
111014	H	C. gar	5	261	31.5	f	149.1	27.02
111014	H	O. nilo	6	170	18.0	m	40.31	7.640
111014	H	O. nilo	7	139	16.5	m	54.81	10.07
111014	H	O. nilo	8	137	17.0	m	8.220	1.570
111014	H	O. nilo	9	119	14.7	f	17.77	3.560
111014	H	O. nilo	10	105	14.8	f	16.73	3.380
111014	H	L. mega	11	126	20.0	f	806.4	149.3
111014	H	L. int	12	139	20.0	m	137.7	25.70
111014	H	L. gorg	13	292	25.5	m	3049	602.8
111014	P	O. nilo	14	157	17.5	m	24.95	4.690
111014	P	O. nilo	15	149	17.0	m	24.42	4.830
111014	P	O. nilo	16	101	15.0		8.820	1.770
111014	C	L. gorg	20	278	26.5	f	1524	308.8
111014	C	L. gorg	21	196	22.0	m	1184	228.9
111014	C	L. gorg	22	127	18.0	m	1058	210.4
111014	C	L. mega	23	418	31.5	m	3053	639.1
111014	C	L. mega	24	236	24.5		1662	309.9
111014	C	O. nilo	25	263	20.5	f	20.10	3.860
111014	C	O. nilo	26	185	18.0	m	13.12	2.320
111014	C	O. nilo	27	144	16.5	m	46.95	8.150
131014	C	L. mega	28	140	20.5	m	1383	275.7
131014	C	L. mega	29	506	33.0	f	2160	472.4
131014	C	L. mega	30	462	32.0	f	1779	380.3
131014	Y	C. gar	31	327	37.5	m	572.6	111.3
131014	Y	C. gar	32	467	37.2	f	364.4	61.87
131014	Y	C. gar	33	277	33.0	m	210.2	35.17
131014	Y	O. nilo	34	163	18.0		5.030	0.890
131014	Y	O. nilo	35	205	18.7		6.120	1.090
131014	Y	L. mega	37	266	27.2	f	1289	233.7
131014	Y	L. int	38	295	26.0	f	104.6	19.05
131014	Y	L. int	39	164	21.5	f	280.3	49.00

Date	Site	Specie name	Code	Total weight (g)	Standard length (cm)	Sex	Hg (ng g^{-1}) dry weight	Hg (ng g^{-1}) wet weight
131014	Y	L. int	40	133	20.1	f	136.6	24.27
131014	H	L. mega	41	146	22.0	f	946.1	168.6
141014	Z	L. mega	42	286	26.7	m	2134	379.1
141014	Z	L. mega	43	544	34.0	m	2814	551.7
141014	Z	L. mega	44	265	26.0	m	1546	272.7
141014	Z	L. mega	45	624	35.5	f	2775	562.6
141014	Z	L. mega	46	232	24.5	f	1662	295.2
141014	Z	L. mega	47	281	28.2	f	2907	565.9
141014	Z	L. gorg	48	173	21.5	m	1157	207.6
141014	Z	L. gorg	49	94.8	18.5	m	1005	182.3
141014	Z	L. int	50	311	26.0	f	215.1	42.65
141014	Z	L. int	51	523	31.5	f	302.9	62.14
141014	Z	L. int	52	209	23.5	m	226.9	34.27
141014	Z	L. int	53	320	28.5	m	319.7	60.80
141014	Z	O. nilo	54	221	19.5	f	39.39	8.290
141014	Z	O. nilo	55	99.2	15.3	m	13.57	2.400
141014	Z	O. nilo	56	246	20.2	m	0.000	0.000
141014	Z	O. nilo	57	108	15.5	m	15.26	2.830
141014	Z	C. gar	58	276	32.0	m	324.6	51.14
141014	Z	C. gar	59	364	34.0	m	340.8	57.18
141014	Z	C. gar	60	380	35.5	m	485.8	83.06
141014	Z	C. gar	61	375	34.5	m	245.9	39.95
141014	Z	C. gar	62	349	35.0	f	449.1	74.76
151014	C	L. mega	64	454	33.0	m	2572	539.7
151014	C	L. mega	65	453	33.6	f	2910	596.9
151014	C	L. mega	66	443	30.5	m	1859	402.1
151014	Y	L. mega	67	545	34.5	f	2082	384.8
151014	Y	L. mega	68	523	33.5	f	1436	259.6
151014	Y	L. int	69	297	26.5	f	129.7	21.80
151014	Y	L. int	70	162	21.5	f	194.0	36.97
151014	Y	C. gar	71	701	42.5	f	816.9	146.3
151014	Y	C. gar	72	171	24.5	f	459.9	73.54
151014	Y	C. gar	73	382	23.0	m	43.02	8.900
151014	Y	C. gar	74	205	19.5	f	80.92	14.33
241014	D	C. gar	75	1135	57.2	f	538.5	96.81
241014	D	L. int	76	168	21.0	f	317.9	62.93
241014	D	L. int	77	106	18.9	m	391.1	84.66
241014	D	L. int	78	175	22.0	m	377.8	77.30
241014	D	L. int	79	117	20.0	f	397.4	73.96

Date	Site	Specie name	Code	Total weight (g)	Standard length (cm)	Sex	Hg (ng g^{-1}) dry weight	Hg (ng g^{-1}) wet weight
241014	D	L. int	80	112	18.9	f	533.1	120.1
251014	G	O. nilo	81	126	16.5	m	29.66	5.410
251014	G	L. int	82	95.0	18.5	f	299.8	55.96
251014	G	L. int	83	88.0	18.2	m	445.3	83.49
251014	G	L. int	84	98.0	18.5	m	237.0	51.75
251014	G	L. int	85	104	19.0	f	333.4	64.15
251014	G	L. int	86	128	20.5	m	164.4	36.93
251014	G	C. gar	87	252	29.5	f	286.4	52.42
251014	G	O. nilo	88	126	17.2	f	9.260	1.750
251014	G	L. mega	89	236	25.5	f	1392	256.6
251014	G	L. mega	90	340	30.5	f	2434	459.9
251014	G	L. mega	91	268	29.5	f	2478	460.8
251014	G	L. mega	92	210	25.2	m	1341	263.2
251014	D	O. nilo	93	235	19.0	f	57.16	11.08
251014	D	O. nilo	94	109	15.2	f	158.3	30.28
251014	D	O. nilo	95	228	24.2	f	70.70	13.12
251014	D	C. gar	96	274	32.5	m	378.9	68.70
251014	D	C. gar	97	189	31.2	m	304.1	48.71
251014	D	C. gar	98	124	23.3	m	216.4	38.24
271014	P	C. gar	99	527	35.2	f	200.0	36.84
271014	P	C. gar	100	265	29.5	f	260.3	47.55
271014	P	C. gar	101	527	36.2	f	174.7	33.45
271014	P	C. gar	102	532	36.3	f	47.68	8.690

APPENDIX 2: ANOVA RESULTS

Table I One way ANOVA for logarithmic Hg concentrations [ng g^{-1}] between species in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
L. megastoma	23	58.76	2.555	0.03103
L. intermedius	20	33.73	1.687	0.04751
L. gorguari	6	14.53	2.422	0.03671
C. gariepinus	26	40.54	1.559	0.14082
O. niloticus	21	12.98	0.6183	0.14976

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	44.94	4	11.24	123.4	6.87E-36	2.472
Within groups	8.284	91	0.09104			
Total	53.23	95				

Table II One way ANOVA for logarithmic Hg concentrations [ng g^{-1}] between sites in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
C	14	30.14	2.153	0.7216
D	12	20.30	1.692	0.1090
G	12	21.61	1.801	0.5361
H	14	17.39	1.242	0.5287
P	7	7.310	1.044	0.3030
Y	17	26.14	1.538	0.5500
Z	20	37.66	1.883	0.4886

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	9.980	6	1.663	3.422	0.004364	2.202
Within groups	43.250	89	0.4859			
Total	53.23	95				

Table III One way ANOVA for logarithmic Hg concentrations [ng g^{-1}] for *L. megastoma* between sites in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
C	7	18.52	2.646	0.01975
G	4	10.16	2.539	0.02058
H	2	4.401	2.200	0.001395
Y	3	7.368	2.456	0.01304
Z	6	15.73	2.621	0.02151

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	0.3668	4	0.09169	4.944	0.007897	2.965
Within groups	0.3152	17	0.01854			
Total	0.6821	21				

Table IV One way ANOVA for differences between logarithmic PFCAs and PFSAs concentrations [ng g^{-1}] between species in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
sum PFCAs	14	-1.229	-0.08778	0.1148
sum PFSAs	14	-7.059	-0.5042	0.2659

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	1.214	1	1.214	6.377	0.01799	4.225
Within groups	4.949	26	0.1903			
Total	6.163	27				

Table V One way ANOVA for differences between logarithmic PFCAs and PFSAs concentrations [ng g^{-1}] between species in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
sum PFCAs	19	-9.136	-0.4809	0.2248
sum PFSAs	19	-22.66	-1.192	0.2319

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	4.815	1	4.815	21.08	5.2E-05	4.113
Within groups	8.222	36	0.2284			
Total	13.03	37				

Table VI One way ANOVA for logarithmic ∑PFASs concentrations [ng g^{-1}] between sites in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
H	3	-2.080	-0.6936	0.004992
P	3	-1.763	-0.5879	0.02443
C	3	-0.5098	-0.1699	0.6815
y	2	0.1917	0.09589	0.3527
G	2	-0.7733	-0.3866	0.01018
D	3	-1.6409	-0.5469	0.02187
Z	3	-0.5504	-0.1834	0.2926

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	1.209	6	0.2015	1.002	0.4672	2.996
Within groups	2.414	12	0.2011			
Total	3.623	18				

Table VII One way ANOVA for logarithmic PFOS concentrations [ng g^{-1}] between species in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
H	3	-5.247	-1.749	0.07121
P	3	-5.709	-1.903	0
C	3	-5.110	-1.703	0.1195
y	2	-3.806	-1.903	0
G	2	-3.806	-1.903	0
D	3	-4.864	-1.621	0.2381
Z	3	-4.414	-1.471	0.1783

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	0.4485	6	0.07475	0.7385	0.6289	2.996
Within groups	1.214	12	0.1012			
Total	1.663	18				

Table VIII One way ANOVA for logarithmic Hg concentrations [ng g^{-1}] between species in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
H	3	2.082	0.6941	0.1897
P	3	1.603	0.5343	0.06160
C	3	1.863	0.6211	0.07526
Y	2	0.1917	0.09589	0.3527
G	2	-0.7733	-0.3866	0.01018
D	3	-1.640	-0.5469	0.02187
Z	2	1.369	0.6847	0.1091

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	4.387	6	0.7311	6.879	0.00312	3.094
Within groups	1.169	11	0.1062			
Total	5.556	17				

Table IX One way ANOVA for logarithmic ∑PFASs concentrations [ng g^{-1}] between sites in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
L. megastoma	3	0.6764	0.2254	0.008426
L. gorguari	2	0.6462	0.3231	0.01959
L. intermedius	3	8	0.001689	0.1115
O. niloticus	3	-0.5504	-0.1834	0.2926
C. gariepinus	3	0.2192	0.07308	0.2349

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	0.4073	4	0.1018	0.6971	0.6128	3.633
Within groups	1.314	9	0.1461			
Total	1.722	13				

Table X One way ANOVA for logarithmic PFOS concentrations [ng g^{-1}] between species in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
L. megastoma	3	-0.3517	-0.1172	0.001716
L. gorguari	2	-0.4685	-0.2342	0.000621
L. intermedius	3	-4.755	-1.585	0.3029
O. niloticus	3	-4.414	-1.471	0.1783
C. gariepinus	3	-5.709	-1.903	0

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	7.445	4	1.861	17.32	0.000295	3.633
Within groups	0.9667	9	0.1074			
Total	8.411	13				

Table XI One way ANOVA for logarithmic Hg concentrations [ng g^{-1}] between species in Lake Tana

Groups	*Count*	*Sum*	*Mean*	*Variance*
L. megastoma	3	7.767	2.589	0.02521
L. gorguari	2	4.578	2.289	0.001584
L. intermedius	3	4.948	1.649	0.01578
O. niloticus	2	1.369	0.6847	0.1091
C. gariepinus	3	5.278	1.759	0.02526

ANOVA

Source	*SS*	*df*	*MS*	*F*	*p-value*	*F-crit*
Between groups	4.883	4	1.220	40.14	2.44E-05	3.837
Within groups	0.2432	8	0.03041			
Total	5.127	12				

APPENDIX 3: CHOSEN FISH SAMPLES FOR PFAS ANALYSIS

Sample	Site/ Specie	weight (g)	strd lenght(m)	C09 PFNA	C10 PFDA	C11 PFUnDA	C12 PFDoDA	C14 PFTeDA	PFBS	PFOS	∑PFCAs	∑PFSAs	**∑PFASs**
14MS-F-H-6	H O.nilo	170.1	18.0	0.0125	0.02584	0.0484	0.03676	0.06456	0.0125	0.03624	0.18806	0.04874	0.2368
14MS-F-H-7	H O.nilo	138.9	16.5	0.0125	0.02584	0.01392	0.00676	0.08711	0.0125	0.0125	0.14614	0.025	0.17114
14MS-F-H-8	H O.nilo	137.1	17.0	0.0125	0.02584	0.05624	0.00676	0.07848	0.0125	0.0125	0.17982	0.025	0.20482
14MS-F-P-14	P O.nilo	157.2	17.5	0.0125	0.17308	0.0027	0.00676	0.14462	0.0125	0.0125	0.33965	0.025	0.36465
14MS-F-P-15	P O.nilo	149.2	17.0	0.0125	0.06754	0.0645	0.00676	0.0894	0.0125	0.0125	0.2407	0.025	0.2657
14MS-F-P-16	P O.nilo	101.0	15.0	0.0125	0.02584	0.0197	0.03715	0.05761	0.0125	0.0125	0.15281	0.025	0.17781
14MS-F-C-25	C O.nilo	263.4	20.5	1.3808	3.36469	0.0027	0.00676	0.00654	1.0052	0.0125	4.76145	1.0177	5.77915
14MS-F-C-26	C O.nilo	185.3	18.0	0.0125	0.29193	0.0027	0.00676	0.00654	0.0125	0.0125	0.32043	0.025	0.34543
14MS-F-C-27	C O.nilo	144.4	16.5	0.0125	0.02584	0.0027	0.03696	0.01471	0.0125	0.04963	0.09272	0.06213	0.15485
14MS-F-Y-34	Y O.nilo	162.9	18.0	0.0125	0.11477	0.30859	0.00676	0.00654	0.0125	0.0125	0.44916	0.025	0.47416
14MS-F-Y-35	Y O.nilo	204.9	18.7	0.0408	0.9099	2.29094	0.00676	0.00654	0.0125	0.0125	3.25489	0.025	3.27989
14MS-F-Z-42	Z L.mega	286.4	26.7	0.0125	0.23574	0.40955	0.1428	0.00654	0.0125	0.72591	0.80714	0.73841	1.54555
14MS-F-Z-44	Z L.mega	264.6	26.0	0.0125	0.23462	0.30346	0.14824	0.00654	0.0125	0.71913	0.70536	0.73163	1.437
14MS-F-Z-47	Z L.mega	280.9	28.2	0.0125	0.49792	0.50618	0.24959	0.00654	0.0125	0.85215	1.27274	0.86465	2.13739
14MS-F-Z-48	Z L.gorg	173.3	21.5	0.3049	1.39914	0.0027	0.00676	0.00654	0.31578	0.60721	1.72003	0.92299	2.64302
14MS-F-Z-49	Z L.gorg	94.8	18.5	0.0125	0.45373	0.62351	0.00676	0.00654	0.0125	0.55989	1.10305	0.57239	1.67544
14MS-F-Z-50	Z L.int	310.9	26.0	0.70609	1.09045	0.0027	0.00676	0.00654	0.49235	0.0125	1.81254	0.50485	2.31739
14MS-F-Z-52	Z L.int	209.2	23.5	0.1129	0.58339	0.0027	0.00676	0.00654	0.13027	0.0125	0.71229	0.14277	0.85506
14MS-F-Z-53	Z L.int	320.1	28.5	0.0125	0.02584	0.33417	0.00676	0.00654	0.0125	0.11228	0.38581	0.12478	0.51059
14MS-F-Z-54	Z O.nilo	221.2	19.5	0.0125	0.35937	0.0027	0.45304	0.00654	0.07451	0.08729	0.83415	0.1618	0.99595
14MS-F-Z-56	Z O.nilo	246.3	20.2	0.34131	0.8178	0.0027	0.00676	0.00654	0.56343	0.0125	1.17511	0.57593	1.75104
14MS-F-Z-57	Z O.nilo	108.1	15.5	0.0125	0.08515	0.0027	0.00676	0.00654	0.0125	0.03531	0.11365	0.04781	0.16146
14MS-F-Z-59	Z C.gar	364.4	34.0	0.0125	0.8684	0.0027	0.00676	0.00654	0.2735	0.0125	0.8969	0.286	1.1829
14MS-F-Z-60	Z C.gar	379.5	35.5	0.0125	0.02584	0.31097	0.00676	0.00654	0.0125	0.0125	0.36261	0.025	0.38761
14MS-F-Z-61	Z C.gar	374.9	34.5	0.16661	0.6966	0.0027	1.50684	0.00654	1.22143	0.0125	2.3793	1.23393	3.61323
14MS-F-G-81	G O.nilo	126.0	16.5	0.0125	0.16725	0.07038	0.00676	0.00654	0.07237	0.0125	0.26343	0.08487	0.3483
14MS-F-G-88	G O.nilo	126.0	17.2	0.0125	0.22212	0.0027	0.06882	0.00654	0.15864	0.0125	0.31268	0.17114	0.48382
14MS-F-D-93	D O.nilo	235.0	19.0	0.0125	0.21701	0.0027	0.00676	0.00654	0.05054	0.0125	0.24551	0.06304	0.30855
14MS-F-D-94	D O.nilo	109.0	15.2	0.0125	0.02584	0.03038	0.01984	0.00654	0.0125	0.08753	0.09511	0.10003	0.19515
14MS-F-D-95	D O.nilo	228.0	24.2	0.0125	0.23814	0.0027	0.00676	0.00654	0.10051	0.0125	0.26664	0.11301	0.37965