



# Distribution of microplastic and small macroplastic particles across four fish species and sediment in an African lake

Lemessa B. Merga<sup>a,b,\*</sup>, Paula E. Redondo-Hasselerharm<sup>a</sup>, Paul J. Van den Brink<sup>a,c</sup>, Albert A. Koelmans<sup>a</sup>

<sup>a</sup> Aquatic Ecology and Water Quality Management Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

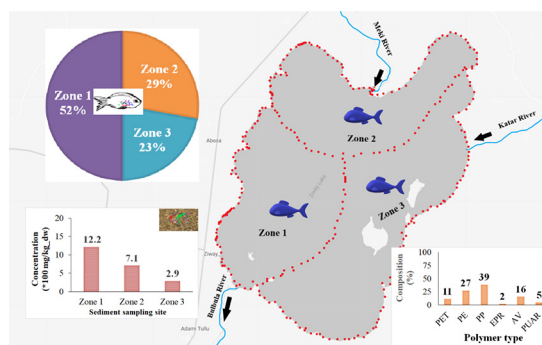
<sup>b</sup> Department of Chemistry, Ambo University, Ambo, Ethiopia

<sup>c</sup> Wageningen Environmental Research, P.O. Box 47, 6700 AA Wageningen, the Netherlands

## HIGHLIGHTS

- We studied microplastic and small macroplastic particles in an African lake.
- We demonstrate temporal and spatial trends on the lake system level.
- We demonstrate ingestion to be higher by benthic/benthopelagic than by pelagic fish.
- Particle size analysis confirms benthic-pelagic transfer from sediment to fish.
- Some of the plastic concentrations in sediment exceed known effect thresholds.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Pollution with microplastics has become an environmental concern worldwide. Yet, little information is available on the distribution of microplastics in lakes. Lake Ziway is one of the largest lakes in Ethiopia and is known for its fishing and drinking water supply. This study aims to examine the distribution of plastic particles, of all sizes (micro- and small macro-plastics) in four of the major fish species of the lake and in its shoreline sediment. The gastrointestinal tracts analysis showed that 35% of the sampled fishes ingested plastic particles. The median number of particles per fish was 4 (range 1–26). Benthic (*Clarias gariepinus*) and benthopelagic (*Cyprinus carpio* and *Carassius carassius*) fish species were found to contain a significantly higher number of plastic particles in comparison to the planktivorous fish species (*Oreochromis niloticus*). More fishes ingested plastic particles in the wet compared to the dry season. The maximum plastic size (40 mm fibre) was found in *C. carpio*. Estimated median mass of plastic particles in fish was 0.07 (0.0002–385.2) mg/kg<sub>ww</sub>. Fish and sediment samples close to known potential sources of plastic particles had a higher plastic ingestion frequency (52% of the fish) and higher plastic concentration compared to the other parts of the lake. The median count and mass of plastic particles measured in sediment of the lake were 30,000 (400–124,000) particles/m<sup>3</sup> and 764 (0.05–36,233) mg/kg<sub>dw</sub>, respectively, the upper limits of which exceed known effect thresholds. Attenuated total reflection (ATR) - Fourier-transform infrared (FTIR) spectroscopy showed that polypropylene, polyethylene and alkyd-varnish were the dominant polymers in fishes and in sediment. The plastic particles size distributions were Log-linear and were identical for plastic particles found in fish and in sediment, suggesting strong benthic-pelagic coupling of plastic particles transfer.

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\* Corresponding author at: Department of Chemistry, Ambo University, Ambo, Ethiopia.  
E-mail address: [lemessa.merga@wur.nl](mailto:lemessa.merga@wur.nl) (L.B. Merga).

## 1. Introduction

Because of unsustainable use and inappropriate management of industrial as well as domestic plastic wastes, plastic debris is widely found in the environment and recently its pollution became an emerging environmental concern all over the globe (Edo et al., 2020; SAPEA, 2019). Once released into the environment, plastic waste generally is persistent and therefore stays for many years (SAPEA, 2019). Its fragmentation and degradation mostly are driven by UV-B radiation, physical stress and microbial action (Galgani et al., 2015; Kooi et al., 2017; SAPEA, 2019), which may enhance sinking of the buoyant polymers (Koelmans et al., 2017). UV radiation and microbially mediated degradation are highly dependent on the chemical constituents of the material and environmental variables such as temperature (Galgani et al., 2015). Depending on size, plastic debris is classified generally as nanoplastic (<1 µm), microplastic (MP, 1 µm–5 mm), and macroplastic (>5 mm) (SAPEA, 2019).

Surface water MP pollution and related impacts on aquatic fauna are a rapidly evolving research issue (O'Connor et al., 2019). Many field observations have demonstrated the occurrence of MP in surface waters (Castañeda et al., 2014; Mintenig et al., 2020) and in sediment (Haave et al., 2019; Imhof et al., 2013; Lorenz et al., 2019; Thompson et al., 2004). Ingestion of MP by aquatic fauna including fish (Lusher et al., 2013; Rummel et al., 2016), mammals (Besseling et al., 2015), and invertebrates (Nel et al., 2018; Scherer et al., 2017) is also documented. Concerns have been raised regarding the potential impacts of MP ingestion by aquatic life, such as internal blockages and disruption of digestion (Cannon et al., 2016), or exposure of organisms to plastic-associated chemicals (O'Connor et al., 2019; Schrank et al., 2019). The implications of trophic transfer of MP through the food web for ecological and human health risks are of additional concern (Carbery et al., 2018; Nel et al., 2018). Empirical data showing impacts of MP on aquatic fauna in situ are scarce (Anderson et al., 2016). Recently, a few experimental studies have illustrated the effect of MP on physiological and behavioural traits including feeding (Cole et al., 2015; Ogonowski et al., 2016), fitness (Ogonowski et al., 2016; Schrank et al., 2019), growth (Redondo-Hasselerharm et al., 2018) and community composition (Redondo-Hasselerharm et al., 2020) of aquatic organisms.

Only little information is available on MP pollution in African lakes (Biginagwa et al., 2016; Madzena and Lasiak, 1997; Nel et al., 2018; Ngupula et al., 2014; Ryan, 1988), whereas sets of field data across species and compartments generally are scarce (Khan et al., 2018). Lake Ziway (Fig. 1 SI) is one of the largest lakes in Ethiopia, situated between 7°51' to 8°07' N and 38°43' to 38°56' E at about 160 km to the south of the capital, Addis Ababa, Ethiopia. Its surface area is 442 km<sup>2</sup> with a shoreline length of 137 km. It is a shallow freshwater lake with average and maximum depths of 2.5–4 m and 7–9 m, respectively. The depth variation of the lake is partially explained by differences in the amount of rain fall between seasons (Merga et al., 2020 in press). Lake Ziway is known by its ecosystem goods and services including fish food and irrigation water supply (Lemma and Desta, 2016; Teklu et al., 2018). The lake is also a source of drinking water for the Batu town population (about 70,436 inhabitants). As a result of urbanization and agricultural activities (Fig. 1 SI), MP pollution is a potential threat to Lake Ziway and to the ecosystem services the lake provides.

The present study aims to examine the occurrence of plastic particles in the gastrointestinal tracts of four major fish species and in shoreline sediment of a large freshwater lake (Lake Ziway). Data on all sizes of plastic particles found were recorded, i.e. including those larger than 5 mm in size. Therefore, we refer to the particles as plastic particles rather than MPs, which is usually defined as plastic with a size smaller than 5 mm only.

## 2. Materials and methods

### 2.1. Fish sample collection and gastrointestinal tract analysis

#### 2.1.1. Sample collection

First, the lake was clustered broadly into three zones (zone 1 to 3) based on the expected level of exposure of the sites to potential sources of plastic particles like urbanization and agricultural activities (Fig. 1 SI). Zone 1 was expected to be influenced by wastes generated from small- and large-scale agricultural activities, and urban areas (e.g. Batu town). Subsistence farms and Meki town (through Meki River) were expected to be the main sources of plastic particles at zone 2. At zone 3 shoreline agricultural activities were rare, thus, urban wastes from Ogocho town via the inflow Katar River could be the main sources of plastic pollution. Fishes used for plastic particle analysis were obtained from active fishery cooperatives in these zones. Fishes were sampled on 24–25 May 2017 and 20–21 November 2017, to include the dry and the wet seasons, respectively.

During each sampling season, 15 individuals per species per zone, i.e. 180 specimens of four commercially important fish species (*Oreochromis niloticus*, *Clarias gariepinus*, *Cyprinus carpio* and *Carassius carassius*), were collected. A total of 360 individual fishes were sampled for analysis over the two seasons. The fish species were selected because they are sources of income for fishermen and widely used for home consumption by the local farmers (Endebu et al., 2015). Therefore, impact to the fish has not only ecological but also economic and possibly human health implications. If plastic particles are in the gastrointestinal tract, the smaller size fractions (e.g. <3 µm) can be translocated into edible fish tissues (Akoueson et al., 2020; Zitouni et al., 2020). The collected fishes were immediately transported in an icebox to the laboratory of Batu Fishery and Other Aquatic Life Research Centre (BFOALRC), located at the western shore of the lake, and stored at –20 °C until further analysis.

#### 2.1.2. Fish gastrointestinal tract analysis

Fish gastrointestinal tracts (GIT) were analysed according to Foekema et al. (2013) with slight modifications. Briefly, in the laboratory, the length and wet weight of fish samples were measured. The entire content of the esophagus, stomach and intestines were collected into clean glass jars using ethanol cleaned scissor and forceps. Each jar was filled with 10% KOH solution (Analytical grade, UNI-CHEM®) in a volume ratio of 3:1 of KOH to biological material. Jars were stored in separate and cleaned cupboards for one month at room temperature to facilitate a complete digestion of the fish GIT matrix. During the process, shaking of the jars was avoided to minimize the damage of the plastic particles due to possible physical scratches by shells and other silica materials. The digested GIT was carefully sieved using a 0.1 mm sieve (i.e. 0.1 mm is the detection limit) and the residue was transferred into a clean glass bottle. Then, plastic particles were visually identified with the help of a 40× stereomicroscope (Premiere SMZ-05, USA) and following previously published procedures (Cannon et al., 2016; Lusher et al., 2016). Criteria included physical characteristics such as unnatural appearance (e.g. shiny particles without visible cellular or organic structures) as described by Lusher et al., 2016, shape of the particles (e.g., fibre, fragment) and colour. Malleability of the particles was checked by squashing with a laboratory stainless dissect needle (micro tip diameter) as stated by Cannon et al., 2016. The number of identified plastic particles was counted per individual fish. The length of the identified plastic particles was measured as the largest cross-section using an ocular micrometer fixed to the eyepiece of the microscope. Colour and shape (fibre, fragment, foam and pellet) were also recorded.

The weight of the plastic particles was estimated using the average density of environmental MP (1.04 g/cm<sup>3</sup>) (Redondo-Hasselerharm et al., 2018) and the estimated volume of each of the plastic particles.

Following Besseling et al. (2019), for fragments, each particle was assumed to have a volume half of the volume of a sphere, with sphere radius taken as half of the measured length of the particles. For fibre plastic particles, the volume was calculated from length and a standard cross-sectional diameter (20  $\mu\text{m}$ ), as fibres usually are assumed to have cylindrical shape (Kooi and Koelmans, 2019). The 20  $\mu\text{m}$  diameter estimate was obtained by taking the median of ten values reported in the literature (Absher et al., 2019; Cincinelli et al., 2017; Cole, 2016; Cole et al., 2014; Edo et al., 2020; Falco et al., 2018; Frias et al., 2010; Napper and Thompson, 2016; Wolff et al., 2019) (Table 3 SI). This diameter is within the range of the environmentally realistic diameter for fibres (10–28  $\mu\text{m}$ ) as reported by Cole (2016). With these assumptions, weights of fragment and fibre plastics were estimated using Eqs. (1) and (2), respectively.

$$\text{Weight of fragment (g)} = \rho * \frac{1}{2} * \left( \frac{4}{3} * \pi * \frac{L^3}{8} \right) \quad (1)$$

$$\text{Weight of fibre (g)} = \rho * \left( \pi * \frac{d^2}{4} * L \right) \quad (2)$$

where “ $\rho$ ” is average density of MP ( $\text{g}/\text{cm}^3$ ), “ $L$ ” length of the plastic particles (cm) and “ $d$ ” is the cross sectional diameter of fibres (cm).

Subsequently, using the weight of the plastic particles and the wet weight (ww) of fish, the mass concentration of plastic particles in fish ( $\text{mg}/\text{kg}_{\text{ww}}$ ) was calculated. To evaluate the field based bio-accumulation of plastic particles through the food chain, Bio-accumulation Factors (BAF) were calculated by dividing the concentration of plastic particles in fish ( $\text{mg}/\text{kg}_{\text{ww}}$ ) to concentration of plastic particles in sediment ( $\text{mg}/\text{kg}_{\text{dry weight (dw)}}$  of sediment) (Su et al., 2016). Note that this BAF is calculated without gut defaecation, because for plastics the GIT is the target organ and thus drives spreading of the particles across the food web.

To reduce air borne contamination, each step during sample preparation and analysis was performed in a laminar flow hood, which was thoroughly cleaned using ethanol as suggested by Foekema et al. (2013) and Hermesen et al. (2018). Plastic made equipment was avoided during the analysis and counting processes. After every sample analysis, all used equipment was scrubbed with ethanol. Furthermore, gloves and cotton lab coats were worn during sample processes and analysis.

## 2.2. Sediment sample collection and analysis

### 2.2.1. Sample collection, transportation and storage

Sediment samples were collected from zone 1 (7 sites), zone 2 (3 sites) and zone 3 (3 sites) regions of Lake Ziway. In total, 13 shoreline sites were investigated (Fig. 1 SI). In addition to the earlier mentioned human activities generating plastic waste, their accessibility was also considered when selecting the sample sites. Surface sediment (0–2 cm) was collected using an Ekman grab sampler (HYDRO-BIOS, surface area = 0.0225  $\text{m}^2$ ) from the selected shoreline sites. Samples were wrapped with aluminium foil and kept in clean wide mouth glass bottles. Three replicates ( $n = 3$ ) were collected in each of the selected sampling sites. Immediately after collection, samples were transported carefully to BFOALRC using an icebox and stored at 4  $^{\circ}\text{C}$  till analysis.

### 2.2.2. Sediment analysis

A density separation technique was used to separate plastic particles from sediment samples following Thompson et al. (2004) with modifications. In brief, 250 mL of wet sediment was dried at 50  $^{\circ}\text{C}$  for 72 h in an oven. The dry weight (dw) of the sediment was measured and the sediment was subsequently added to a glass beaker containing 500 mL saturated NaCl solution (354 g/L, sieved by a 0.1 mm sieve). The solution was stirred slowly for 15 min to avoid damage to the plastic particles.

The stirred sample was left to settle for 3 h to enhance the separation of plastic particles from fine mineral particles, followed by careful filtration using a 0.1 mm sieve. The residue was transferred into a clean glass bottle (wide mouth) and examined for plastic particles applying the same procedure used for the fish GIT analysis. Concentrations were expressed as numbers of plastic particles per sediment volume (particles/ $\text{m}^3$ ) and per dry weight of sediment (particles/ $\text{kg}_{\text{dw}}$ ), and weight of plastic particles per dry weight of sediment ( $\text{mg}/\text{kg}_{\text{dw}}$ ). Furthermore, the numerical abundance of plastic particles per surface area (particles/ $\text{m}^2$ ) was calculated by dividing the number of plastic particles counted by the area of sediment sampled by the Ekman grab sampler (0.0225  $\text{m}^2$ ). Colour and shape of the identified plastic particles were recorded. To avoid airborne fibre contamination, the same practice mentioned earlier for GIT analysis was applied.

## 2.3. Characterization of the plastics

Attenuated Total Reflectance (ATR) - Fourier Transform Infrared (FTIR) spectroscopy was used to characterize the polymer identity of the plastic particles detected in the sediment and in the GIT of the fishes. The analysis was performed at Wageningen University Research. A total of 4.4% of the particles extracted from sediment and 3.2% of the particles from fishes were examined. The particles were analysed with a Scimitar series 1000 ATR-FTIR spectroscope (Varian, Agilent technologies Inc., USA) as described by Hermesen et al. (2018). Polymer identification was performed by comparing the measured spectra (650–4000  $\text{cm}^{-1}$ ) with the reference spectra. A reference database and free software developed by Aalborg University, Denmark and Alfred Wegener Institute, Germany (SiMPle; <https://simple-plastics.eu/index.html>) was used for comparison.

## 2.4. Data analysis

The non-parametric Wilcoxon test was used to assess significant differences in the concentration of plastic particles in sediment (particles/ $\text{m}^3$ ) and percent of fish (%) that ingested plastic particles between the dry and wet seasons. Furthermore, Chi-Squared test (Roch et al., 2019) was employed to test the significance differences between the four fishes in burden of plastic particles and between the three zones of the lake in frequency of fish (%) that found with plastic particles.

To estimate plastic particles size distribution below the limit of detection (i.e. 0.1 mm) of this study, we performed a particle size distribution analysis according to Roch et al. (2019). The plastic particles were grouped into 47 size bins ranging from 1 to 52,169  $\mu\text{m}$  where the size of each next bin was increased by a factor of 1.26. Various parameters were calculated including size bin boundaries ( $l_i, l_{i+1}$ ), size of each particle size bin, ( $\Delta l_i$ ), volume equivalent diameter ( $l_{i+}$ ), size of each particle size bin to volume equivalent diameter ratio ( $\Delta l_i/l_{i+}$ ), number of plastic particles per size bin ( $\Delta N_i$ ) and particle frequency per size bin ( $\Delta N_i/\Delta l_i$ ). A linearized (log-log) graph of volume equivalent diameter ( $l_{i+}$ ) versus particle frequency per size bin ( $\Delta N_i/\Delta l_i$ ) were plotted. To extrapolate particle frequency for sizes < 200  $\mu\text{m}$  the regression function obtained from the linearized log-log graph was used.

Analysis of covariance model was used to test the significance of differences between the calculated linear regressions for size distributions of fish plastic particles and sediment plastic particles. Condition index (K) was calculated for fishes with and without plastic particles using a length-weight relationship equation ( $K = 100 * (W/L^3)$ ) as described by Foekema et al. (2013), where W is wet weight (g) and L is total length (cm) of fish. A Mann-Whitney test was used to test the significance of the difference between the condition index of fish with and without plastic particles. All analyses were performed using SPSS software package version 25 (IBM Corp., NY) and a critical  $p$ -value < 0.05 was selected.



### 3. Results

#### 3.1. Occurrence of plastic particles in GIT of fishes and sediments

The mean length and wet weight of the studied fish species were  $20.1 \pm 4.5$  cm and  $163 \pm 96.2$  g (*O. niloticus*),  $36.4 \pm 7.8$  cm and  $352 \pm 190$  g (*C. gariepinus*),  $27.4 \pm 7.1$  cm and  $338 \pm 206$  g (*C. carassius*), and  $34.4 \pm 7.3$  cm and  $504 \pm 288$  g (*C. carpio*), respectively. From the 360 examined individual fishes, plastic particles were found in the GIT of 125 (35%) individuals (Table 1 SI). All four species were found with plastic particles in their GIT. The fish species with the highest percentage of individuals with ingested plastics was *C. gariepinus* (41%) and the lowest was *O. niloticus* (22%). For *C. carpio* and *C. carassius*, plastics were found in 39% and 37% of the individual species, respectively. The number of fishes (70) that contained plastic particles in their GIT was significantly higher during the wet season than in the dry season (55) (Wilcoxon test;  $p = 0.042$ ).

As for spatial variability, the fish GIT analysis indicated that a significantly (Chi-Squared test;  $p < 0.001$ ) higher frequency of fish with ingested plastic particles was collected from the western part (zone 1 = 52%) compared to the northern (zone 2 = 29%) and south-eastern (zone 3 = 23%) parts of Lake Ziway. Moreover, of the 560 quantified plastic particles in the GIT of 125 fishes, a significantly (Chi-Squared test;  $P < 0.001$ ) higher proportion (68%) of the particles was identified in fishes collected from the zone 1 sampling site, while fishes collected from zone 2 and zone 3 contributed only for 20% and 12% particles, respectively (Fig. 2a).

*C. carpio* and *C. gariepinus* contained significantly more plastic particles than *O. niloticus* and *C. carassius* (Chi-Squared test;  $P < 0.001$ ). The count based median concentrations of plastic particles in fish was 4 (1–26) (particles/fish) (Fig. 2b), but the burden value is increased to 6.3 when the number of extrapolated MP included. The weight based median concentrations of plastic particles in fish were 0.07 (0.0002–385.2) mg/kg<sub>ww</sub> (Table 1a). The highest number of plastic particles was quantified in *C. carpio* (benthopelagic), sampled from zone 1. The calculated weight based average BAF was 0.0048 ( $\pm 0.0051$ ) and ranged from 0.00027–0.0152. Furthermore, we found no significant differences between the condition factors of fishes with and without plastic particles ( $p > 0.05$ ).

Plastic particles were detected in all sediment samples taken at the shoreline sites of Lake Ziway (Fig. 1; Table 2 SI). In the total of 78 sediment samples collected from the 13 sites during the dry and wet seasons, 649 plastic particles were counted. Contrary to the seasonality of the numbers of plastic particles in fish, a significantly higher (Wilcoxon test;  $p < 0.001$ ) number of plastic particles was observed for the dry season sediment (427 plastic particles) compared to the wet season samples (222 plastic particles). The count and weight based median of plastic particle concentrations in sediment of the lake were 30,000 (400–124,000) particles/m<sup>3</sup> and 764 (0.05–36,233) mg/kg<sub>dw</sub>, respectively. Higher plastic particle concentrations were observed at sampling sites found at the western and northern parts of the lake (Fig. 1; Table 1b). Particularly, sediments collected from Kidanemihiret (Dry =  $74,667 \pm 29,029$  particles/m<sup>3</sup>, 12,294 (915.9–36,233.2) mg/kg<sub>dw</sub>), Korekonch ( $57,333 \pm 29,139$  particles/m<sup>3</sup>, 4957.5 (198.6–13,309.2) mg/kg<sub>dw</sub>) and Meki-River (Dry =  $48,667 \pm 14,841$  particles/m<sup>3</sup>, 1269 (36–5142.3) mg/kg<sub>dw</sub>) sampling sites were found with the highest plastic particle concentrations (Fig. 1; Table 1b; Table 2 SI). The lowest concentrations were quantified at the Reference sampling location ( $6000 \pm 3347$  particles/m<sup>3</sup>, 66.5 (0.26–152.5) mg/kg<sub>dw</sub>).

#### 3.2. Size, shape and colour of plastic particles

The minimum and maximum sizes of the quantified plastic particles in the GIT of the studied fishes were 0.2 mm and 40 mm, respectively, with highest abundance at lower sizes (Fig. 3). The longest size

(40 mm) was measured for a fibre, observed in *C. carpio* sampled during the wet season at zone 1. The observed median and mean length values were 3.3 mm and 4.9 mm, respectively. Of the 560 quantified plastic particles, the majority (74%) were found to be in the MP particle size range of 0.2–5 mm (Fig. 4a), with 146 particles being larger than 5 mm. The MP percentage was increased to 83% when the extrapolated number of MP < 0.2 mm included (Fig. 3). MP abundances per species (plastics < 5 mm) were 77%, 61%, 71% and 69% in *O. niloticus*, *C. carassius*, *C. gariepinus* and *C. carpio*, respectively. No significant differences were observed between the four species with respect to the size of the ingested plastic particles (Fig. 2 SI). However, we found differences in the longest size of ingested plastic particles between *O. niloticus* (15 mm), *C. carassius* (35 mm), *C. gariepinus* (31.5 mm) and *C. carpio* (40 mm). However, in general, no strong correlation was observed between the size of the plastic particles and length of fish for each species where the plastic particles size-fish length  $R^2$  ranged from 0.0001–0.0372 (Fig. 3 SI). The plastic particles were dominated by fragments (57.5%), followed by fibres (42.5%). Plastic particles with blue (37%) and transparent white (36%) colours were dominant in the GIT of the fishes. Red, green, black and pink coloured plastic particles were also quantified in the range of 3.9–6.6%.

The minimum and maximum sizes of plastic particles quantified in sediment samples were 0.15 mm and 45 mm, respectively with highest abundance at lower sizes (Fig. 3). The largest size (45 mm) of fibre plastic particle was found in a sediment sample collected from the Floriculture site during the wet season. The plastic particle size distribution observed in sediment was not significantly different from the size distribution measured in GIT of the fishes ( $p = 0.233$ ). The observed median and mean size values of sediment plastic particles were 3.8 mm and 5.3 mm, respectively. And 70% (46% fragment and 24% fibre) of plastic particles quantified in sediment had a size in the range of 0.15–5 mm (Figs. 1, 4a). But, when the extrapolated size (<0.15 mm) included, the percentage of MP in sediment rise to 80%. Similar to the plastic particles found in the GIT of the fish species, the dominant shape and colours in sediment samples of Lake Ziway were fragments (62%), and transparent white (43%) and blue (36%). Plastics with red, green, black and pink colours were also found, but their percentage was low, ranging from 3.6 to 9.1%.

#### 3.3. Polymer identity of the sorted plastic particles

ATR-FTIR analysis revealed that 93% (27 pieces) of the particles sorted from sediments were plastics, while 2 of them were non-plastic organic matter particles. Similarly, 94% (17 pieces) of the particles sorted from the GIT of the fishes were confirmed to be plastics. Synthetic and semi-synthetic polymers such as polyethylene (PE), polypropylene (PP) and alkyd-varnish (AV) were predominantly found in both fish and sediment samples (Fig. 4b). Polyethylene terephthalate (PET), ethylene-propylene rubber (EPR) and polyurethane\_acrylic\_rasin (PUAR) were identified in sediment in lower quantities only, as shown in Fig. 4b.

### 4. Discussion

#### 4.1. Ingestion of plastics by fishes of Lake Ziway

This study shows that four fish species of Lake Ziway were contaminated by plastic particles of various polymer types and sizes (Table 1 SI; Fig. 4). As the species are commercially important and are the subsistence food source for many people in the region (Endebu et al., 2015), the contamination with plastic may pose a risk on human health due to possible translocation of plastic particles into edible tissues of fish as observed by Collard et al. (2018) in freshwater *Squalius cephalus* species from Marne and Seine Rivers, France.

It is difficult to make comparisons across MP studies due to the differences in methods (Markic et al., 2019) and in the level of quality

**Table 1**

Plastic particles mean, minimum (min.) and maximum (max.) concentrations in fish (a) and sediment (b) samples of Lake Ziway.

a. Fish sample					b. Sediment sample			
Sample location	Fish species	Concentration (mg/kg_ww)			Sampling site	Concentration (mg/kg_dw)		
		Mean	Min.	Max.		Mean	Min.	Max.
Zone 1	<i>O. niloticus</i>	1.1	0.001	6.4	Bochesa	226.1	0.046	446.2
Zone 1	<i>C. carassius</i>	3.8	0.001	51.4	Bulbula	543.7	116.4	1296.6
Zone 1	<i>C. gariepinus</i>	34.1	0.0007	170.7	Floriculture 2	849.1	89.4	2552.7
Zone 1	<i>C. carpio</i>	56.3	0.0005	385.2	Floriculture 1	3895.2	452.7	11,892.6
Zone 2	<i>O. niloticus</i>	1.3	0.004	9.7	Korekonch	4957.5	198.6	13,309.2
Zone 2	<i>C. gariepinus</i>	5.4	0.004	34.1	Kidanemihiret	12,294.0	915.9	36,233.2
Zone 2	<i>C. carassius</i>	8.3	0.013	35.1	Edo-Kontola	2509.0	324.2	9732.7
Zone 2	<i>C. carpio</i>	11.2	0.0002	51.3	Abosa	2644.0	88.6	5528.8
Zone 3	<i>O. niloticus</i>	3.9	0.01	15.5	Tepho-Choroke	1354.1	556.0	3380.0
Zone 3	<i>C. gariepinus</i>	23.9	0.003	106.2	Mekidela	3181.8	126.2	9613.6
Zone 3	<i>C. carassius</i>	2.4	0.0005	19.1	Meki-River	1269.0	36.0	5142.3
Zone 3	<i>C. carpio</i>	3.0	0.005	21.0	Reference	66.5	0.26	152.5
					Katar-River	4200.6	289.1	11,525.3

control/quality assurance used by researchers (Hermesen et al., 2018). Still it is useful to reflect on the present data in the light of earlier work. The observed percentage of fish containing plastic particles (35%) was similar to previously reported values for marine fishes from the North Pacific Central Gyre (Boerger et al., 2010) and the English Channel, UK (Lusher et al., 2013) as depicted in Table 2. The reported value (20%) by Biginagwa et al. (2016) for *O. niloticus* fish species sampled from the southern part of Lake Victoria (Africa), was also comparable with our result (22%) for the same species (Table 2). Our results appear to be higher than the values reported for fishes in freshwater French rivers (Collard et al., 2018; Sanchez et al., 2014), in the North Atlantic (Lusher et al., 2016) and in the North Sea (Foekema et al., 2013; Hermesen et al., 2018) (Table 2). Differences in studied fish species and in regional sources of plastics may also contribute to this variation.

Our results (Table 1 SI) showed that a larger number of benthic (*C. gariepinus*) and benthopelagic (*C. carpio* and *C. carassius*) fishes ingested plastic particles compared to the surface feeding planktivorous *O. niloticus* species. Furthermore, a significantly larger number of plastic particles was found in the GIT of *C. carpio* and *C. gariepinus* compared to the other two species. This shows that species mainly feeding on sediment can be exposed to plastic particles to a higher extent than surface feeding planktivorous fish species, which was also reported by Jabeen et al. (2017) for fishes sampled from Lake Taihu (China). Thus, feeding behaviour and feeding habitat are important factors in plastic particle studies in aquatic biota.

The differences observed in the frequency of fish that ingested plastic particles between various locations of Lake Ziway can possibly be explained from shoreline human activities, particularly urban influence (Peters and Bratton, 2016). A significantly higher number of fish containing plastic particles was observed at zone 1, which is close to Batu town.

The average number of plastic particles per fish measured in our study ( $4.4 \pm 3.6$ ) was comparable to the results recorded for fish from the North Pacific Central Gyre ( $2.1 \pm 5.78$ ) (Boerger et al., 2010), the Lake Taihu and Yangtze estuary of China ( $3.7 \pm 1.5$ ) (Jabeen et al., 2017) and the Balearic Islands of Spain ( $3.75 \pm 0.25$ ) (Nadal et al., 2016). But, there are differences in measured size windows between these literature data and our study. Jabeen et al. (2017) and Nadal et al. (2016) considered plastic particles  $\geq 0.005$  mm and 0.001–5 mm, respectively, whereas in the present study plastics  $\geq 0.1$  mm were counted. This shows that plastics with lower sizes ( $<0.1$  mm), which have a profound contribution (e.g. up to 95%) in sediment samples (Haave et al., 2019), were not quantified in our study. This was indicated in our extrapolation result (Fig. 3) for plastic particle size  $<0.1$  mm.

Studies have reported that fishes may prey intentionally on plastic particles that possess colour (e.g. transparent white, blue and green) similar to their natural food items such as planktons (Boerger et al., 2010; Nadal et al., 2016). These colours, particularly transparent white (36%) and blue (37%) plastic particles, were found dominantly in the

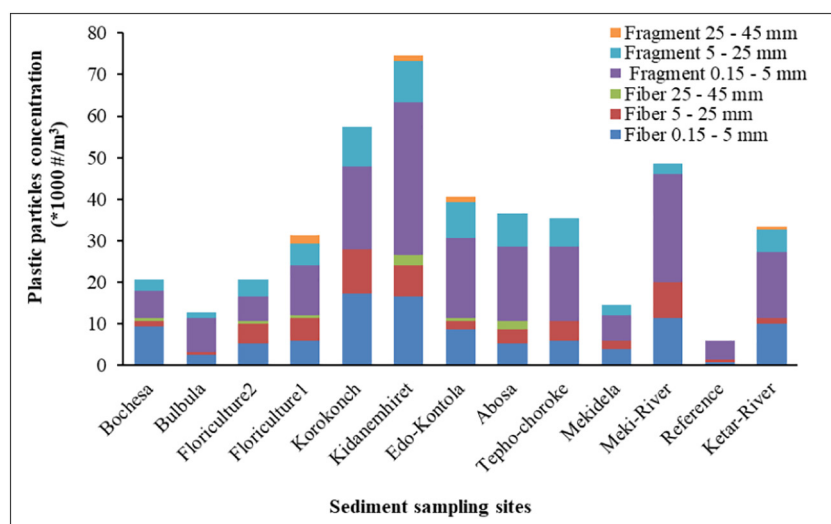


Fig. 1. Plastic particle concentrations (particles/m<sup>3</sup>) in sediment samples collected from different shoreline sites of Lake Ziway. The particles are grouped according to shape and size.

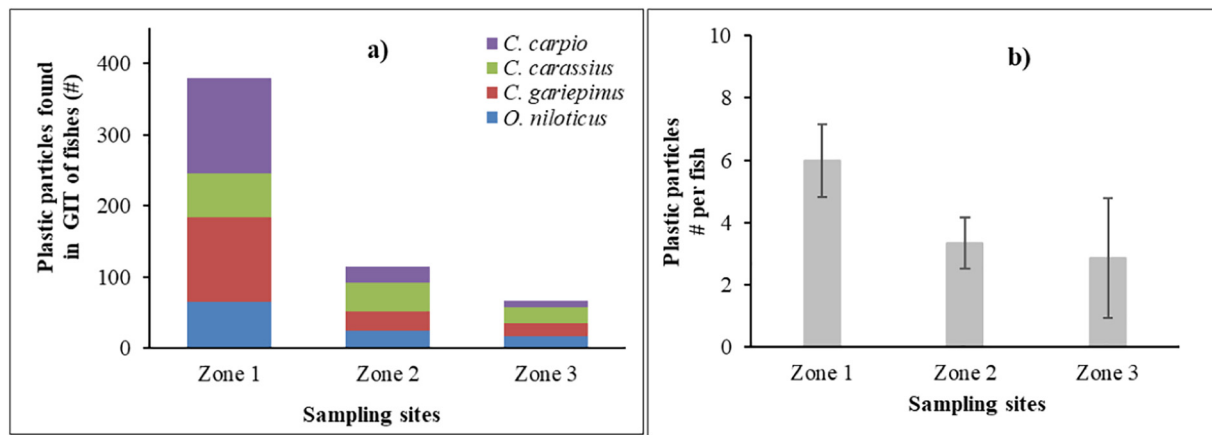


Fig. 2. Total number of plastic particles counted in GIT of fishes (a) and average number of plastic particles per fish that ingested the particles (b).

GIT of the studied fishes. However, our results do not suggest the intentional preying of plastic particles by the studied fishes as the colours were also similarly abundant in sediment samples (transparent white (43%) and blue (37%)). Unintentional ingestion of the particles attached to their food (Nadal et al., 2016) and secondary ingestion via prey items (Cannon et al., 2016) are the possible major sources of the plastic particles we found in the GIT of the fishes.

A recent allometric study (Jäms et al., 2020) showed a positive correlation between body length of organisms and size of ingested plastic particles, however in our data no strong relationship was observed for the studied fishes (Fig. 3 SI). Furthermore, the size distribution of plastic particles measured in fishes and in sediment samples (Fig. 3) were comparable and the difference was not significantly different ( $p = 0.233$ ). This suggests that plastic particles found in the GIT of fishes just reflect the plastic particle characteristics of those detected in the sediment.

Similar to some other studies (Cannon et al., 2016; Possatto et al., 2011; Romeo et al., 2015; Rummel et al., 2016), fragments were the dominant (57.5%) shape of the plastic particles found in GIT of fishes of Lake Ziway (Table 2), with 42.5% being fibres. This indicates that fragmentation of larger plastic debris into smaller pieces (Rummel et al., 2016) may be the key source of the particles in the lake, rather than other sources including effluents from wastewater treatment

plants and laundry machines that mainly generate fibre plastic particles (Edo et al., 2020; Falco et al., 2018; Fischer et al., 2016). However, our result differs to the result reported by Peters and Bratton (2016), who found fibres to be dominant (96%) in the stomach of *Lepomis macrochirus* and *Lepomis megalotis* fishes. Peters and Bratton (2016) suggested that these fish species may reject fragments as the plastic particles do not easily adhere into organic food items while fibres plastic particles do. Therefore, differences in investigated fish species and in exposure concentrations of plastic particles with different shape may explain the variation.

#### 4.2. Concentration and distribution of plastic particles in shore sediments

There was variation in the concentration of plastic particles in sediment between the sample locations (Fig. 1; Table 1; Table 2 SI). As evidenced by several previous studies (Castañeda et al., 2014; Fischer et al., 2016), the abundance of plastic particles in shoreline sediment of surface waters, was mainly explained by urban activities. For Lake Ziway, wastewater drainages (e.g. from Batu town), rivers which cross towns (e.g. Meki River and Katar River), and surface runoffs upon heavy rain are likely the main routes through which plastic particles enter the lake. The recorded high concentrations of plastic particles in sediment samples

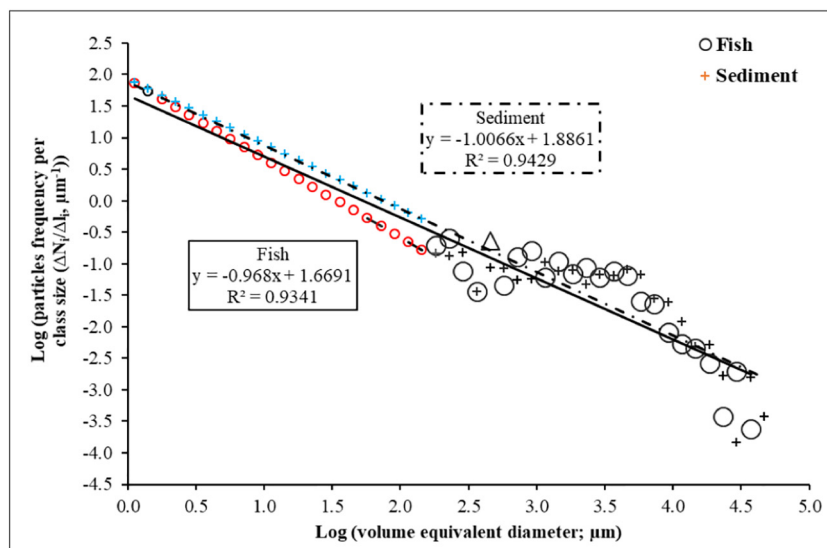
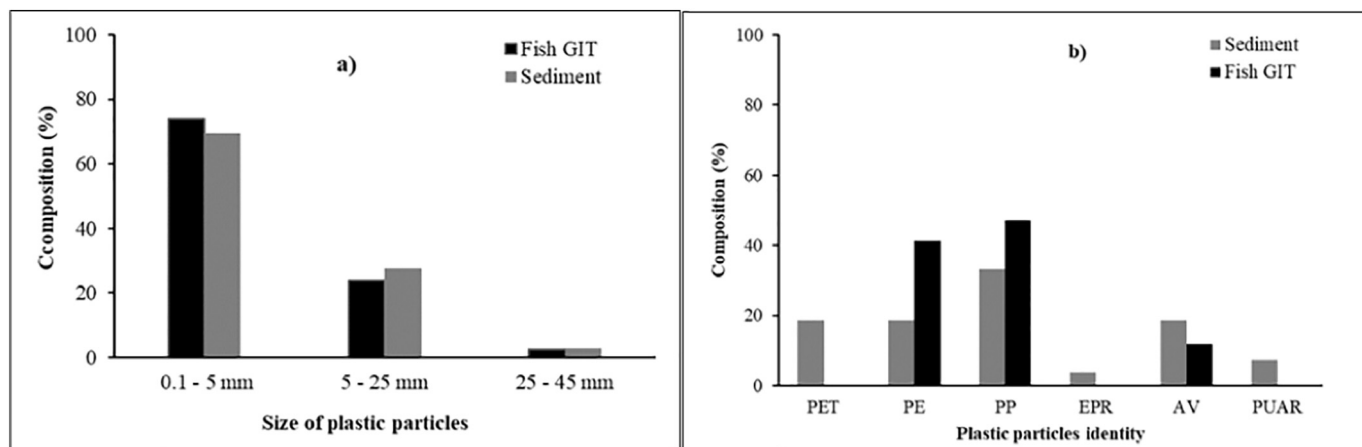


Fig. 3. Particle size distribution analysis of plastic particles in sediment and fish. The values in red and blue colours are for the extrapolated microplastics < 0.1 mm.



**Fig. 4.** Percent composition of plastic particles according to their size class in fish and in sediment samples of Lake Ziway (a). Identity and percent composition of plastic particles collected from GIT of fish and from sediment samples (b).

collected from Korekonch and Kidanemihiret (receiving urban waste from Batu town), and Meki-River (receiving Meki town's litter through the inflow Meki River) sites are indicative for the entry pathways and major origins of the particles in the lake (Fig. 1; Table 1).

Fishing and tourism activities are also sources of plastic litter to aquatic ecosystems (Karthik et al., 2018). Recreation related activities such as boating, restaurants, resorts and fishing (commercial and subsistence use) are among the possible key contributors for the observed high concentration of plastic particles in sediment samples collected from Korekonch, Kidanemihiret and Tepho-Choroke shoreline sites. Rivers are another important entry route transporting plastic debris from a catchment area into receiving water bodies such as lakes, estuaries and marines (Constant et al., 2020; Karthik et al., 2018). The observed sediment plastic particles concentration at the mouths of Meki River and Katar River reflect the contributions of the inflowing rivers.

The rivers transport plastics from the catchment area originating from towns and agricultural areas that the rivers pass through.

Surface runoff from agricultural lands provides another flux of plastic particles to surface waters (Fischer et al., 2016; Sanchez et al., 2014; Zhang, 2017). The smallholder vegetable farmers in the central rift valley region widely use polypropylene made plastic ropes to support tomato plants (Merga et al., 2020 in press). These ropes may constitute a major source of plastic particles for the sediments collected from Abosa and Edo-Kontola sampling sites. Plastic particle concentrations in sediment samples at Floriculture1 and Floriculture2 sites indicate the contribution of the proximate flower farms, but relatively low compared to the aforementioned sources. At the north-eastern part of the lake (i.e. the Reference site), where agricultural and urban influence was minimal, we have observed the lowest concentration of plastic particles in sediment.

**Table 2**

Reported literature values of frequency of fish ingested plastic particles (%), number of ingested plastic particles per fish (mean  $\pm$  SD), concentration of plastic particles in fish (mg/kg<sub>ww</sub>), size of ingested plastic particles (mm), and dominant identity and shapes of the plastics for various surface waters around the world.

Water body from where the studied fishes collected	Analysed matrix	Fish ingested plastics (%)	particles per fish (mean $\pm$ SD)	Concentration (mg/kg <sub>ww</sub> )	Ingested size (mm)	Dominant polymer	Major shape	References
North Pacific Central Gyre	SC	35%	2.1 $\pm$ 5.78	NR	1–2.79	Not reported	Fragments	Boerger et al. (2010)
Goiana Estuary, Brazil	SC	23%	NR	NR	NR	Nylon	Fragments	Possatto et al. (2011)
English Channel, UK	GIT	36.5%	1.9 $\pm$ 0.1	NR	0.13–14.3	PA, Rayon, PES	Fibres	Lusher et al. (2013)
Northern and southern parts of North Sea	GIT	2.6%	NR	NR	0.04–4.8	PE, PP, PET, SA	NR	Foekema et al. (2013)
French rivers, France	DT	12%	NR	NR	NR	Not reported	NR	Sanchez et al. (2014)
North Sea and Baltic Sea	SC	18.2%	1.3 ( $\pm$ 0.2)	0.002–93.9	0.63–164.5	Not reported	Fragments	Romeo et al. (2015)
Southern shore of Lake Victoria	GIT	20%	NR	NR	NR	PE, PUR, PES, PE/PP cop, SR	NR	Biginagwa et al. (2016)
Balearic Islands, Spain	GIT	68%	3.75 $\pm$ 0.25	NR	<5	Not reported	Fibres	Nadal et al. (2016)
Brazos River Basin, USA	SC	45%	NR	NR	NR	Not reported	Fibres	Peters and Bratton (2016)
Southern Hemisphere	GIT	5.5%	1.4 ( $\pm$ 0.5)	NR	0.18–500	PE, PP, PA	Fragments	Rummel et al. (2016)
North Sea and Baltic Sea	GIT	0.3%	2 (–)	0.0031	0.58–0.84	ACR	Fragments	Cannon et al. (2016)
Tokyo Bay, Japan	DT	77%	2.3 (1–15)	NR	0.1–7	PP, PE	Fragments	Tanaka and Takada (2016)
North Atlantic	DT	11%	1.2 $\pm$ 0.54	NR	0.5–11.7	NR	Fibres	Lusher et al. (2016)
Northeast Atlantic, Scotland	GIT	47.7%	1.8 ( $\pm$ 1.7)	NR	0.1–15	PA	Fibres	Murphy et al. (2017)
Lake Taihu and Yangtze Estuary, China	GIT	98%	3.7 $\pm$ 1.5	NR	0.04–24.8	CPH, PET, PES	Fibres	Jabeen et al. (2017)
Southern part of North Sea	GIT	0.25%	NR	NR	0.4	PMMA	Spherical	Hermesen et al. (2018)
Marne and Seine Rivers, France	SC	15%	NR	NR	0.39–7.38	PP, PE	Fibres	Collard et al. (2018)
Lake Ziway, Ethiopia	GIT	35%	4.4 $\pm$ 3.6	17.8 $\pm$ 46.8	0.2–40	PE and PP	Fragments	This study

**Abbreviations:** polypropylene (PP), Polyethylene (PE), polyurethane (PUR), polyester (PES), Polyethylene/polypropylene copolymer (PE/PP cop), silicone rubber (SR), Polyethylene terephthalate (PET), styrene acrylate (SA), polymethylmethacrylate (PMMA), Polyamide (PA), Cellophane (CPH), acrylic resin (ACR), stomach contents (SC), gastrointestinal tracts (GIT), digestive tract (DT) and not reported (NR).



The plastic particle number concentrations measured in sediment in this study (33,282 (5333–97,333) particles/m<sup>3</sup>) were comparable to values reported for freshwater lakes (Imhof et al., 2013; Su et al., 2016), freshwater rivers (Di and Wang, 2018; Nel et al., 2018) and marine sediment (Browne et al., 2010) (Table 3); and sometimes lower than other values reported (Castañeda et al., 2014; Klein et al., 2015; Leslie et al., 2017; Wang et al., 2017) (Table 3). However, mass concentrations of plastic particles in the present study are higher than those reported by Klein et al. (2015), which is one of the few studies reporting mass concentrations of plastic particles in sediment. This difference might be due to the larger particles included in our data. Differences in targeted size window applied for plastic particle quantification is a major cause of variation between results of studies (Koelmans et al., 2019; Lorenz et al., 2019; Mintenig et al., 2020). In addition to differences in regional sources of plastic particles, the variations we observed between our result and results of other studies are likely due to differences in targeted size window for detection. In our present study only plastic particles with  $\geq 0.1$  mm size were investigated in sediment. The studies by Leslie et al. (2017), Fischer et al. (2016) and Klein et al. (2015) included smaller size plastic particles (<0.1 mm).

Differences in used quality assurance/quality control are also a cause for the results variability (Hermesen et al., 2018; Koelmans et al., 2019; Mintenig et al., 2020). Given this fact, comparison between studies is difficult. As this has been addressed already by several authors (Hermesen et al., 2018; Koelmans et al., 2019; Markic et al., 2019), the problem requires the establishment of standard sampling, extraction, identification and quality control protocols for different environmental matrices.

Furthermore, we have observed a large mean size ( $5.3 \pm 6.0$  mm) of plastic particles and a low fraction (70%) of MP (size < 5 mm) compared to other studies. For example, in sediment samples from the St. Lawrence River of Canada (Castañeda et al., 2014), the southern North Sea (Lorenz et al., 2019), the Rhine River of Germany (Mani et al., 2019) and the Byfjorden coast of Norway (Haave et al., 2019) plastic particles < 5 mm (100%), <0.5 mm (99.96%), <0.075 mm (96%) and <0.1 mm (95%) were reported, respectively. Being able to detect plastic particles in environmental samples depends on the targeted size range and on the sample volume (amount) (Koelmans et al., 2019). The above mentioned studies used high amounts (1.2–2 kg) of sediment and reported a small maximum size window ( $\leq 5.033$  mm). The present study analysed comparably large sample quantities (e.g. 630 g) and used a large maximum size window (45 mm). We hypothesize that the larger sample sizes enabled us to find larger particles that occur at a lower frequency.

Remarkably, the highest mass concentration measured in sediment of the studied lake was 36,233 mg/kg<sub>dw</sub>, i.e. 3.62% on a dry weight basis. To our knowledge, this is the highest plastic particle mass concentrations in sediment reported to date (Redondo-Hasselerharm et al., 2020; Schell et al., 2020). Redondo-Hasselerharm et al. (2018) found an EC<sub>10</sub> of 1.07% and an EC<sub>50</sub> of 3.57% for the growth of *Gammarus pulex*, and a long term (15 month) benthic community effect LOEC of 5% (Redondo-Hasselerharm et al., 2020). Effects on emergence and on body weight of *Chironomus riparius* upon chronic exposure (28 day) to a concentration of 2% of microplastic were observed by Scherer et al. (2020). Furthermore, effects on larval growth (10-d LOEC = 0.25%) and on imagoes emergence (10-d LOEC = 0.15%) of *C. riparius* were reported by Silva et al. (2019). These imply that the highest mass concentration measured in sediment samples from the lake exceed the currently known effect thresholds for MP in sediment, thus indicating that long term in situ benthic community effects cannot be excluded.

The possible reason for the observed significantly lower number of plastic particles in sediment samples collected during the wet

season compared to the dry season samples (Table 1 SI) could be a result of the resuspension of plastic particles from bottom sediment due to heavy rain and runoff (Fischer et al., 2016; Mintenig et al., 2020). Plastic particles are likely to reside in the overlying water for considerable time as plastic particles only slowly settle from the water column to the bottom sediment (Nel et al., 2018). The identified polymer types in sediment samples were mainly PP and PE (Fig. 4b). These polymers generally have low densities that also enhance the resuspension of the particles from bottom sediment upon heavy rain during wet season. Though plastic particles in the water column were not quantified in our study, we hypothesize that the concentration of the particles in overlying water of the lake could be higher in the wet season than the dry season, as in the rainy season plastic particles enter into the lake via runoff from terrestrial ecosystem (Zhang, 2017).

#### 4.3. Identity and potential sources of the plastic particles

We have identified different type of polymers (Fig. 4b). Similar to previous studies reporting polymer types for sediments (Karthik et al., 2018; Lorenz et al., 2019; Wang et al., 2017) and for fishes (Biginagwa et al., 2016; Karthik et al., 2018; Rummel et al., 2016), PP and PE were the most frequently found polymers in sediment and in the GIT of fishes of Lake Ziway (Tables 2, 3). The percentage of PET and AR found in sediment samples was also high (18.5%) compared to polyurethane-acrylic resin (7.4%) and ethylene-propylene rubber (3%). The contribution of alkyd-varnish (AV) in both fish (11.8%) and sediment (18.5%) was also considerable (Fig. 4b). These polymers are likely present in the lake due to urban wastes including plastic bags, packaging materials and disposable bottles that end up in the lake through various entry pathways including wastewater drainages, town crossing inflowing rivers, and heavy rain causing urban and agricultural land runoff. Similarly, the quantified EPR in sediment samples was likely originates from water hoses or electrical insulation waste (Haave et al., 2019). As reported by Wang et al. (2017) for sediment of the Beijiang River of China, and by Haave et al. (2019) for sediment at the Byfjorden coast of Norway, the identified synthetic resins such as polyurethane-acrylic resin and alkyd-varnish in sediment and fishes samples of this study were potentially originating from workshop wastes (e.g. wood and metal) and paint of boats.

#### 5. Conclusion

To our knowledge this is the first study to report plastic particle abundance and characteristics both in the GIT of fishes and in sediment for an African shallow freshwater lake. Our results indicate that fishes and shoreline sediments sampled near to towns were more contaminated with plastic particles, compared to the samples taken from shore sites with a lower urban and agriculture activities, and exceed currently known threshold effect concentrations. In addition, there was a significant difference between the wet and dry seasons with respect to the frequency of fishes found with ingested plastic particles, as well as the plastic particle concentration in the sediment of the lake. The studied fish species have a significant economic and ecological roles in the region (Endebu et al., 2015). Because of their role as ecosystem engineers as well as ecosystem service providing units, it is important to study the impact of ingestion of plastic particles on these species. Furthermore, assessment of human health impacts caused by consumption of plastic particles contaminated fishes (Carbery et al., 2018) as well as drinking water (WHO, 2019) is needed. As confirmed by ATR-FTIR, mainly urban related domestic waste was among the major sources for the plastic pollution in the studied lake. Mitigation measures such as implementing proper domestic waste management practices (Khan et al., 2018) by municipalities of the nearby towns and encouraging tomato producing farmers to use natural fibres made of degradable



**Table 3**

Reported literature values of numerical abundance over an area (particles/m<sup>2</sup>), concentrations (particles/kg\_dw, particles/L, particles/m<sup>3</sup> and mg/kg\_dw), and dominant polymers and shapes documented in studied sediments of various surface waters around the world. The \* indicates the concentration is reported as particles/kg\_wet weight.

Source of studied sediment sample	Abundance (particles/m <sup>2</sup> )	Con. (particles/L)	Con. (particles/kg_dw)	Con. (particles/m <sup>3</sup> )	Con. (mg/kg_dw)	Dominant polymer	Dominant shape	References
Tamar Estuary, UK	NR	<60–160	NR	NR	NR	PVC, PES, PA	Fibres	Browne et al. (2010)
Lake Garda, Italy	8.3–1108	NR	NR	NR	NR	PS, PE, PP	Fragments	Imhof et al. (2013)
Lake Erie, North America	1.5 (0.36–3.7)	–	NR	NR	NR	PE, PP	Fragments	Zbyszewski et al. (2014)
Lake St. Clair, North America	1.7 (0.18–8.38)	–	NR	NR	NR	PE, PP	Fragments	Zbyszewski et al. (2014)
Lake Huron, North America	9.5 (0–34)	–	NR	NR	NR	PE	Pellets	Zbyszewski et al. (2014)
St. Lawrence River, Canada	13,832 (0–136,926)	NR	NR	NR	NR	PE	Beads	Castañeda et al. (2014)
Rhine-Main rivers, Germany	1800–30,000	NR	228–3763	NR	21.8–932	PP, PE, PS	Fragments	Klein et al. (2015)
Lake Bolsena, Italy	1922 (1903–1941)	NR	112 (109–117)	NR	NR	NR	Fibres	Fischer et al. (2016)
Lake Chiusi, Italy	2117 (1772–2462)	NR	234 (205–266)	NR	NR	NR	Fibres	Fischer et al. (2016)
Taihu Lake, China	NR	NR	11–234.6	NR	NR	CPH, PET	Fibres	Su et al. (2016)
Amsterdam canal, Netherlands	NR	NR	2071 (<68–10,500)	NR	NR	NR	Fibres	Leslie et al. (2017)
Dutch North Sea coast, Netherlands	NR	NR	100–3600	NR	NR	NR	Fibres	Leslie et al. (2017)
Beijiang River, China	NR	NR	312.5 (178–544)	NR	NR	PE, PP	Fibres	Wang et al. (2017)
Bloukrans River, South Africa	NR	NR	13.3–563.8	NR	NR	NR	NR	Nel et al. (2018)
Yangtze River, China	NR	NR	82 (25–300)*	NR	NR	PS, PP, PE	Fibres	Di and Wang (2018)
Lake Ziway, Ethiopia	378 (59–1081)	33 (5–97)	40 (6.3–115.9)	33,282 (5333–97,333)	764 (0.05–36,233)	PE, PP, AV, PET	Fragments	This study

**Abbreviations:** polyester (PES), acrylic resin (ACR), polypropylene (PP), polyethylene (PE), polystyrene (PS), cellophane (CPH), polyethylene terephthalate (PET), polyvinylchloride (PVC), polyamide (PA), Alkyd-Varnish (AV) and not reported (NR).

ropes instead of using plastic ropes are highly recommended to abate the problem.

### CRedit authorship contribution statement

**Lemessa B. Merga:** Investigation, Project administration, Writing - original draft, Formal analysis. **Paula E. Redondo-Hasselerharm:** Investigation, Writing - review & editing. **Paul J. Van den Brink:** Conceptualization, Supervision, Writing - review & editing, Project administration. **Albert A. Koelmans:** Conceptualization, Resources, Writing - review & editing.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.140527>.

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