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Assessment of Microplastics in Water, Sediment, and Fish of Ikpoba Rivers of Edo State, Nigeria

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ABSTRACT: This study presents a comprehensive assessment of microplastics in water, sediment, and fish species (*Clarias gariepinus* and *Oreochromis niloticus*) of the Ikpoba River. Water samples were collected using twenty-five-litre stainless steel buckets, sediment samples were obtained using a Van Veen grab sampler, and fish samples were collected using gill nets and hand nets respectively. Microplastics were extracted from water and sediment samples using a density separation method, employing a saturated sodium chloride solution. Fish samples were dissected, and the gastrointestinal tracts (GIT) were examined for the presence of microplastics. Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy (FTIR) was employed to confirm the polymer composition of select particles. The results indicate the widespread presence of microplastics in the Ikpoba rivers ecosystem with a high prevalence of polypropylene (PP), polystyrene (PS), and polyethylene (PE) in surface water and Polyethylene terephthalate (PET) and Polyvinyl chloride (PVC) in sediment samples. In fish samples, *C. gariepinus* accumulated the highest concentration of microplastics compared to *O. niloticus*. The polymer PE was highest in both fish species followed by PC. Most MP shapes identified in this study consist of fiber, film, foam, and fragments in water, sediment, and fish. Therefore, this study quantitatively demonstrates the presence of microplastic contamination in the Ikpoba River and thus raises significant concerns about the vulnerability of the local fish population to microplastic ingestion.

Keywords: Microplastics, Freshwater Pollution, FT-IR Spectroscopy, Polymer types.

Introduction

Microplastics are a potential threat to the global environment and are closely tied to the overall plastics disposal and life cycle issues. They are emerging contaminants that have gained considerable attention in the last decades due to their adverse impact on living organisms and the environment (Mammo *et al.*, 2020). MP particles enter the aquatic environment in various forms, shapes, and colour, derived from several different sources and pathways, such as wind advection, stormwater runoff, and illegal plastic waste dump, among others, and they can be transported across environmental compartments with different residence times (Sutton *et al*., 2016; Wagner and Lambert, 2018). The continuous increase in synthetic plastic production and the inadequate management of waste in facilities has led to a tremendous increase in aquatic environments (Woodall *et al*.,

2014). Freshwater ecosystems have remained an important conduit typically transporting MPs from land-based sources to estuaries and the open ocean (Mani *et al*., 2015; Luo *et al*., 2019).

MPs have become contaminants of global concern due to their wide distribution in every environmental compartment and matrix (Bellasi *et al*., 2020). They are believed to be persistent, bioaccumulated, and biomagnified throughout the food web, and their bioaccumulation potential increases with decreasing size (Frias *et al*., 2019; Tongo *et al*., 2022). Owing to their small size, MPs may be ingested by a range of organisms as food such as bivalves, zooplankton, mussels, shrimps, oysters, copepods, lugworms, whales, seabirds, fish, and even mammals (von Moos *et al*., 2012; Watts *et al*., 2014; Vikas *et al*., 2019). The trophic transfer of MPs has been documented in both freshwater (Setälä *et al*. 2014; Santana *et al*. 2017) and marine systems (Batel *et al*. 2016). Globally, plastic production has increased exponentially exceeding 390 million tons in 2021 due to their high durability, malleable features, and ease of use as packaging materials (PlasticsEurope, 2022). MPs are dominated by six types of polymers such as polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyethylene terephthalate (PET) polystyrene (PS), and polyurethane (PU) (Lithner *et al*. 2011; PlasticsEurope 2015; Munari *et al*., 2021).

In recent times, the study of the environmental occurrence and effects of MPs has shifted from marine ecosystems to inland waters (Wagner and Lambert, 2018) as they contribute to the diminishing aesthetic beauty of the aquatic environment and biodiversity loss (Thompson *et al*., 2009; Gall and Thompson, 2015). While significant progress has been made in understanding microplastic contamination in the marine ecosystem, there are still many unanswered questions and research gaps in the freshwater environment. As a result, the body of knowledge on the accumulation and effects of plastics in freshwater and terrestrial ecosystems is much less than in marine ecosystems (Thompson *et al*., 2009; Wagner *et al*., 2014). Until recently the distribution of microplastics in freshwater systems was unknown. However, in the last few years studies have shown that MPs have been identified in freshwater ecosystems across continents (Lu *et al*., 2021).

Plastic pollution is indeed a concern in Africa as statistics have shown that five African countries (Nigeria, Ghana, Egypt, South Africa, and Morocco) form part of the top twenty highest contributors to plastic marine debris worldwide (Sambyal, 2018). In addition, the Niger and Nile rivers are listed among the ten rivers worldwide that carry approximately 90% of plastic waste into the oceans, as they carry about 35,196 and 84,792 tons, respectively, every year (Okeke *et al*., 2022). While some MPs may be perceived as impediments to the achievement of the UN Sustainable Development Goals (SDGs), there is no specific mention of targets aimed at explicitly reducing MP pollution in the aquatic ecosystems based on their threat to biodiversity (SDGs 6.3, 6.6,) and Life Under Water (SDG 14.1). Therefore, this study aims to provide an in-depth analysis of microplastic contamination in water, sediment, and fish of the Ikpoba river of Edo state. Understanding the concentration distribution, and ecological impacts of MPs is essential for developing effective mitigation strategies and policies to address their effects on aquatic ecosystems and potential human health implications.

Materials and methods

Site description: The research was conducted in a stretch of the Ikpoba River (Fig. 1) a fourth-order stream situated within the rainforest belt of Edo State, southern Nigeria; flowing in a south-westerly direction in a steeply incised valley and through sandy areas before passing through Benin City and joining the Ossiomo River (Atuanya *et al*., 2012; Odigie, 2015). The Ikpoba River lies within Latitude 6.5°N and Longitude 5.8°E and is surrounded on both sides by the sloppy terrain of the Ikpoba slope (Atuanya *et al*., 2012). The river is dendrite in the upper reaches and its headwaters originate from the Ishan Plateau in the east coastal plain to the northeast of Benin City, with an elevation of about 230m above sea level (Odigie, 2015). The Ikpoba River drains an area of about 730.20 km^2 according to Odemerho (1992). The river has a maximum stage-discharge of 320 cm and a minimum of 191cm (Owena River Basin Authority, 1996). Typically, the region has the characteristic features of a humid tropical wet and dry climate governed primarily by rainfall. The vegetation of Ikpoba River consists of rainforest which is secondary in nature and has been subjected to deforestation and other anthropogenic activities.

Figure 1: Map showing sampling locations investigated in the Ikpoba River

Sample Collection: Surface water, sediment, and fish samples were collected from July to December 2020 from 3 sampling locations of Ikpoba River [University of Benin (UNIBEN), Upper Lawani, and Ikpoba Slope]. The points were selected systematically considering the anthropogenic inputs visualized on-site (Ogbomida and Emeribe, 2013). The procedures employed for microplastic sampling were adapted from Masura *et al*. (2015). Surface water was collected from each sampling point using a clean 25 L stainless steel buckets previously rinsed and cleaned with deionized water and 70% ethanol at a depth of 1 m and then filtered on-site with a 0.48 µm stainless steel sieve (Wang *et al*., 2017; Zhao *et al*., 2014). Before laboratory analysis, the samples were fixed in 5% formalin at 4 °C (Lattin *et al*., 2004). To maximize sample homogeneity, each sample collected from sampling sites was taken in two replicates at 5 m intervals pooled into a single composite sample, and labeled as the W series such as W1, W2, and W3.

The sediment samples were obtained from the riverbed according to the method of Claessens *et al*. (2011). 2 kg wet weight (ww) sediment was collected from sampling locations using a Van Veen grab (0.25 m^2 sampling surface) of the top (≈10 cm depth) at each site. The sediment samples were placed in a glass jar and then preserved at 4 °C before microplastic extraction and analysis (Dahms *et al*., 2020). Composite samples of two replicates were taken at each site and labeled as the S series such as S1, S2, and S3.

Fish samples for MP analysis were caught from the Ikpoba River by local fishermen using sets of gill nets and hand nets (Connell *et al*., 2020). 3 individuals per species per station, of two commercially important fish species (Tilapia (*Oreochromis niloticus*, Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822) were immediately dissected on-site to recover the entire gastrointestinal tract (buccal cavity to anus). The dissected fish were cleaned and returned to the fishermen for sale. GITs and their contents were then individually preserved in 96% ethanol and transported to laboratory facilities at the National Centre for Energy and Environment, University of Benin and stored at 4 °C (Lattin *et al*., 2004) until further analysis.

Microplastic extraction: To dissolve natural organics, all the water-filtered samples fixed in 5% formalin at 4 °C were treated with 30%, v/v H_2O_2 for 12 h to remove visible organisms from the samples (Liebezeit and Dubaish, 2012). A ferrous sulphate solution was used as a catalyst. Density separation was conducted using a zinc chloride solution (1.5 g/cm^3) to remove sand and minerals and the supernatants were collected in the density separator and then filtered through a 0.22 µm pore size GF/C filter (Membrane Solutions LLC., Kent, WA, USA). Since natural air drying can curl the filter, all the filters were placed in a covered glass dish and dried in an oven set at 60 °C before microscopic observation and analysis (Wang *et al*., 2017).

The microplastics in the sediments were extracted by applying a two-step density separation method (Nuelle *et al*., 2013; Thompson *et al*., 2004) with some modifications. First, 1 L of saturated sodium chloride solution was added to 500 g of wet sediment in a glass beaker (2 L), stirred for 2 min, and settled for 10 min. Then, the supernatant was poured through a 0.48 µm stainless steel sieve and the microplastics intercepted by the sieve were washed into a beaker, which was subsequently covered with tin foil. The filtered sodium chloride solution was recycled, and the extraction process was performed three times for each sample. The purpose of this

preliminary extraction was to reduce the sample mass for the next step since sodium iodide is expensive and not eco-friendly. The second step aimed to further the extraction of high-density MPs. After the first extraction step, the remaining sediment was collected and transferred to a triangular flask (500 mL), and a 60% sodium iodide solution was added to three-fourths of the flask. The mixture was then shaken for 2 min at 200 rpm on a shaker and permitted to stand for 10 min. After stratification, the supernatant treatment was the same as the first step. For each sample, the process of refilling, shaking, precipitation, and decantation was repeated twice. The suspension obtained from this two-step extraction was then treated together with 30%, v/v H_2O_2 to digest the natural organics. The remaining procedure was the same as the water samples.

Also, fish GITs were analyzed according to Foekema *et al*. (2013). The GIT tissues were placed in 300 mL digestion glass bottles containing an appropriate volume of 10% KOH (Analytical grade, UNI-CHEM®) solution until the organs were submerged and placed in the oven for 24 h at 60°C. After extraction, the sample was filtered with a 0.48 µm filter paper (WhatmanTM, UK). Each filter paper was placed in a petri dish and labeled to observe MPs in the sample.

Microplastic observation and identification: Microplastics were visually identified in a clean, rinsed glass Petri dish using a dissecting microscope with a digital camera (M165 FC, Leica, Germany) according to Cannon *et al*. (2016) and Lusher *et al*. (2016). Criteria for microplastic characterization included physical characteristics such as unnatural appearance (e.g., shiny particles without visible cellular or organic structures) as described by Lusher *et al*. (2016), the shape of the particles (e.g., fibre, fragment), and colour (MERI 2015; Rochman *et al*., 2015; Windsor *et al*., 2019). The identification of microplastics followed a step-by-step guide of elimination, to determine conservative estimates for microplastics as described by Hidalgo-Ruz *et al*. (2012) and the Marine and Environmental Research Institute's guide to microplastic identification MERI (2015). The malleability of the plastic particles was checked by squashing them with a laboratory stainless dissect needle (micro-tip diameter) as stated by Cannon *et al*. (2016). Colour and shape (fiber, fragment, foam, and film) were recorded. Only if an item had passed all the previously named checkpoints, was it counted as a microplastic (Hidalgo-Ruz *et al*., (2012); Lusher *et al*., 2017). For microplastic abundance in the water samples, the unit of calculation was the number of microplastics per cubic meter, whereas, for sediments, it was the number of microplastics per kilogram w/w. The chemical composition of all suspected plastics was identified non-destructively by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy.

Statistical analysis: Statistical analysis was performed using JMP Statistical Discovery version 17. Microplastic counts were log-transformed to allow the data to be more interpretable due to the extremely high and low counts detected between the matrices. A Spearman's rank correlation test was conducted between the various reporting units showing significant correlations ($p < 0.01$) to determine any correlations between total microplastics. The Mann-Whitney *U* test was conducted to determine any significant differences.

Results

Quantification of MPs in water: MPs were prevalent in all water samples from the three stations UNIBEN Site, Upper Lawani, and Ikpoba Slope, with polymer abundances ranging from 2.67±0.58 particles/m^3 to 8.00±1.00 particles/m^3 (Table 1). The highest concentration of total microplastics was detected at UNIBEN Site (Station 1) followed by Upper Lawani (Station 2) and the lowest at Ikpoba Slope (Station 3). In station 1 PE polymer was predominant while stations 2 and 3 recorded PS polymer as the most ubiquitous. The most abundant microplastic shapes found were fibers (86.7%), followed by Film (52.0%) Foam (48.3%), and Fragment (45.9%) [Figure 1].

Table 1: Abundance of microplastics in surface water samples

Microplastic Polymers	**S1 (UNIBEN Site) Mean ± SD**	**S2 (Upper Lawani) Mean ± SD**	**S3 (Ikpoba Slope) Mean ± SD**
CP	6.67 ± 3.21	2.67 ± 0.58	3.83 ± 1.15
PET	3.67 ± 1.15	5.33 ± 2.52	4.50 ± 2.65
PVC	5.67 ± 2.89	3.67 ± 1.53	3.17 ± 1.53
PC	4.00 ± 1.73	4.00 ± 1.00	3.17 ± 0.58
PS	4.33 ± 1.15	6.67 ± 1.53	4.67 ± 2.52
PE	8.00 ± 1.00	4.67 ± 1.15	5.67 ± 1.53
PU	3.33 ± 0.58	4.00 ± 1.00	4.33 ± 1.00
PP	7.00 ± 3.61	2.67 ± 0.58	3.67 ± 0.58

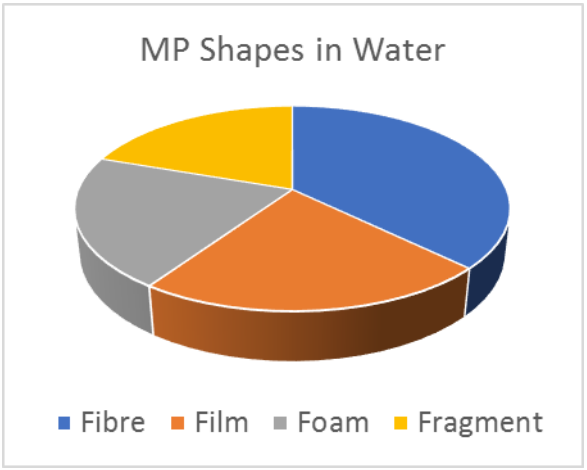

Figure 1: Percentage distribution of MP Shapes in water sample of Ikpoba River\

Quantification of MPs in sediment: MPs in sediment samples revealed values of 55±16.8 particles/kg in UNIBEN Site (station 1), 40±13.4 particles/kg Upper Lawani (Station 2), and 53.33±18.1 particles/kg Ikpoba Slope (Station 3). High values of polymers ranged from PET 12.67±6.66 particles/kg to PS 1.33±0.58 particles/kg for station 1, 9.67±3.79 particles/kg to 1.33±0.58 particles/kg for station 2, and 9.33±2.52 particles/kg to 5.33±1.53 particles/kg station 3 Table 2. MP shapes in sediment were classified into four types: films, fragments, fibers, and foams**.** The MP shapes observed in sediments revealed fibers (89.02%), fragments (46.00%), foam (42.56%), and films (35.70%) Figure 2. The particle shape distribution follows a clear trend along the course of the river.

Table 2: Abundance of microplastics in sediment samples

Microplastic Polymers	**S1 (UNIBEN Site) Mean ± SD**	**S2 (Upper Lawani) Mean ± SD**	**S3 (Ikpoba Slope) Mean ± SD**
CP	6.33 ± 2.52	3.67 ± 0.58	6.00 ± 2.65
PET	10.00 ± 6.24	12.67 ± 6.66	5.33 ± 1.53
PVC	8.33 ± 1.15	9.67 ± 3.79	9.33 ± 2.52
PC	6.67 ± 1.15	8.00 ± 2.65	6.33 ± 2.52
PS	3.67 ± 0.58	1.33 ± 0.58	5.67 ± 1.15
PE	7.33 ± 2.08	4.67 ± 1.53	6.00 ± 2.65
PU	3.67 ± 1.15	6.00 ± 2.00	7.67 ± 1.53
PP	6.33 ± 1.53	4.00 ± 1.73	7.00 ± 3.61

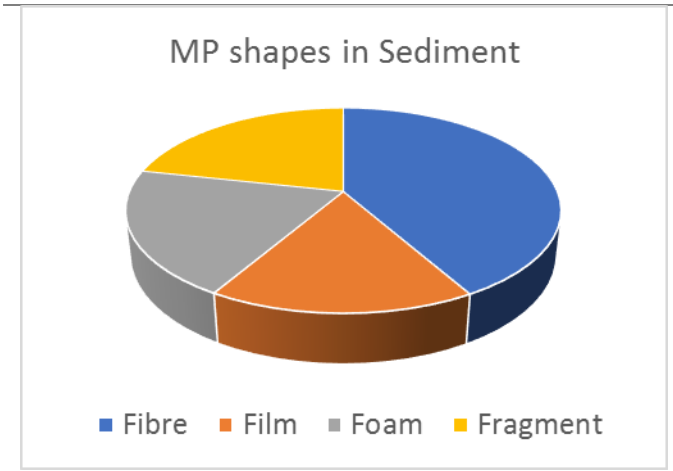

Figure 2: Percentage distribution of MP Shapes in sediment sample of Ikpoba River

Quantification of MPs in fish species: The GIT assessment of fish species for MPs showed that the total mean value Mean ± SD ranged from 3.00 ± 1.00 particles per fish to 7.33±2.08 particles per fish in *C. gariepinus* and 2.33 ± 0.58 particles per fish to 6.67 ± 0.58 particles per fish *O. niloticus* respectively Table 3. The highest amount of microplastics was recorded in *C. gariepinus*.

The shape of the MPs determined in the fish species were dominated by fibers 79.51%), followed by fragments (42.21%), film (40.16%), and foam (34.02%) in *C. gariepinus* while *O. niloticus* the MP shapes were in order of fibers 84.24%, followed by fragments (40.89%), foam (44.83%) and film (38.42%) Figure 3.

Table 3: Abundance of Microplastics in fish species

Microplastic Polymers	***C. gariepinus***			***O. niloticus***		
	S1 - UNIBEN Site	**S2 - Upper Lawani**	**S3 - Ikpoba Slope**	**S1 - UNIBEN Site**	**S2 - Upper Lawani**	**S3 - Ikpoba Slope**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
PET	5.00 ± 2.65	6.33 ± 2.08	5.33 ± 2.08	3.67 ± 1.15	2.33 ± 0.58	4.67 ± 2.08
PC	6.33 ± 1.53	7.00 ± 2.00	4.00 ± 1.00	4.67 ± 0.58	6.33 ± 1.53	4.00 ± 1.00
PS	3.00 ± 1.00	6.67 ± 0.58	5.00 ± 1.00	3.33 ± 0.58	6.00 ± 2.65	5.00 ± 2.65
PE	5.67 ± 1.53	7.33 ± 2.08	6.67 ± 2.52	5.00 ± 1.00	4.33 ± 1.53	6.67 ± 0.58
PP	4.33 ± 1.15	3.67 ± 1.53	5.00 ± 1.00	3.67 ± 1.53	4.33 ± 0.58	3.67 ± 1.53

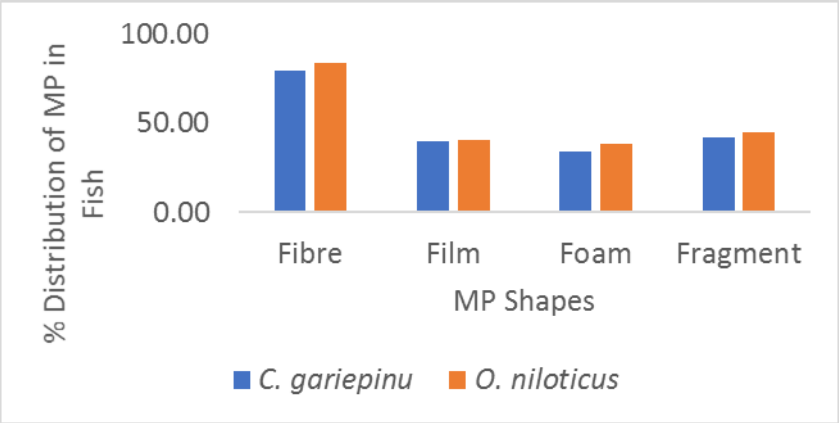

Figure 3: Percentage morphotypes of microplastics identified from GIT from different fish species in Ikpoba River

Discussion

The assessment of microplastics in water, sediment, and fish is a pressing environmental issue with far-reaching ecological and potential human health implications. In this study a total of 8 microplastic polymers were detected in water, sediment, and fish which include CP, PET, PVC, PC, PS, PE, PU and PP. In the water samples of Ikpoba River, PP, PET, PS, and PE were the dominant types of MPs recorded. This result conforms with Bordos *et al.* (2019), Liu *et al.* (2021), and Garcés-Ordóñez *et al.* (2022) who reported PE and PP in various aquatic ecosystems. PE and PP have been identified as common thermoplastics used in packaging products such as film, shopping bags, bottles, toys, houseware, juice containers, milk containers, crates, plastic packaging, fibers, and textiles. The predominance of PP, PET, PS, and PE polymers in surface water is due to their density relative to the water which is lower than the density of water (Cincinelli *et al*., 2017; Song and Andrary, 1991). PP, PS, and PE are lighter than water due to their neutral buoyancy and hence float on the surface and then are easily ingested by aquatic organisms in the different food chains. The occurrence, composition, and identification of MPs in the environment are highly dependent on the sources of the plastic wastes. Also in the water sample fibers were dominant shapes of MPs which is consistent with the studies of Su *et al*. (2019) and Clere *et al.* (2022).

The ubiquity of MPs in sediment samples showed that plastics sink into deeper water and PET and PVC were major polymers samples because of their densities higher than water. According to Kowalski *et al*. (2016) size, density, and shape determine the rate at which plastics sink into deeper water and sediments. Also, fibers were found to be the most abundant MP type within the sediment samples contributing about 89.01%. A recent study by Yin *et al*. (2020) also reported fibers as a dominant type of MPs in freshwater. The same highest proportions of fibers were documented by Sembiring *et al*. (2020) and Zhang *et al*. (2019) in sediments. Overall, MPs such as PE, PP, PS, and PET have been reported to induce numerous negative impacts on aquatic organisms (De Sá *et al*., 2018). Reports suggest that the existence of MPs in the sediment samples could reflect long-term

contamination for both terrestrial and aquatic environments resulting in long-term ecological pollution and impacts (Nel *et al*., 2018; Smith *et al*., 2018).

Fish is an essential biological component of freshwater ecosystems with great nutritional and economic importance. About 94% of all freshwater fisheries occur in developing countries (FAO, 2007), providing food and a livelihood for millions of the world's poorest people, and contributing to the overall economic well-being of rural communities. Therefore, the occurrence of MPs in *C. gariepinus* and *O. niloticus* may lead to a variety of negative health impacts and biodiversity loss. In this study, *C. gariepinus*, a benthic fish accumulated the highest concentrations of MPs in the GIT suggesting the potential for bioaccumulation in the aquatic food chain. This may be attributed to their proximity to the sediment than the surface water (Skelton, 2001). MP particles are also easily ingested by fish species in unintended ways due to their small size and similarity to natural food items (Crawford and Quinn, 2017). This study is potentially important as it can help identify those species at particular risk from microplastic contamination that are also of high conservation concern (Parker *et al*., 2021). Accumulation of MP in fish has a wide range of negative impacts such as decreased feeding activity, impeded growth, energy interruption, oxidative stress, and even genotoxicity (Lu *et al*., 2016; Hassan *et al*., 2023). MPs hinder fish metabolism by lowering the amount of energy needed for growth and delaying ovulation (Wright *et al*., 2013). MP may lead to obstructing the gastrointestinal tract or intestinal blockage producing distorted satiation and internal abrasion and posing numerous ecotoxicological effects which may severely affect swimming and/or survival ability. Following a similar trend observed in water and sediments, fibers were also dominant MP shapes in both fish species. Studies demonstrated that the toxicity of MP fibers is greater than that of other MP particles (Ziajahromi *et al*., 2017), which may be related to the longer duration of fiber in the intestinal tract (Au *et al*., 2015; Lei *et al*., 2018) as well as its ability to adsorb other persistent and toxic chemical pollutants (Au *et al*., 2015; Re *et al*., 2019). Moreover, the higher abundances of fragments, films, and fibers in the Ikpoba River may pose a higher MPs encounter potential in the inhabiting freshwater organisms (Fang *et al*., 2018; Phuong *et al*., 2018).

Conclusion

This study provides important preliminary data on the distribution of microplastics in the Ikpoba River of Edo State, Nigeria. The widespread occurrence of microplastics in water, sediment, and fish samples underscores the need for continued monitoring and the development of mitigation strategies to reduce microplastic pollution in these important freshwater systems. Further research is required to assess the long-term impacts of microplastics on aquatic ecosystems and human health in the region.

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