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A Preliminary Investigation of Microplastic Levels in a Commercially Important Clariid Fish Species from Ikpoba River, Benin City, Nigeria

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ABSTRACT

Microplastics (MPs) in natural water resources and their inclusion in food webs has become a critical issue globally. The paucity of risk assessment data on the levels of MPs in Ikpoba River, Benin City, Nigeria, warranted this research which was done via Fourier Transform Infrared (FTIR) Spectroscopy. The mean levels of MPs in *Clarias gariepinus* (mean total length 32.74 ± 1.58 cm, mean weight 885 ± 1.76 g) according to stations ranged from 0.37 in June at station 1 to 5.23 in August at station 3 with a total ranging from 2.97 at station 2 to 6.18 at station 3. Significant differences (P¡0.05) were observed in the mean levels of MPs between the months across stations 1 and 3. The mean plastic load in *C. gariepinus* according to stations ranged from 0.31 in June at station 1 to 6.34 in August at station 3 with a total ranging from 3.22 at station 2 to 7.41 at station 3. Significant differences (P¡0.05) were observed in the mean plastic load between the months across stations 1 and 3. The dominant types of MP particles found in *C. gariepinus* were fragments and filaments of Polyethylene. The estimated daily intake (EDI) for MPs (No./person/day) ranged from 0.00154 at station 2 to 0.00321 at station 3. *C. gariepinus* must therefore be consumed with caution in order to avert health problems associated with polymer pollution in the long run.

Keywords: *Clarias gariepinus*, Fourier Transform Infrared Spectroscopy, Microplastics, Polyethylene

1 INTRODUCTION

Plastic production has increased substantially since large scale industrial manufacture started many decades ago such that all aspects of daily human life involve plastics hence their persistence in aquatic ecosystems globally (Lusher *et al.,* 2017). The National Oceanic and Atmospheric Administration (NOAA), has defined microplastics (MPs) as small fragments of plastic measuring less than 5 mm in length that are hazardous to aquatic bodies and their resources (NOAA, 2023). These polymers could have either primary or secondary sources. Primary microplastics are plastics directly released into the environment in the form of small particulates e.g. cosmetics, shower gels, abrasion of large plastic objects, wear of tyres and abrasion of synthetic textiles. Secondary microplastics on the flipside are microplastics originating from the degradation (e.g. photodegradation and weathering processes) of larger plastic items into smaller plastic fragments (International Union for Conservation of Nature, 2017). The occurrence of MPs in natural water resources and their inclusion in food webs made possible by their minute size, has become a critical issue around the world especially in recent times (Kamau *et al.,* 2023; Pellicer and Domingo, 2023). It has been observed that a host of hydrobionts including fish, readily ingest MPs while grazing for food and these particles end up making their way into other parts of the body including edible tissue which is cherished by man (My *et al.,* 2023). The marine environment has been the focus of MP research in recent times (Wagner *et al.,* 2017; Selvakumar *et al.,* 2023). However, the searchlight has shifted to freshwater bodies which are now known to contain a cocktail of MPs including Polyethylene (PE), Polyamide (PA), Polyester (PES), Polypropylene (PP) and Polystyrene (PS) especially in their sediments thereby making benthic fish veritable targets of risk (Boskovic *et al.,* 2023). Mangrove ecosystems have also been scrutinized for MPs around the world in order to examine the contamination of flora, fauna

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and abiotic compartments (Mendes *et al.,* 2023). It has been observed that an increase in anthropogenic activities over the years has led to the influx of synthetic fibres of MP into the aquatic environment which is fast overshadowing the presence of natural fibres in such recipient waters (Mancuso *et al.,* 2023). The negative impacts of MPs on aquatic life (including fish) and human health has been well documented (Kamau *et al.,* 2023). Microplastics remain a threat in the aquatic environment as more polymer-based products such as polymer electrodes and polymer-based ionic conductors are being manufactured (Arof, 2023). The Ikpoba river in Benin City, Nigeria, has been under a lot of anthropogenic pressure over the years which has resulted in a plethora of researches encompassing the impact of both inorganic pollutants (Oronsaye *et al.,* 2010; Wangboje *et al.*, 2014) and organic pollutants (Wangboje and Oronsaye, 2012; Wangboje and Oguzie, 2013). There is however paucity of risk assessment data on the levels of MPs in this particular ecosystem which this current research attempts to provide thereby expanding the pollutant base profile of the river. The fish species of choice, *Clarias gariepinus* (Burchell, 1822), was particularly targeted as it is available in the Ikpoba river all year round and it is of high commercial appeal in Benin City and environs. The fish species can attain a length of over a metre with a weight of over 7 kg. It is commonly found in swamps, streams, rivers and lakes. It is an omnivorous bottom feeder, feeding on detritus, plankton, gastropods, crustacean, worms and small fishes such as *Tilapia* and *Alestes* species (Idodo-Umeh, 2003).

2 Materials and Methods

2.1 Description of Study Area

This research was carried out on the Ikpoba River in Benin City (Latitude 6°20'00" N and Longitude 5°37'20" E) Edo state, Nigeria (Fig. 1). The City is located within the Tropical rainforest ecological zone of south-south Nigeria. Further details of the study area have been published by Wangboje and Momoh (2023). The stations established for this research were Upper Lawani (Station 1), Reservoir (Station 2), and Bridge (Station 3). The Upper Lawani station is located within Latitude 6°22'32" N and Longitude 5°38'45" E. Human activities carried out at this point include fishing and traditional religious activities. In the area are several retail stores, sawmills, and woodwork industries. The Reservoir station is located within Latitude 6°22'37" N and Longitude 5°38'23" E. Economic activities in the vicinity include retail stores, sawmills, woodwork industries, and tyre vulcanizing. The Bridge station is located within Latitude 6°21'5" N and Longitude 5°38'49" E. The most prevalent anthropogenic activities at this station are car wash, rug wash, and the operation of slaughterhouses.

Figure 1: Map of the study area (Source: Survey Department, Benin City)

2.2 Collection of Fish Specimens

Fishes were caught between 7:00 am and 9:00 am on sampling days between June and August 2023, with the assistance of artisanal fishermen. Fishes that did not visually resemble the target species were released immediately upon capture. Retained samples of fishes were rinsed with river water in order to remove extraneous materials. In-situ, the identities of the fish species were verified and confirmed using a key (Idodo-Umeh, 2003) and a field guide (Olaosebikan and Raji, 2013). Total length (cm) measurements were taken using a water-proof measuring board while weight (g) of fish samples was ascertained using an electronic Scale (Mettler® PM4800 Delta Range Series). The mean total length was 32.74 ± 1.58 cm while the mean weight was 885 ± 1.76 (n=27). Samples were placed in labeled zip-lock bags and conveyed to the laboratory in a Thermolineo® insulated ice chest within 24 hours for further studies.

2.3 Ex-situ Procedures and Laboratory Assay

2.3.1 Isolation of MPs from Organic Matter and Microscopy

The modified isolation procedure by Abidin et al. (2021) was applied. Briefly, fifty grams (50 g) of the excised intestine of fish was treated with 10% KOH solution (at three times the volume of tissue) followed by incubation for 24 hours. Excision was carried out using a stainless steel lancet via the ventral aspect of whole fish. The Wet Peroxide Oxidation process was applied by adding 30 mL of 0.05 M Fe (II) oxide (FeO) and 30 mL of 20% Hydrogen peroxide (H2O2), followed by heating on a Binatone® Hotplate (Model ECP-207, rated at 2,500W) at 75°C for 45 minutes to eradicate organic matter from the fish. All reagents used were of analytical grade (SIGMA, Germany). The remaining filtrates were treated via heating, followed by filtration with stainless steel sieves of 45 m mesh size. The dried filtrate was placed on a slide under a UNIC® compound binocular electric microscope, in order to visually account for the numerical strength of fibres, filaments, fragments, and pellets.

2.3.2 Additional Verification Procedures for MPs

Additional verification of MPs was achieved by applying the tagging method as described by Maes et al. (2017) and the hot needle test or hot point test as described by De Witte et al. (2014).

2.3.3 Application of Fourier Transform Infrared (FTIR) Spectroscopy for Polymer Differentiation

Polymer differentiation was achieved using FTIR Spectroscopy. The specific device used was a Schimadzu® FTIR 8400S Spectroscope which was correctly programmed and conditioned according to the manufacturer's standard prior to use.

2.3.4 Determination of Plastic Load (PL)

The mean amount of MP per fish is known as the plastic load (PL). PL = Total number of MP particles ÷ Total number of fish species examined (Zhang et al., 2021).

2.3.5 Determination of Frequency of Occurrence (FO) for MP

Frequency of occurrence (FO) denotes the percentage of fish with at least one piece of MP. FO = Number of fish with at least one MP particle ÷ Number of fish sampled (Riaz et al., 2023).

2.3.6 Contamination Circumvention

In order to avert contamination of samples, non-plastic clothes were worn (Cotton laboratory coat) while sampling and laboratory instruments made from non-plastic components, such as glass, were used as recommended by Cutroneo et al. (2020). All work benches and surrounding areas were thoroughly wiped with absolute alcohol (99.9%) and disposable Cotton sheets before, in between, and after analysis.

2.3.7 Determination of Estimated Annual Intake (EAI) and Estimated Daily Intake (EDI) of MPs

- EAI (No./person/year) =
  Number of MP items in fish × Per capita figure ÷ Adult body weight (Assumed to be 70 kg)
  Where: Per capita figure is 13.3 kg/person/year for Nigeria (World Fish Center, 2023)

- EDI (No./person/day) = EAI ÷ 365 days.

2.4 Statistical Analysis

GENSTAT software (version 12.1) was used for statistical analysis. Analysis of Variance (ANOVA) was used to determine significant differences between mean values of MPs at a 5% level of probability, while significant means (P¡0.05) were separated using New Duncan Multiple Range Test.

3 Results

As presented in Table **??**, the mean levels of MPs in *C. gariepinus* according to stations ranged from 0.37 in June at Station 1 to 5.23 in August at Station 3, with a total figure ranging from 2.97 at Station 2 to 6.18 at Station 3. Significant differences (P¡0.05) were observed in the mean levels of MPs between the months across Stations 1 and 3. The mean plastic load in *C. gariepinus* according to stations ranged from 0.31 in June at Station 1 to 6.34 in August at Station 3, with a total figure ranging from 3.22 at Station 2 to 7.41 at Station 3. Significant differences (P¡0.05) were observed in the mean plastic load between the months across Stations 1 and 3 as shown in Table **??**. The mean frequency of occurrence of MPs in *C. gariepinus* according to stations ranged from 0.31 in June at Station 1 to 0.85 in August at Station 3, with a total figure ranging from 1.54 at Station 3

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Table 1: Table 1: Mean level of microplastics in Clarias gariepinus according to stations

Month	Station 1	Station 2	Station 3
June	0.37 ± 0.55a	1.35 ± 1.32a	0.38 ± 0.26a
July	0.66 ± 0.48a	1.25 ± 0.77a	5.23 ± 1.65b
August	0.66 ± 0.48a	1.25 ± 0.77a	5.23 ± 1.65b
Mean	3.38	2.97	6.18

Mean values with the same superscripts are not significantly different (P<0.05). Vertical comparison only.

Table 2: Table 1: Mean level of microplastics in Clarias gariepinus according to stations

Month	Station 1	Station 2	Station 3
June	0.31 ± 0.55a	1.37 ± 1.41a	0.35 ± 0.46 a
July	2.65 ± 1.25b	0.52 ± 0.56a	0.72 ± 1.25a
August	0.71 ± 0.43a	1.33 ± 0.58a	6.34. ± 1.62b
Mean	3.38	3.22	7.41

Mean values with the same superscripts are not significantly different (P<0.05). Vertical comparison only.

Table 3: Table 1: Mean frequency of occurrence of microplastics in Clarias gariepinus according to stations

Month	Station 1	Station 2	Station 3
June	0.31 ± 0.04a	0.75 ± 0.03c	0.32 ± 0.04a
July	0.78 ± 0.13c	0.62 ± 0.03a	0.37 ± 0.03a
August	0.49 ± 0.02b	0.57 ± 0.05b	0.85 ± 0.02b
Mean	1.58	1.94	1.54

Mean values with the same superscripts are not significantly different (P<0.05). Vertical comparison only.

to 1.94 at Station 2. Significant differences (P¡0.05) were observed in the mean frequency of occurrence of MPs in *C. gariepinus* between the months across all stations as presented in Table **??**. The type of MP particles found in *C. gariepinus* according to morphological classification is presented in Table **??**, where fragments and filaments were found in *C. gariepinus* in June, fragments, filaments, pellets, and fibres in July, and foam, fragments, filaments, and pellets in August. A typical FTIR spectrum for MP particles obtained from *C. gariepinus*, showing absorbance bands at different wave numbers, is presented in Figure 2. Peaks at 2910 cm^{-1}, 2820 cm^{-1}, 1445 cm^{-1}, and 710 cm^{-1} are characteristic of polyethylene (PE) compounds. The estimated annual intake (EAI) values (No./person/year) for MPs ranged from 0.564 at Station 2 to 1.174 at Station 3 (Fig. 3) while the estimated daily intake (EDI) values (No./person/day) ranged from 0.00154 at Station 2 to 0.00321 at Station 3 (Fig. 4). As presented in Figure 5, the percentage quota of abundance for MPs in *C. gariepinus* by stations ranged from 23.70% at Station 2 to 49.32% at Station 3. Figure 6 shows the proportion of the type of microplastics in *C. gariepinus*, with the highest proportions being for filaments and fragments (33.33%) and the least proportion for fibre (3.70%). As shown in Figure 7, the temporal percentage quota of types of microplastics peaked for filaments in August (55.55%) while there was a peak for fragments in June, July, and August (33.33%).

4 Discussion

Microplastic particles were found in *C. gariepinus*, clearly implying that the fish species consumed such particles while grazing for prey. The mean level of MPs in *C. gariepinus* according to Stations reached a zenith at Station 3 and was the lowest at Station 1. Similarly, the mean plastic load in *C. gariepinus* peaked at Station 3 and was the lowest at Station 1. Significant differences (P ¡ 0.05) were observed between months for Stations 1 and 3 in the mean level of MPs, and similar significant differences were observed in the mean plastic load between months for Stations 1 and 3. These significant differences indicate variations in the MP profile in the investigated fish species across the research period. Mendes et al. (2023) observed that both temporal and spatial variations in MPs are to be expected when sampling for MPs in environmental matrices. Additionally, particle density, water surface area, water surface depth, wind action, and water flow patterns could further influence the distribution and consequent levels of MPs in aquatic bodies (Sau et al., 2023).

The mean frequency of occurrence of MPs in *C. gariepinus* by stations was at a peak at Station 3, while it was lowest at Station 1. Significant differences (P ¡ 0.05) in the mean frequency of occurrence of MPs in *C. gariepinus* were observed between months across all stations. This variability

Table 4: Mean frequency of occurrence of microplastics in Clarias gariepinus according to stations

Month		June			July			August	
Station Code	1	2	3	1	2	3	1	2	3
R1	N	T	N	Z, T	N	N	N	Z, P	Z, T, P
R2	T, Z	T	N	Z, P	P	N	N	Z	Z, P
R3	N	N	Z	T	N	B, T	T	N	Z,T, P

R1, R2, R3= Replicates Key: N = No plastic, Z = Filament, T = Fragment, P = Pellet, B = Fibre

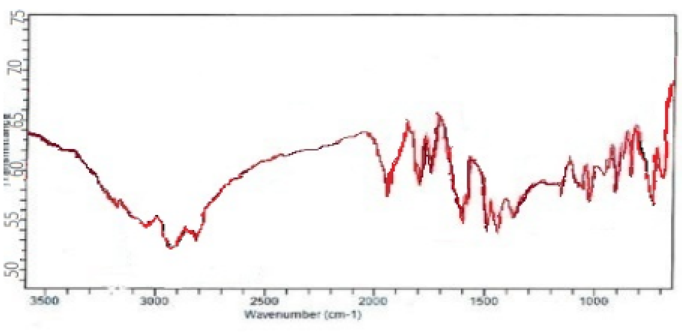

Figure 2: A typical FTIR spectrum for microplastic particles obtained from *Clarias gariepinus* showing absorbance bands at different wave numbers.

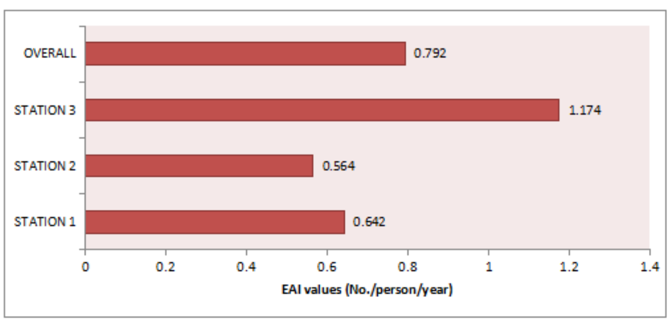

Figure 3: Estimated annual intake (EAI) values for microplastics from *Clarias gariepinus*.

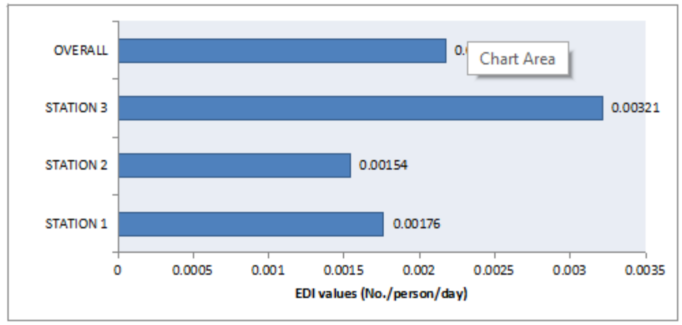

Figure 4: Estimated daily intake (EDI) values for microplastics from *Clarias gariepinus*.

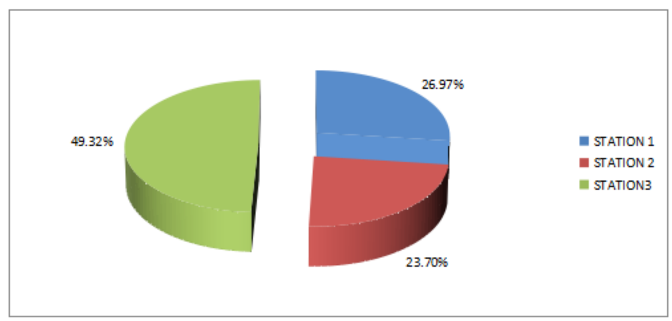

Figure 5: Percent (%) quota of abundance for microplastics in *Clarias gariepinus* by station.

indicates a significant fluctuation in the frequency of occurrence of MPs in the target fish species. The types of MP particles found in *C. gariepinus* included fragments, filaments, fibers, and pellets, with filaments and fragments being the dominant types. However, the temporal distribution revealed that filaments and pellets were prominent in August. The varying types of MP particles observed could reflect different degradation mechanisms, such as photo-oxidation, ultraviolet radiation, hydrolysis, and biodegradation, leading to various shapes and forms (Gewert et al., 2015). The temporal distribution indicates the occurrence of these MP types within the sampling period, considering the respective months. MP levels in aquatic resources can vary by water body, location, and season (Boskovic et al., 2023).

Fourier Transform Infrared (FTIR) Spectroscopy confirmed the presence of Polyethylene in *C. gariepinus*. According to Unnimaya et al. (2023), specific band widths from spectrometric readings define the type of plastic in examined samples. Boskovic et al. (2023) observed that Polyethylene is the most common plastic in rivers worldwide, along with Polypropylene, resulting from anthropogenic impact. These plastics are widely used in the packaging industry, which may account for their

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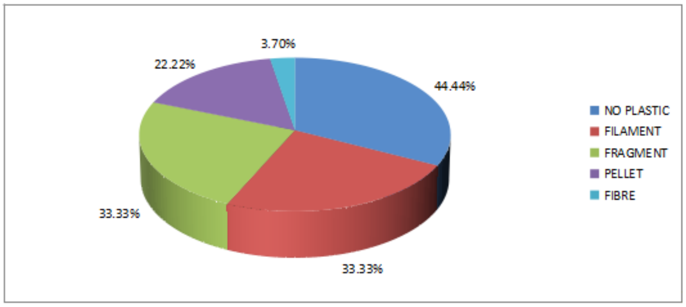

Figure 6: Proportion of the type of microplastics in *C. gariepinus*.

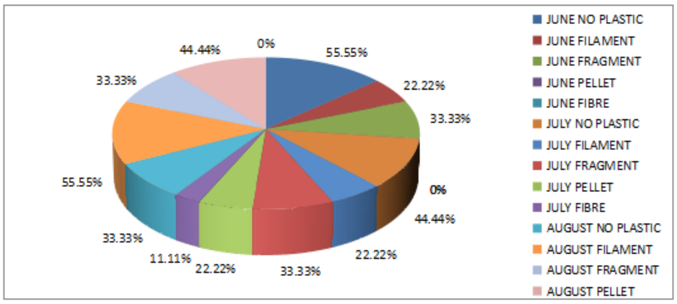

Figure 7: Temporal distribution quota of the type of microplastics in *C. gariepinus*.

prevalence in both terrestrial and aquatic ecosystems. In Nigeria, plastic and nylon waste pollution remains a problem (Bolaji et al., 2021). Mancuso et al. (2023) noted the dominant presence (52%) of Polyester in the Antarctic fish *Trematomus bernacchii* from the Terranova Bay Area.

The EAI and EDI values were highest at Station 3 and lowest at Station 2. This was supported by the percentage quota of abundance for MPs in *C. gariepinus* by station, where Station 3 accounted for 49.32 % of the total. This indicates that *C. gariepinus* at Station 3 (Bridge) were more impacted by MPs, while the least impact was at Station 2 (Reservoir). This implies that *C. gariepinus* from Station 3 for human consumption may have a greater impact compared to fish from other stations due to the heavier MP load. It could be inferred that Station 3 (Bridge) is the most polluted regarding MPs. The prevalent anthropogenic activities at the Bridge station, including car washes, rug washes, and slaughterhouses, contribute to the MP burden of the river. Ng et al. (2022) observed that abattoir wastewater contains proteins, fats, microbes, organic matter, and emerging pollutants including MPs. Rugs, carpets, and car parts, including tyres, contain plastics as components (Aydin et al., 2023). Moreover, the Bridge station, situated downstream of the other two stations, receives additional MPs from upstream due to the river's lotic nature and high anthropogenic impact in the busy city.

5 Conclusion

This research was a pilot investigation into the levels of microplastics (MPs) in *C. gariepinus*, particularly sourced from the Ikpoba River in Benin City, Nigeria. The presence of MPs in the aforementioned fish species is a concerning development due to the potential primary risk to the fish species and the potential secondary risk to end-of-the-line consumers, including humans. To address the ongoing influx of MPs into the Ikpoba River ecosystem, it is recommended that stringent legislation on the disposal of plastic products into aquatic environments be enforced. Additionally, a robust monitoring and enforcement system should be established to ensure that the plastic load in the river and its natural resources does not reach critical levels. Furthermore, *C. gariepinus* should be consumed with caution to avoid health problems associated with polymer contamination in the long run.

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