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Accumulation of Microplastics in African Sharptooth Catfish (*Clarias gariepinus*) from Ikpoba Reservoir, Benin City, Nigeria

Wangboje Oiseoje Michael* and Oshodin Eghe

Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, P.M.B. 1154, Benin City, Nigeria.

Corresponding Author: oiseoje.wangboje@uniben.edu

ABSTRACT

Microplastics (MPs) are minute pieces of plastic of synthetic origin. The potential threat posed by MPs to human health warranted this research on the Ikpoba Reservoir in Benin City, Nigeria which was achieved by Fourier Transform Infrared Spectroscopy (FTIR) following the extraction of organic matter from the intestinal tract of *Clarias gariepinus* fish. The mean levels of accumulated MPs in *C. gariepinus* (mean total length 29.95 ± 2.76 cm, mean weight 798 ± 2.15 ranged from 0.91 (June) at the Okhoro station to 1.45 (August) also at the Okhoro station with no observed significant difference (p>0.05) in the levels of MPs in *C. gariepinus* between stations while the mean plastic load ranged from 0.31 (June) at the Okhoro station to 0.97 (July) at the Low lift pump station with an observed significant difference (p<0.05) in the plastic load in *C. gariepinus* between months (June, July and August) at the Midpoint and Okhoro stations. Polyethylene pellets dominated the microplastic particles having the highest quota of 33.33%. The estimated daily intake values (Mg/person/day) for MPs ranged from 0.00054 (Okhoro station) to 0.00059 (Midpoint station) with an overall value of 0.00059. A triad approach to contain the menace of MPs was suggested to include routine monitoring, enforcement of stringent laws on the disposal of plastics and public enlightenment in order to safeguard the integrity of this ecosystem and to protect public health.

Keywords: *Clarias gariepinus,* Microplastics, Pellets, Polyethylene

INTRODUCTION

Microplastics (MPs) are particles of plastic measuring less than 5 mm in size and are of synthetic origin (Compa *et al.,* 2023).The threat posed by microplastic pollution to the integrity of the aquatic environment and to human health has continued to ignite a plethora of researches around the world such that scientists have discovered MPs in about 1,300 diverse aquatic creatures to date, ranging from minute free-living plankton to large whales (Hailstone, 2024). In addition, it has been observed that fish from aquaculture facilities accumulate less MPs and potentially toxic elements compared to fish from the wild (Simionov *et al.,* 2023). According to Jarf *et al.,* (2024), sources of MPs include clothing, vehicle parts, packaging materials, agriculture, construction and electrical tools.

Many nations around the world have recognized the menace of MPs in the environment and have accordingly adopted one strategy or another to curtail the impact of these pollutants such as the establishment of inter-ministerial committees as has been done in Malaysia (Tan and Vethasalam, 2024). The identification, characterization and effects of MPs on aquatic organisms has been documented in the wake of their widespread distribution in Oceans, Rivers and Arctic regions (Alak *et al.,* 2022). Documented adverse effects of MPs in man include oxidative stress, DNA damage, neurotoxicity, metabolic disorders, reproductive toxicity and organ dysfunction (Li *et al.,* 2023).

The Ikpoba Reservoir located in Benin City, Nigeria, is a valuable source of consumable water and fishes to dwellers in the City and has attracted the attention of environmental toxicologists over the years owing to its urban location and attendant anthropogenic pressure (Wangboje, 2021). Available

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literature has shown that the Ikpoba River system and its Reservoir contain both organic pollutants (Wangboje and Oguzie, 2013) and inorganic pollutants (Olele *et al.,* 2013). However, there is lack of scientific data on the accumulation of MPs particularly in fish from the Reservoir which this research sought to provide thereby providing baseline data and filling an existing gap in knowledge.

Besides, fishes ingest MPs while grazing for food and when people consume such fish, they may also ingest MPs which makes this research relevant in present times. The target fish species, *Clarias gariepinus* (Burchell, 1822), is of commercial appeal and is widely distributed in the Ikpoba River ecosystem (Olele *et al.,* 2013). The principal objectives of this research therefore, were to determine the levels and types of MPs in *C. gariepinus* from the Ikpoba Reservoir and to estimate the daily intake of MPs via the potential consumption of the target fish species.

MATERIALS AND METHODS

Description of the Study Area

This research was carried out within the confines of the Ikpoba Reservoir (Figure 1 and 2) in Benin City (Latitude 6°20’ 00’’N and Longitude of 5 37’ 20’’ E) Edo state, Nigeria The Ikpoba River was impounded by the Ikpoba weir to form the Ikpoba Reservoir which separates the downstream from the upstream sections of the River. The Ikpoba weir was constructed between March 1977 and May 1982 and has a length of 60 M. The Reservoir is situated about 3.75 Km South-East of the Ugbowo campus of the University of Benin, Benin City and it was commissioned in September 1987.

Its storage capacity is 1.5 million M^3 and the water supply per day is 90,000 M^3 with a minimum discharge of 1474 M^3/Hr and a maximum discharge rate of 4422 M^3/Hr. The Reservoir covers a surface area of 107.5 hectares (1.1 million square meters) with a crest level of 36.8 M. Its depth is irregular and varies from 0.5 to 4 M (Wangboje, 2013). Human activities at the Reservoir include bathing, laundry and fishing. In the vicinity are sawmills, a rubber processing factory and a quarry. There is a run off drainage channel that empties into the Reservoir at the Okhoro bank and solid wastes including tyres, beverage cans and plastic bags are visible in the Reservoir.

The three stations established for the research were the Okhoro Station (Station 1), Midpoint Station (Station 2) and Low Lift Pump Station (Station 3). The total distance between the Okhoro and Low Lift Pump Stations is approximately 750 M with the Midpoint Station situated in the middle of the other two Stations, hence its name. It is at the Low Lift Pump Station that water from the Reservoir is pumped out for treatment by the Edo State Urban Water Board Authorities, who are the managers and operators of the Reservoir.

Collection of Fish Samples

Fish were netted by local fishermen between 7:00 am and 9:00 am from June to August 2023. Sampling occurred three times in each of these months within the first three consecutive weeks. The identities of the fish species were authenticated using a fish identification key by Idodo-Umeh (2003) and a field guide for freshwater fish by Olaosebikan and Raji (2013).

Total length of fish was determined to be 29.95 ± 2.76 cm using a water-proof measuring board, while weight (798 ± 2.15 g, n=27) was determined with a portable electronic scale. The fish samples were enclosed in labeled vacuum-cleaned bags and immediately dispatched to the laboratory in an ice chest.

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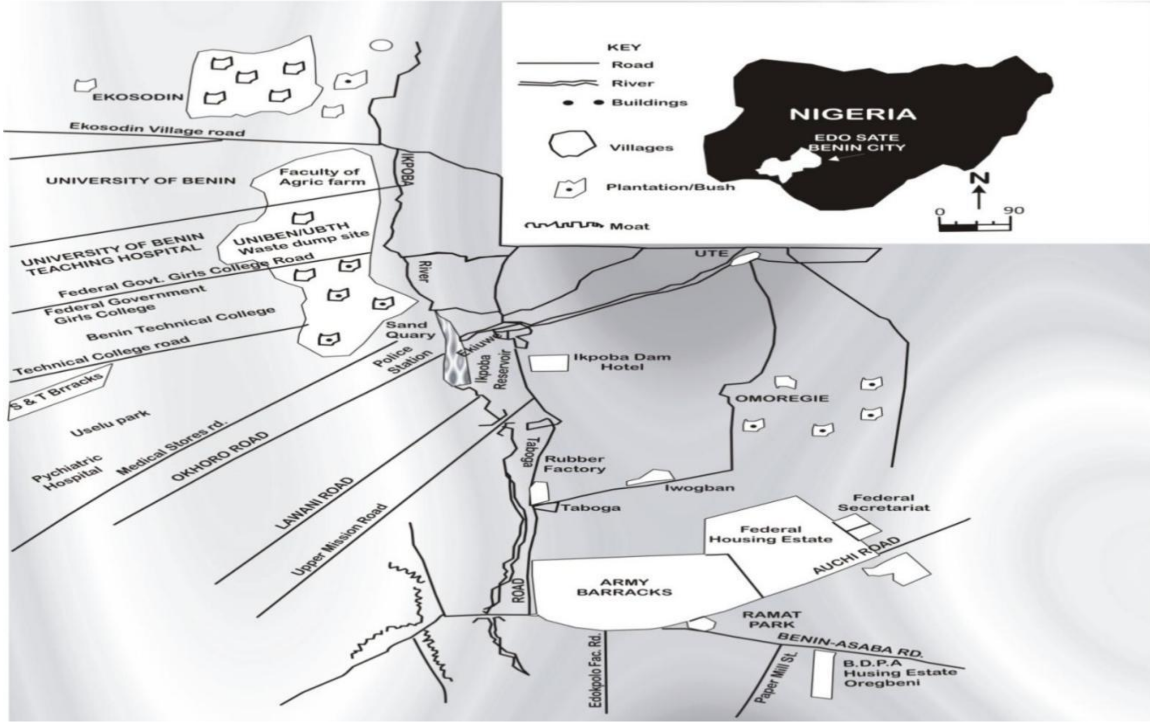

Figure 1: Map of Ikpoba River showing the Ikpoba Reservoir.

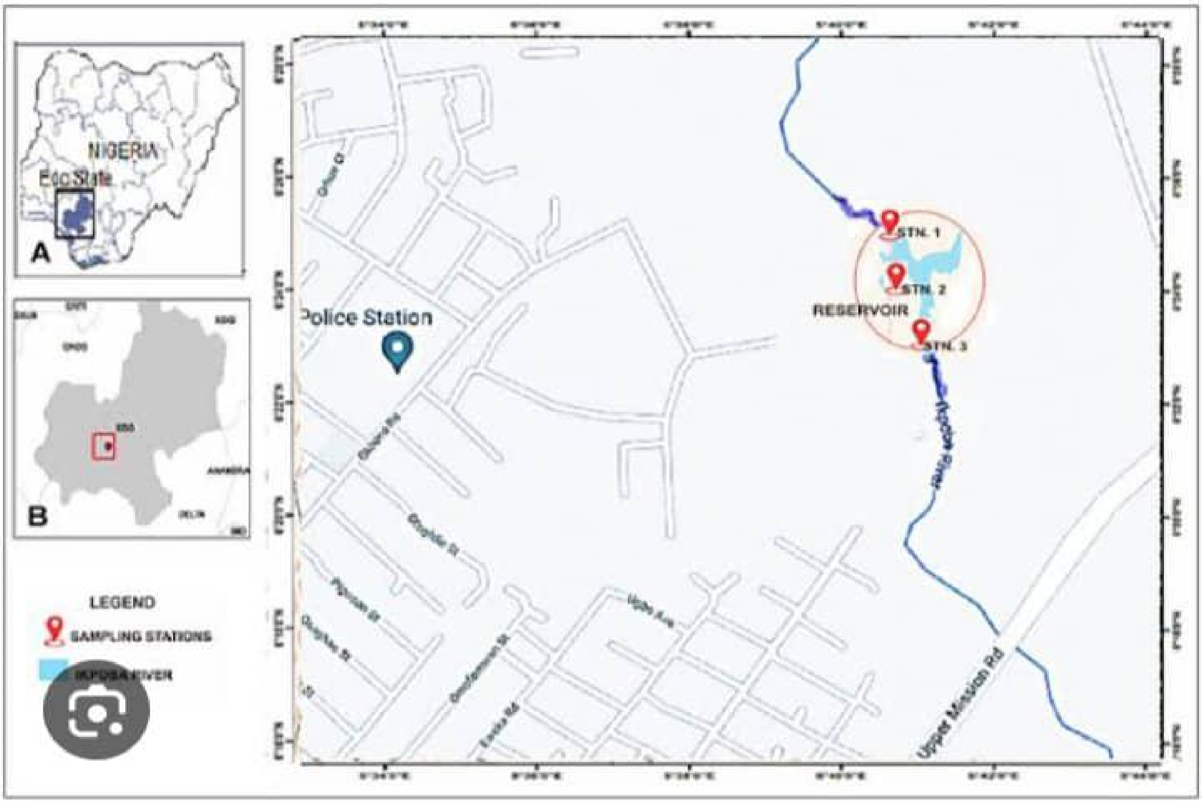

Figure 2: Map of the Ikpoba Reservoir with the sampled stations.

Laboratory Assay

Digestion of intestinal tract and microscopy

The intestinal tract of fish was excised and thereafter digested using the method as outlined by Abidin *et al.,* (2021) in order to separate the MPs for further processing while microscopy of the final filtrate was determined using a UNIC® compound binocular electric microscope, in order to

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ascertain the actual number of polymer particles present (Primpke *et al.,* 2020).

Additional verification of microplastics

To confirm the presence of MPs, the tagging method as detailed by Maes *et al.,* (2017) was employed in tandem with the hot needle test as detailed by De Witte *et al.,* (2014).

Identification of specific types of Polymer

Specific types of polymers were identified using Fourier Transform Infrared (FTIR) spectroscopy, utilising the Schimadzu® FTIR 8400S Spectroscope.

Derivation of plastic load in fish

The concept of plastic load in fish was derived by Zhang *et al.,* (2021) as the average amount of microplastics per fish sampled, known as the plastic load (PL).

PL=

$$\text{Total number of microplastic particles} \Big/ \text{Total number of fish species examined}$$

Calculation for frequency of occurrence

Frequency of occurrence (FO) is defined as the percentage of fish with at least one piece of microplastic according to Riaz *et al.,* (2023).

FO=

$$\text{Number of fish with at least one microplastic particle} \Big/ \text{Number of fish sampled}$$

Prevention of contamination

Contamination prevention strategies as recommended by Cutroneo *et al.,* (2020) such as adorning of non-plastic clothes and using non-plastic equipment were implemented to abate contamination. To ensure cleanliness, work surfaces and surrounding work space were cleaned with absolute alcohol and disposable Cotton sheets before, during and after analysis.

Evaluation of estimated annual intake (EAI) and estimated daily intake (EDI) of MPs

The estimated annual intake (EAI) and estimated daily intake (EDI) of MPs were evaluated as follows;

The EAI expressed as Number of MPs/person/year is determined by dividing the per capita figure of 13.3 kg/person/year for Nigeria by an assumed adult body weight of 70 kg.

EAI=

$$\text{Number of MP items in fish} * \text{ Per capita figure} \Big/ \text{Adult body weight of 70 kg}$$

Where: Per capita figure is 13.3 kg/person/year for Nigeria (Word Fish Center, 2023)

The EDI expressed as Number of MPs/person/day is determined by dividing the EAI by 365 days. The EDI is calculated to determine the daily intake of MPs per person.

EDI (No. of MPs /person/day) =

$$\text{Estimated annual intake} \Big/ \text{365 Days}$$

Statistical Analysis

The GENSTAT software (version 12.1) was used for conducting statistical analysis. To identify significant differences between the average values of MPs with a 5 % level of probability, Analysis of Variance (ANOVA) was utilised. The New Duncan Multiple Range Test was used to separate significant means ($p<0.05$). Microsoft Excel was employed for creating all graphical designs and presentations.

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RESULTS

The mean levels of microplastics in *Clarias gariepinus* from Ikpoba Reservoir ranged from 0.91 in June at the Okhoro station to 1.45 in August also at the Okhoro station with no observed significant difference (p>0.05) in the levels of microplastics in *C. gariepinus* between stations as shown in Table 1.

Table 1: Mean level of microplastics in *C. gariepinus* from Ikpoba Reservoir.

Month	Okhoro station	Midpoint station	Low Lift Pump station
June	0.91 ± 0.09^a	1.33 ± 0.58^a	0.97 ± 0.05^a
July	0.92 ± 0.75^a	0.93 ± 0.08^a	1.37 ± 0.057^a
August	1.45 ± 0.53^a	1.29 ± 0.61^a	1.04 ± 0.14^a

Means with the same superscript in the same column are not significantly different (p>0.05).

The mean plastic load ranged from 0.31 in June at the Okhoro station to 0.97 in July at the Low lift pump station with an observed significant difference (p<0.05) in the plastic load in *C. gariepinus* between months at the Midpoint and Okhoro stations as presented in Table 2.

Table 2: Mean plastic load in *C. gariepinus* from Ikpoba Reservoir.

Month	Okhoro station	Midpoint station	Low Lift Pump station
June	0.31 ± 0.08^a	0.63 ± 0.05^a	0.77 ± 0.22^a
July	0.63 ± 0.05^b	0.68 ± 0.01^a	0.97 ± 0.25^a
August	0.92 ± 0.11^c	0.88 ± 0.11^b	0.95 ± 0.15^a

Means with the same superscript in the same column are not significantly different (p>0.05).

The frequency of occurrence of microplastics in *C. gariepinus* ranged from 0.32 in August at the Midpoint station to 0.94 in June also at the Midpoint station with observed significant differences (p<0.05) in the frequency of occurrence of microlastics in *C. gariepinus* between months at the Okhoro and Midpoint stations as shown in Table 3.

Table 3: Frequency of occurrence of microplastics in *C. gariepinus* from Ikpoba Reservoir.

Month	Okhoro station	Midpoint station	Low lift pump station
June	0.37 ± 0.04^a	0.94 ± 0.09^a	0.67 ± 0.30^a
July	0.63 ± 0.04^b	0.65 ± 0.04^b	0.91 ± 0.1^a
August	0.92 ± 0.08^c	0.32 ± 0.03^c	0.93 ± 0.05^a

Means with the same superscript in the same column are not significantly different (p>0.05)

The specific type of microplastic particles in *C. gariepinus* based on physical classification were fragments, pellets, fibres and filaments as shown in Table 4.

Table 4: Physical classification of microplastics particles in *C. gariepinus*.

M	June			July			August		
S	Station1	Station 2	Station 3	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
R1	D	D	D	A	B	A	A	C	B, E
R2	E	B	D	E, A	E	B, E	A	C	B, E, A
R3	C	B	E	B	D	B	C	D	B,C

Key: A = Fragment, B = Pellet, C = Fibre, D = No plastic, E = Filament.

Microplastic pellets had the highest quota of 33.33% in C. gariepinus as shown in Figure 3.

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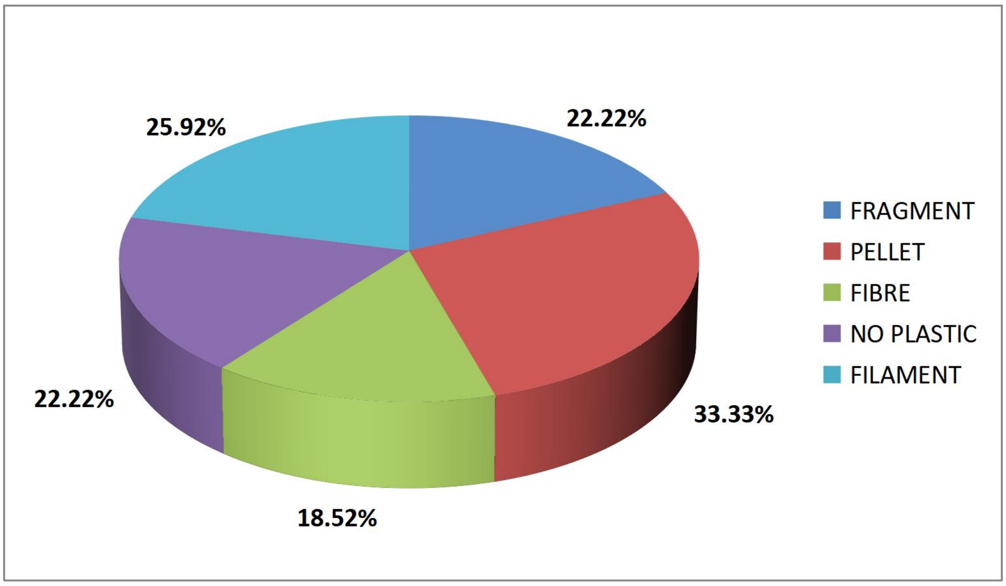

Fig. 3: Percent (%) quota of microplastic particles in *C. gariepinus* from Ikpoba Reservoir.

The typical FTIR spectrum obtained from the research shows absorbance bands at different wave numbers with peaks of 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} as shown in Figure 4.

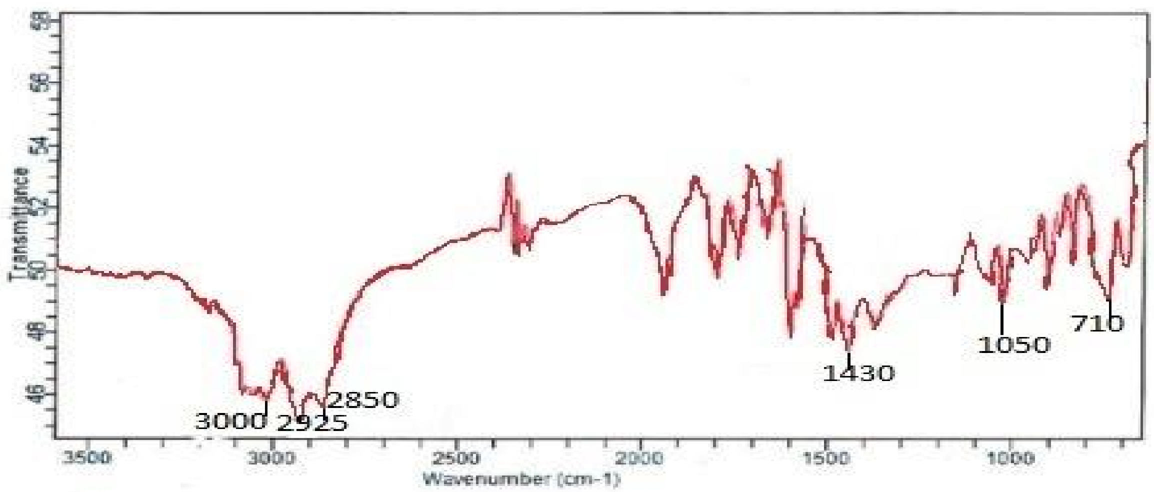

Figure 4: A typical FTIR spectrum for microplastic particles in *C. gariepinus* from Ikpoba Reservoir.

The estimated annual intake (EAI) values (Mg/person/year) for microplastics ranged from 0.20 at Okhoro station to 0.22 at the Midpoint station with an overall value of 0.22 (Figure 5) while the estimated daily intake (EDI) values (Mg/person/day) for microplastics ranged from 0.00054 at Okhoro station to 0.00059 at the Midpoint station with an overall value of 0.00059 as presented in Figure 6.

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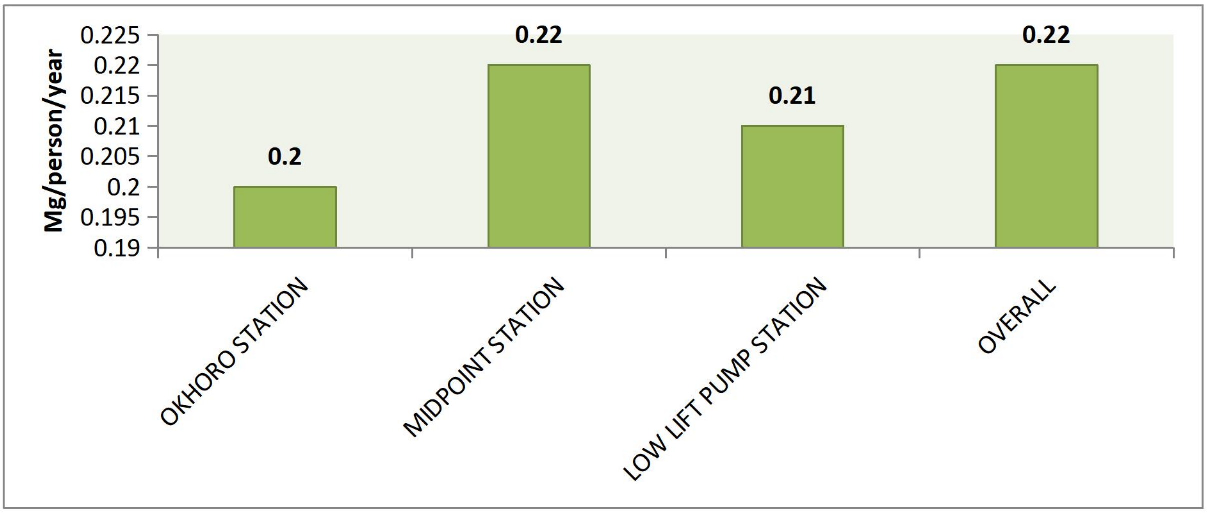

Figure 5: Estimated annual intake (EAI) of microplastics from *C. gariepinus.*

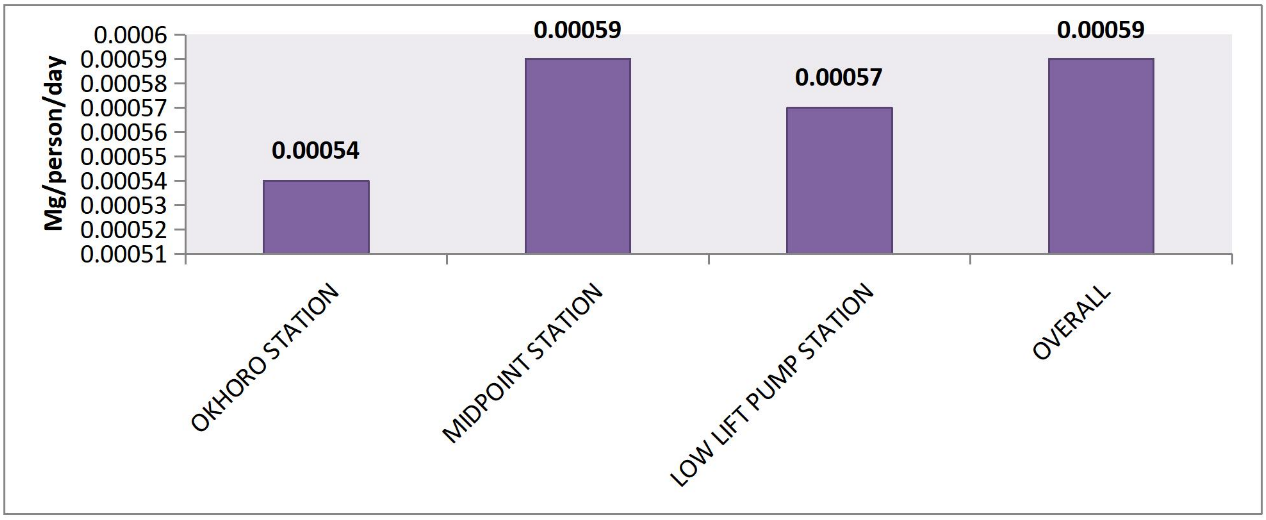

Figure 6: Estimated daily intake (EDI) of microplastics from *C. gariepinus.*

DISCUSSION

Microplastics (MPs) were discovered in *C. gariepinus* at all stations within the Ikpoba Reservoir with no observed significant differences ($p>0.05$) in the mean levels of MPs in *C. gariepinus* between stations, which gives an indication that the levels of MPs in fish remained relatively stable all through the stations, although a longer sampling campaign may have produced a varied scenario. The general microplastic rank profile was Midpoint station > Low lift pump station > Okhoro station, implying that fish species at the Midpoint station were conceivably more impacted with MPs compared directly to fish species in the other stations.

There were observed significant differences ($p<0.05$) in the mean plastic load of MPs in *C. gariepinus* between months at the Midpoint and Okhoro stations, suggesting that there was a variation in the ratio of the total number of MPs to the total number of fish species examined. There were observed significant differences ($p<0.05$) in the mean frequency of occurrence of MPs in *C. gariepinus* between months at the Midpoint and Okhoro stations, indicating that there was a variation in the ratio of the number of fish species with at least one microplastic particle to the total number of fish species sampled. The fact that MPs were present in this fish species is confirmation that these particles were consumed by the fish in its ambient water medium either directly or via contaminated prey.

This observation conforms with the findings by Jarf *et al.,* (2024) that MPs can be consumed mistakenly by fish which confuse these particles for food items such as fish eggs and tadpoles. In addition, Jeong *et al.,* (2023) observed that MPs in water and prevailing biological conditions could co-influence the microplastic levels present in hydrobionts such as fish. On the basis of physical classification, diverse types of particles of MPs were found in *C. gariepinus* which took the rank profile in terms of abundance, Pellets > Filaments > Fragments > Fibres with Pellets dominating the particle profile with 33.33 % while on the flipside, Fibres were least represented with 18.52 %.

Plastic pellets due to their minute size and light weight, are easily distributed in water where they can leach toxic additives as well as absorb toxic chemicals including heavy metals and persistent organic pollutants such as pesticides (Leviker *et al.,* 2024).The presence of the various types of particles gives a glaring indication into the mechanisms of breakdown that larger pieces of polymer materials reaching the Reservoir were subjected to as corroborated by Lindberg (2024).

The typical FTIR spectrum showed absorbance bands at different wave numbers with a peak at 2925 cm^{-1} attributed to absorption of asymmetric CH_2 stretching. There was another peak at 2850 cm^{-1} attributed to symmetric CH_2 stretching. The peak at 1430 cm^{-1} was attributed to CH_2 scissoring while the peak at

710cm^{-1} was attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430 cm^{-1} and 710 cm^{-1} are absorbance wave numbers range used to identify Polyethylene (PE) meaning that PE particles (Pellets, Filaments, Fragments and Fibres) were present in *C. gariepinus.* Unnimaya *et al.,* (2023) worked on the identification of MPs using a custom built micro-Raman Spectrometer and observed that. absorbance wave number ranges are used to identify specific types of polymers in environmental media.

Polyethylene is formed through the polymerization of ethylene and is the most widely used plastic in the world, being widely applied in food wrappers, shopping bags, detergent bottles and vehicular fuel tanks (Britannica, 2024). Dao *et al.,* (2023), observed that other types of plastics found in aquatic bodies around the world include Polypropylene, Polyester, alkyd and Polyethylene terephthalate acrylate.

The EAI and EDI values were both at a peak at the Midpoint station and the least at the Okhoro station. The significance of this observation is that *C. gariepinus* captured from the Okhoro station would present a lower microplastic load to potential consumers even though the total absence of MPs in fish is desirable. It is pertinent to note at this point that it may not be totally possible to eradicate MPs from fish but any strategy or action that would keep their levels as low as reasonably possible are welcome in order to mitigate and contain their menace.

The presence of MPs in *C. gariepinus* in this research is a clear indication of the anthropogenic pressure that the Reservoir is being subjected to with regard to polymer pollution. The issue is further compounded by the fact that the Reservoir is located within the very busy city of Benin. In this light, it has been observed that MPs are present more in aquatic animals dwelling in waters closer to cities or urban areas than in those living in waters in the rural areas as a result of greater anthropogenic impact linked to higher human population strengths (Jeong *et al.,* 2023).

CONCLUSION

The findings of this research revealed the presence of microplastics (MPs) in *C. gariepinus* from the Ikpoba Reservoir, Benin City, Nigeria thus providing baseline data on the subject matter. Polyethylene pellets dominated the MP profile in *C. gariepinus* while the estimated daily intake values (Mg/person/day) for MPs peaked at the Midpoint Station of the Reservoir. Going forward from the results obtained, a triad approach to contain the menace of MPs in the Reservoir is suggested to include routine monitoring for polymer pollution, enforcement of stringent laws on the disposal of plastics and public enlightenment in order to safeguard the integrity of this ecosystem and to protect public health.

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