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Baseline Investigation of Microplastic Levels in the Nile Tilapia (Oreochromis Niloticus, Linnaeus, 1758) from River Okhuo, Benin City, Nigeria

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ABSTRACT

Microplastic (MP) contamination of natural aquatic media has been recognized as a serious ecological problem globally. The paucity of scientific data on MP in *Oreochronmis niloticus* (mean total length, 22.52 ± 0.93 cm, mean weight 591.67 ± 1.18 g) from River Okhuo in Benin City, Nigeria, warranted this research which was done using Fourier Transform Infrared (FTIR) Spectroscopy. The mean values of MPs in fish ranged from 0.67 in July at station 2 to 2.50 in June at station 1 with no observed significant difference ($p>0.05$) in the mean levels of MPs between months at each station. The plastic load ranged from 0.06 to 2.21 in July at stations 3 and 1 respectively with an observed significant difference ($p<0.05$) in the plastic load between months at station 1. The frequency of occurrence (FO) of MPs ranged from 1.35 in July at station 2 to 2.45 in June at station 3 with an observed significant difference ($p<0.05$) in the FO between months at station 2. The physical classification of MPs revealed that fragments were found at all stations while the FTIR absorbance wave numbers identified the presence of polyethylene. The estimated daily intake (No./person/day) for MPs ranged from 0.0005 at station 2 to 0.001 at station 1 while the percent (%) quota of occurrence of microplastic particles ranged from 3.70% for fibres to 48.15% for fragments. In order to stem the tide of plastic pollution, it was recommened that a continuous monitoring programme be put in place along with effective public enlightenment that would ensure that the River Okhuo and its resources are protected from the negative impacts of MPs.

Key words: *Oreochromis niloticus,* Fourier Transform Infrared Spectroscopy, Microplastics

INTRODUCTION

Microplastics (MPs) have been recognized to be common anthropogenic pollutants consisting of minute plastic particles up to 5 mm in size (Seetapan & Prommi, 2023). Microplastics have been recognized to be emerging contaminants that adversely affect natural ecosystems worldwide (Li *et al.*, 2023). The presence of microplastics in fish is not desirable owing to potential human health risk issues. A lot of aquatic species around the world have been shown to ingest a wide range of MPs including particles and fibres and macroplastics including plastic sheets, fishing gear and household items as a result of anthropogenic impact (Yang *et al.*, 2023). According to Maaike *et al.* (2023), MPs being persistent substances, can accumulate in various niches of the environment such as soil, freshwater, marine and the atmosphere. Microplastic contamination in water supply sources has been recognized as an serious ecological problem as these particles have the propensity to migrate through the food chain and accumulate in biota. They can also contain harmful contaminants on their surfaces thus becoming secondary sources of contamination (Teeratitayangkul *et al.*, 2023). According to Laelandi *et al.* (2023), it is important for people around the world to learn about pollution

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abatement methods especially as it regards MPs owing to their increasing menace in the environment. Karmakar (2023) has documented the adverse effects of MPs on aquatic organisms. For example, MPs can physically damage the digestive organs of recipient fish, leading to internal injuries and potential mortality. In addition, the absorbed pollutants on the MPs, such as pesticides and heavy metals, can transfer into the bodies of the organisms upon ingestion, leading to bioaccumulation and biomagnification of toxic substances within the food chain. Other effects of MPs include loss of biodiversity, altered trophic interactions and disrupted ecosystem functioning which can have both ecological and socio-economic implications (Manohar & Sonune, 2023). The fact that MPs have been absorbed and detected in aquatic ecosystems at high concentrations makes them a real threat to the environment (Wiradana *et al.*, 2023). The impact of MPs has been investigated for a number of prey species. The evidence presented is somewhat contradictory as some studies suggest no effects and while others reveal behavioural, physiological and toxicological effects (O'Brien & Rippon, 2023). According to Tabl *et al.* (2023), human exposure to MPs is a global problem and variability and lifetime accumulation of MPs remain unresolved depending on their size, shape, and functional group chemistry. The River Okhuo, flows through areas that have diverse anthopogenic activities which may impact negatively on this aquatic ecosysem. Besides from extensive literature search using Google scholar, there has been no specific research or investigation on MPs on this ecosysyem which this research has attemped to present thus filling an existing gap in knowledge while providing baseline data. The Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1758), inhabits rivers and their tributaries, lakes, estuaries and large ponds. It can survive in salinity of up to 35 ‰ but thrives better at about 29 ‰. *O. niloticus* has an omnivorous diet feeding on algae, diatoms, insect larvae, fish eggs, detritus and fry of fish (Idodo-Umeh, 2003). A preliminary investigation on the river revealed that this fish species is commonly found along with sister cichlids which are usually present in the catch of artisanal fishermen.

MATERIALS AND METHODS

Description of the Study Area

River Okhuo is located within Latitude 6º 34' 24'' N and Longitude 5º 32' 10'' E (Figs. 1 and 2). It flows along the northern boundary of the Nigerian Institute for Oil Palm Research (NIFOR), situated approximately 23 km northwest of Benin City (Latitude 6°20' 00''N and Longitude of 5 37' 20'' E), Nigeria. The river is located within the Tropical rain forest belt with a bi-modal seasonal pattern and has an elevation of 31 metres (102 feet). Localities that are adjacent to the river include Ogbiyeye and Ehenomeghele.Three sampling stations were established characterized with slow flowing water and visible solid wastes. Station 1 is situated downstream at the Nigerian Institute for Oil Palm Research (NIFOR). Fish can be seen in the transparent water while thickets of *Bambusa vulgaris* flourish along the bank of the river. The visible river bed is partly weedy and the soil is composed of coarse sand. Emergent vegetation and floating aquatic flora are present while laundry activities take place at this point. Station 2, is located westwards of station 1 and is noticeably devoid of trees. Human activities include laundry, swimming and fishing. Station 3 is located further downstream at Okodobo village. Human activities include laundry and fishing.

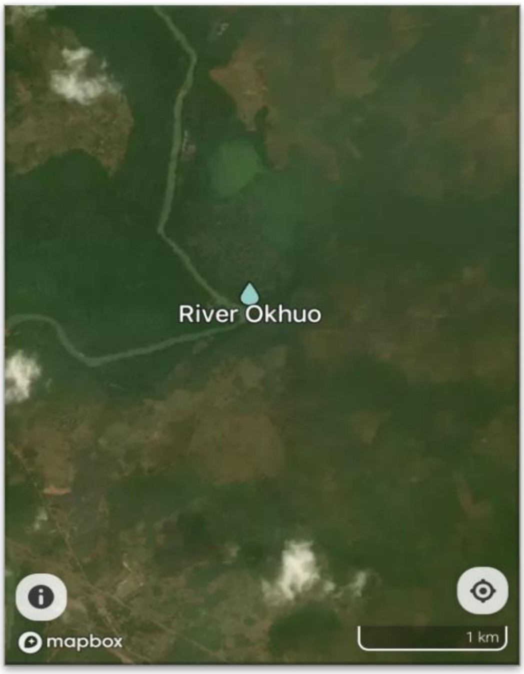

Figure 1: Satellite map of the study area (Mapcarta.com, 2023)

Figure 2: Road map of the study area (Mapcarta.com, 2023)

Collection of Fish Samples

Fish were caught between 7:30 am and 9:30 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 carefully released immediately upon capture. Retained fish samples were rinsed with river water in order to remove extraneous materials while *in-situ* the identities of the fish species were verified and confirmed using a key (Idodo-Umeh, 2003) and a field guide (Olaosebikan & Raji, 2013). Total length (cm) measurements were taken using a wooden measuring board while weight (g) of fish samples was ascertained using a battery-powered compact electronic scale (A & D, EK-AEP series). The mean total length was 22.52 ± 0.93 cm while the mean weight was 591.67 ± 1.18 g (n=27). Samples were placed in labeled vacuum-cleaned zip-lock bags and conveyed to the laboratory in a Thermolineo® ice chest within 24 hours for further studies.

Segregation of MPs from Organic Matter

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 and 30 mL of 20% Hydrogen peroxide (H_2O_2) continued by heating on a Binatone® Hotplate (Model ECP-207, rated at 2,500W) at 75℃ for 45 minutes to eradicate organic matter from 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 μm 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 various plastic particles. All procedures conformed to standard scientific research guidelines including the ARRIVE® guidelines for experimental animals and the American Fisheries Society/American Institute of Fishery Research Biologists/American Society of Ichthyologists and Herpetologists guidelines for the use of fishes in research (2004). Ethically speaking, all specimens used were lifeless on arrival in the laboratory and thus there was no need to sacrifice them.

Further Verification Procedures for MPs

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

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 specification prior to use.

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).

Frequency of Occurrence (FO) for MP

Frequency of occurrence (FO) is a figure that 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).

Contamination Mitigation Procedures

The immediate work area was air-vacuumed to eliminate the possible existence of plastic particles. In addition, non-plastic clothes (Cotton apparels) were worn while laboratory instruments made from non-plastic components (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.

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

EAI (No./person/year) = Number of MP items in fish x Per capita figure ÷ Adult body weight (Assumed to be 70 kg)

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

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

Statistical Analysis

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

RESULTS

The mean levels of MPs in *O. niloticus* ranged from 0.67 in July at station 2 to 2.50 in June at station 1 with no observed significant difference ($p>0.05$) in the mean levels of MPs between months at each station as presented in Table 1. As shown in Table 2, the plastic load ranged from 0.06 to 2.21 in July at stations 3 and 1 respectively with an observed significant difference ($p<0.05$) in the plastic load between months at station 1. The frequency of occurrence (FO) of MPs ranged from 1.35 in July at station 2 to 2.45 in June at station 3 with an observed significant difference ($p<0.05$) in the FO between months at station 2 as presented in Table 3. Table 4 shows the physical classification of MPs in which filaments, fragments, foam, pellets and fibres were all present with fragments occurring at all stations throughout the study period.

Table 1: Mean level of microplastics from *O. niloticus* according to stations and months

Months	Station 1	Station 2	Station 3
June	2.50 ± 1.35^a	1.05 ± 0.55^a	1.07 ± 0.76^a
July	2.33 ± 1.52^a	0.67 ± 1.15^a	0.95 ± 0.16^a
August	1.06 ± 0.58^a	1.33 ± 0.57^a	1.67 ± 0.53^a
Σ	5.89	3.05	3.69

Vertically arranged means with the same superscripts are not significantly different ($p>0.05$)

Table 2: Plastic load of *O. niloticus* according to stations and months

Months	Station 1	Station 2	Station 3
June	1.83 ± 0.16^b	1.16 ± 0.15^a	1.14 ± 0.15^a
July	2.21 ± 0.11^a	0.71 ± 0.06^a	0.06 ± 0.05^a
August	1.16 ± 0.15^b	0.74 ± 0.05^a	1.70 ± 0.04^a
Σ	5.20	2.61	2.90

Vertically arranged means with the same superscripts are not significantly different ($p>0.05$)

Table 3: Frequency of occurrence of microplastics according to stations and months

Months	Station 1	Station 2	Station 3
June	2.33 ± 0.57^a	1.67 ± 0.58^a	2.45 ± 1.04^a
July	3.02 ± 052^a	1.35 ± 0.57^b	1.36 ± 0.57^a
August	2.35 ± 0.68^a	2.56 ± 0.58^a	2.42 ± 1.09^a
Σ	7.70	5.58	6.23

Vertically arranged means with the same superscripts are not significantly different ($p>0.05$)

Table 4: Physical classification of microplastic particles according to stations and months

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

Note: R= Replicate; A = No plastic, B = Filament, C = Fragment, D = Foam, E = Pellet, F = Fibre

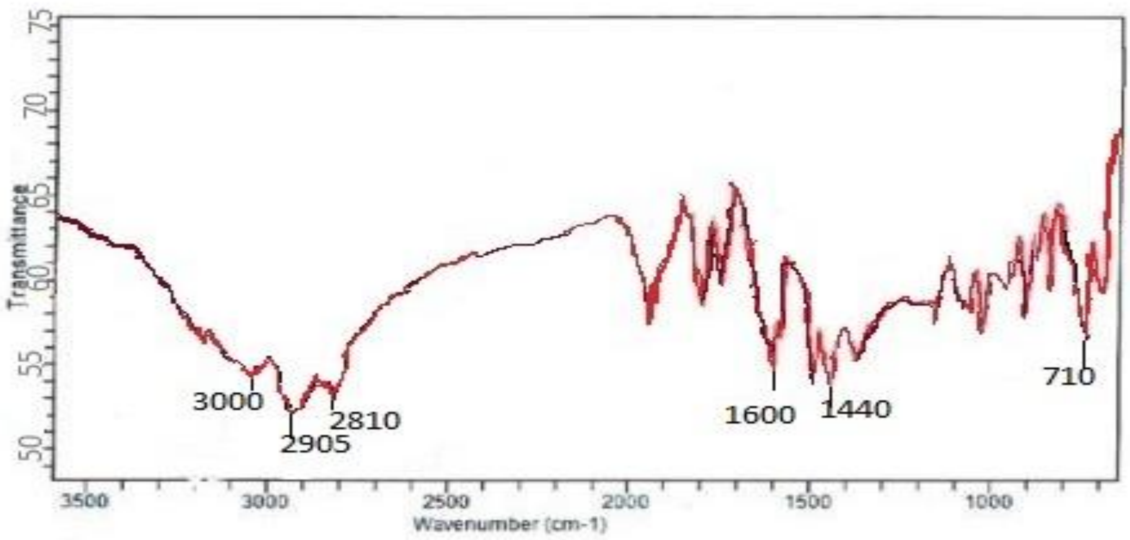

Figure 3: A typical FTIR spectrum for microplastic particles showing absorbance band at different wave numbers

The FTIR spectrum shows absorbance band at different wave numbers. There is a peak at 2905cm^{-1} which is characteristics absorption of asymmetric CH_2 stretching. There is also, a peak at 2810cm^{-1} which is a characteristic of symmetric CH_2 stretching. A peak at 1440cm^{-1} is a characteristics CH_2 scissoring. There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking. The peaks at 2905cm^{-1}, 1440cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers (Figure 3).

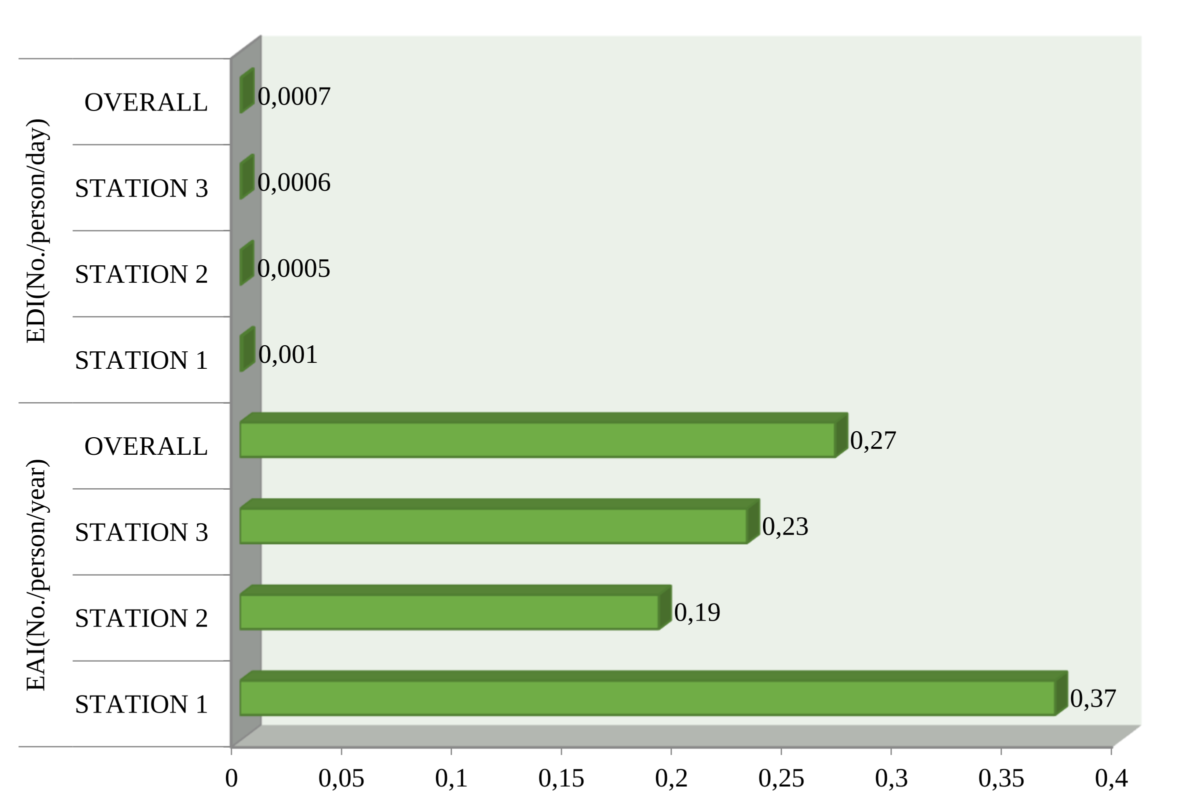

Figure 4: Estimated annual intake (EAI) and estimated daily intake (EDI) values for microplastics

As shown in Figure 4, the EAI (No./person/year) ranged from 0.19 at station 2 to 0.37 at station 1 while the EDI (No./person/day) ranged from 0.0005 at station 2 to 0.001 at station 1. The percent (%) quota of occurrence of microplastic particles ranged from 3.70% for fibres to 48.15% for fragments as shown in Figure 5.

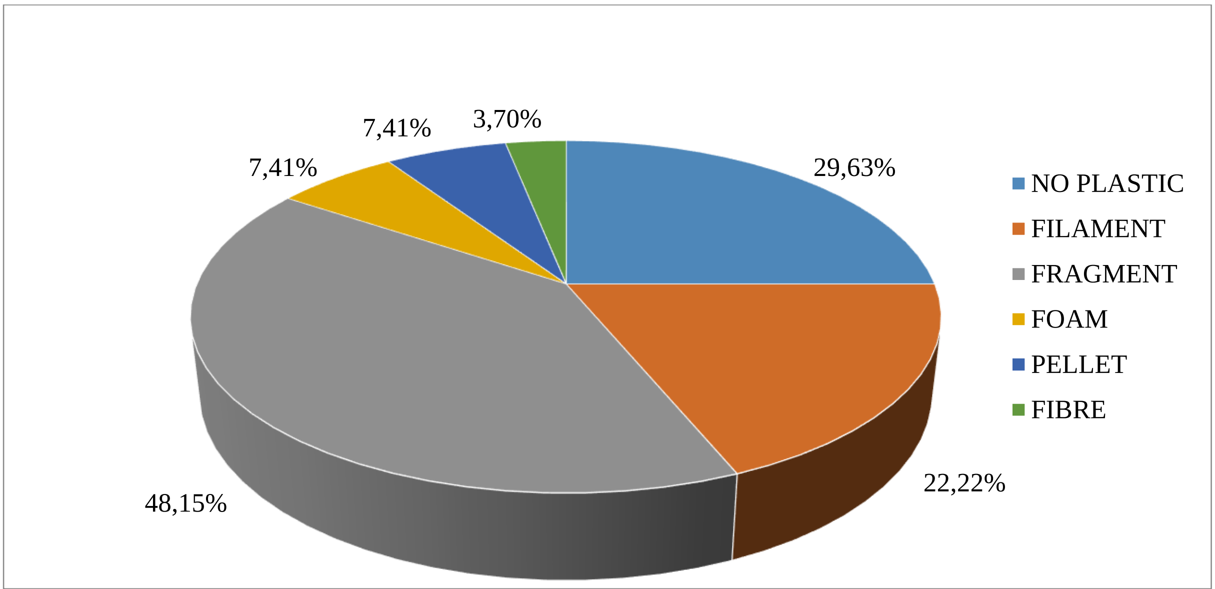

Figure 5: Percent (%) quota of occurrence of microplastic particles

DISCUSSION

Interestingly, MPs were indeed found in *Oreochromis niloticus* in this research. The mean level of MPs in the target fish species according to stations took the profile of station 1> station 3 > station 2 with no observed significant difference (p>0.05) in the mean levels of MPs between months at each station. This observation is a pointer to the fact that each station may have been equally impacted by MPs with very slight variations between stations. The plastic load (PL) also took the same pattern of station 1> station 3 > station 2. However, there was an observed significant difference (p<0.05) in the PL between months at station 1, indicating that station 1 was clearly ahead in terms of the PL rating when compared directly to the other stations. The frequency of occurrence (FO) of MPs also took the rank of station 1> station 3 > station 2 with an observed significant difference (p<0.05) in the FO between months at station 2, suggesting that station 2 had a relatively different FO profile compared to the stations. Regarding the physical classification of MPs, filaments, fragments, foam, pellets and fibres were all present with fragments occurring in the fish species at all the stations and accounting for almost half (48.15%) of the quota of occurrence. This observation gives an inroad into the diverse distribution of MP particles especially fragments. It can be inferred that the greater distribution of fragments could be linked to a higher distribution potential when compared to the other MP particles. In addition, the different MP particles certainly do not present the same densities, sizes, shapes and weights which are crucial in their distribution and eventual availability to biota including fish as corroborated by Uchida *et al.* (2016) and Kooi *et al.* (2019). The ubiquity and consequent distribution of MPs is further confirmed by their wide presence in aquatic and terrestrial ecosystems (Do Amparo *et al.*, 2023). The FTIR spectrum analysis with absorption wave numbers generally confirmed the presence of Polyethylene **(**PE) as the type of plastic material found in *O. niloticus.* Unnimaya *et al.* (2023), confirmed that the absorption wave numbers from the FTIR are used to identify specific plastic types as different plastics exhibit different absorption patterns which is important for their differentiation. According to the Food and Agriculture Organization of the United Nations (FAO), PE is widely used globally especially for packaging purposes (FAO, 2017). It is interesting to note that station 1 is situated downstream at the Nigerian Institute for Oil Palm Research (NIFOR) which uses PE bags for holding Oil Palm seedlings. Discarded PE bags can easily be washed into the river thereby constituting a menace to aquatic organisms by becoming secondary sources of MPs. The situation becomes compounded against the backdrop that MPs do not easily degrade in the environment thus persisting in water and sediment and exhibiting a high affinity for potentially toxic elements (Xiong *et al.,* 2023). Apart from PE, other types of MPs polymers that have been found in environmental compartments around the world include Polypropylene, Polyamide, Polyester and Polystyrene (Abisha *et al.*, 2024). In another research, Polyethylene microplastics were observed to impair the gonodal development in Carp fish (Zhu *et al.*, 2023). Such MPs have also been linked in reducing the accumulation of toxic Polychlorinated biphenyls (PCBs) in some terrestrial biota (Li *et al.*, 2024). The EAI and EDI figures were highest and lowest at stations 1 and 2 respectively, implying that in principle *O. niloticus* caught for consumption at station 1 would have a higher MP load and thus netting or catching fish from station 2 would be desirable owing to its obvious lower MP load. It is pertinent to note that it may not be possible to totally eradicate MPs from fish caught in the wild but attempting to keep the levels of MPs in such biota as low as reasonably possible would definitely reduce the incidence of risk. The occurrence and fate of MPs in water supplies in eastern China was investigated by Han *et al.* (2024) who observed worrisome EDI values for infants in the range of 45.5 to 75 items/Kg/day, further highlighting the menace of MPs in the environment. Elsewhere in Indonesia (North Maluku region), the skipjack tuna fish (*Katsuwonus pelamis*) collected from market sources contained 948 microplastic particles while the Brown-marbled grouper fish (*Epinephelus fuscoguttatus*) contained 594 microplastic particles in its digestive tract (Ahmad *et al.*, 2023). The aforementioned number of MP particles were far higher than what was observed in this study suggesting a much higher anthropogenic impact in such studies. The presence of MPs in *O. niloticus* as revealed in this research implies the potential risk of these particles ultimately reaching man through the food chain as corroborated by Saikumar *et al.* (2024).

CONCLUSION

Polyethylene particles were found in *O. niloticus* in this research suggesting that the River Okhuo is under the influence of anthropogenic impact with regard to the influx of materials of plastic origin. Therefore, in order to stem the tide of plastic pollution, it is recommended that a continuous monitoring programme be put in place along with effective public enlightenment that would ensure that the River Okhuo and its resources are protected from the impacts of MPs.

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