

## Investigation of Microplastics Contamination in African Catfish *Clarias gariepinus* and Nile Tilapia *Oreochromis niloticus* fish species in Owe River Ile-Oluji, Ondo State, Nigeria

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### ABSTRACT

The primary aim of this study is to investigate the presence of microplastics (MPs) contamination in surface water and two fish species (*Clarias gariepinus* and *Oreochromis niloticus*) in Owe River. One Litre (1 L) of water samples were collected from four different locations along the river in triplicates, samples were digested to remove organic matters. Eighteen (18) numbers of fishes were collected for each species. Samples collected were dissected and the guts excised were digested with 10% Potassium hydroxide and 10% Hydrogen Peroxide. Both digested water and fish samples were filtered using 0.45µm membrane through a vacuum filtration apparatus. The filter papers were then observed under digital microscope (1600X RoHS China) for MPs identification. MPs were classified into colours and shapes. A total of 104 items was found in the gut of both fish species with an average of  $4.33 \pm 1.71$  and  $1.44 \pm 0.70$  items/individual for *Clarias gariepinus* and *Oreochromis niloticus* respectively. Fiber was seen to be dominant in fish species compared to other MPs shapes identified. The abundance of MPs in the water samples ranged from  $203 \pm 50.64$  to  $724.33 \pm 129.89$  Items/L with a total of  $1898 \pm 198.34$  Items/L. Most of the recovered MPs from river water samples were fragments. Attenuated total reflectance–Fourier transform infrared (ATR – FTIR) spectroscopic analyses led to the identification of four (4) plastic polymer types, with polyethylene (PE) and polypropylene (PP) jointly constituting 82 % of the plastic particles. The enormous anthropogenic activities and indiscriminate disposal of plastic themed wastes along the river course are notably contributing factors to the MPs contamination of Owe River. A serious monitoring of waste disposal and necessary mitigation process is advised.

**Keyword:** *Microplastics, Fragments, Polymer, contamination, gut, shapes*

## 1. INTRODUCTION

Microplastics (plastic smaller than 5 mm) have become one of the most recognized pollutants globally in recent times. They are found everywhere in our environment and in all the continents[1]. In the developing part of the world like Nigeria, microplastics (MPs) abundance is due to the fact that plastics debris is found in an unprecedented large amount in the environment[2]. Plastics present in the environment will degrade or breakdown into tiny particles (MPs) when it is subjected to ultraviolet radiation, hydrolysis, or mechanical forces[3]. The degradation will make the plastic to undergo changes in its physical properties, such as discoloration, loss of tensile strength, elasticity, and later becoming brittle and easily broken [4]. These tiny particles (MPs) can enter into the environment through various sources such as industrial wastes discharge, surface runoff, granite blasting media, municipal solid waste landfill, washing clothes, disposable trash and wastewater treatment plants. Hence, there is continuous accumulation of MPs in our aquatic environments, including rivers, lakes, and marine environments, leading to increased concern on their potential risks for aquatic organisms including humans [5]. The chemical (additives) incorporated during plastics production to enhance and improve the quality of plastics are toxic if ingested[6]. These additives also have the ability to leach and proven transfer potential across the aquatic food chain to cause harm to aquatic organisms that ingest them[7]. In clear scruples, aquatic organisms tend to mistaken MPs for food causing toxic effects and as well act as a vector for further pollutants such as heavy metals, persistent organic pollutants (POPs) which could lead to various forms of toxicity [8]. Studies have shown that MPs are ingested by aquatic organisms from various parts of the world including Nigeria. Ingestion of MPs have been reported to cause internal blockage or damage, impede growth rate, block enzyme production, restraint mobility, affect reproduction, loss of appetite oxidative stress, as well as carcinogenic, teratogenic and mutagenic effects on organisms [9]. In addition, human being may also be affected; in a study by [10]several mechanisms of microplastics effect on human health such as exaggerated inflammatory response, genotoxicity, and oxidative

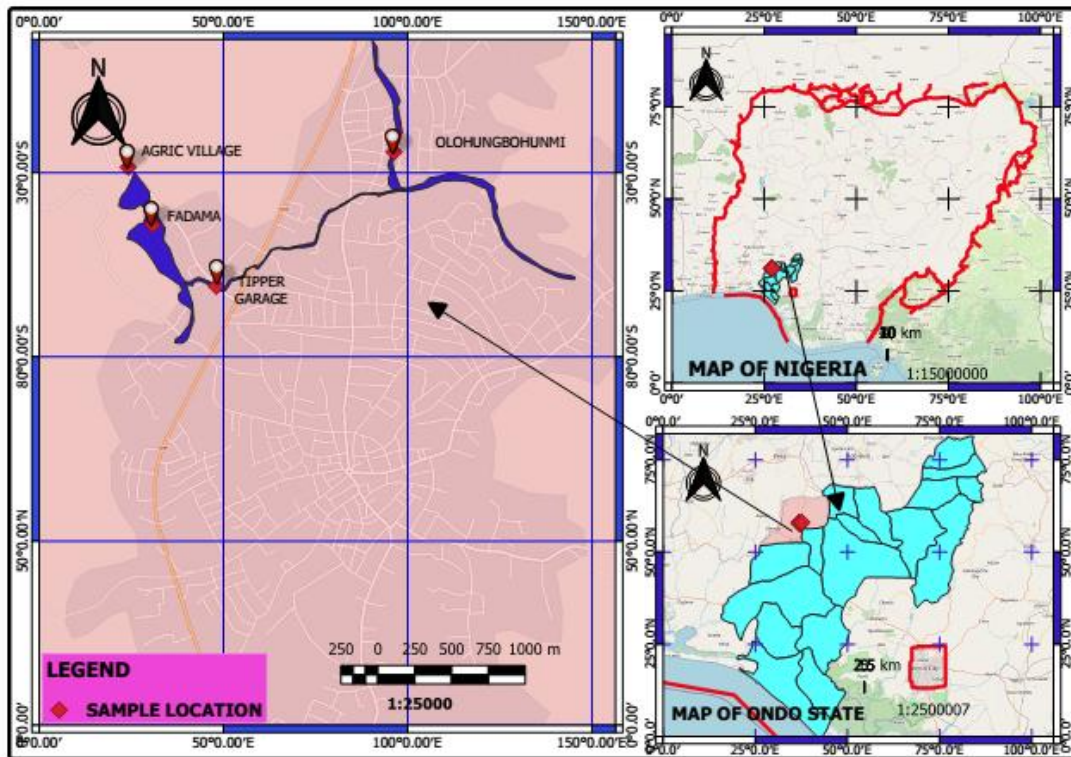
stress resulting in cell and tissue damage, fibrosis, and potentially carcinogenesis have been reported. The study further explains that polyvinyl chloride is a proven carcinogen and causes angiosarcoma of the liver [10]. In recent years, microplastics have been found in the human body [11].

It is worth knowing that the study of microplastics in freshwater environments has gained numerous attentions by researchers since the last years [12][4][13]. Recently, microplastics studies have gained attention in Nigeria some of which are highlighted in Table 1. However, there is still need for more of our aquatic systems to be enumerated. Therefore, the main objective of this study was to assess the presence of microplastics contamination in surface water and two most abundant fish species (African catfish *Clarias gariepinus* and Nile Tilapia *Oreochromis niloticus*) in Owe River Ile-Oluji, Ondo State, Nigeria.

## **2.0 MATERIAL AND METHODS**

### **2.1 Study area**

This study was conducted in Ileoluji / Okeigbo, a local government Area in Ondo State Nigeria (Fig.1). Its headquarters are in the town of Ileoluji. It has an area of 698m<sup>2</sup> and a population of 172,870 at 2006 census. Basically, it is an agrarian area and people are seriously involved in farming of various types of crops especially cocoa and river owe is also the main river that residents use for fishing and also to feed their local ponds for aquaculture. Four different locations along river owe namely; Olohunghunmi (OWR1), Tipper garage (OWR2), Fadama (OWR3) and Agric Village (OWR4) were considered as representatives of the area for the study because of the different activities at each sampling location. Samples were collected in August, 2023. A global positioning system was used to determine the location of the sampling site.



**Fig. 1: Map of study area showing the sampling locations**

## 2.2 Samples collection

Fish samples (Nile Tilapia *Oreochromis niloticus* and African catfish *Clarias gariepinus*) were captured using assorted fish traps from local fisher men. A total number of 36 fish specimens were obtained for microplastic analysis (fish per species  $n = 18$ ). Samples were transported on ice and kept frozen until they were ready for analysis at the laboratory of the Department of Fisheries Technology of the Federal Polytechnic Ile-Oluji, Ondo state Nigeria. In the laboratory, fish samples were cleaned with pre-filtered distilled water to remove any externally adhered plastic as described by [14]. Water samples (1 L) were collected from four different locations along the river for microplastics analysis in triplicates using glass bottle containers cleaned with pre-filtered distilled water. The containers were tilted in the surface water allowing the water to enter and then put upright as the water fills in to remove air bubbles. Samples were treated in triplicate. All samples were properly labelled and kept in ice-chest coolers prior to transportation to the laboratory where further preservation in

refrigerators continued before analysis. Sample collection points were geo-referenced with a Global Positioning System (GPS).

### **2.3 Isolation of microplastics from sample water**

The microplastics from water were isolated as explained by [15]. Prior to vacuum filtration, 9 mL of 50% H<sub>2</sub>O<sub>2</sub> solution was added to each 1 L water sample to digest organic materials and the samples were kept away from light for 48 hr at room temperature. Water samples were then filtered sequentially through a membrane filter with a nominal pore size of 0.45 µm with the aid of a glass aperture vacuum filtration apparatus. Each filter paper was placed in a glass petri dish and placed in a desiccator under room temperature. To isolate and count microplastics, the dried filter papers were examined under a light digital camera microscope (Digital camera 1600X RoHS China). Pictures of the viewed microplastic particles were taken and classified into four dominant shapes; fiber, film, fragment and pellet; six colours (green, black, white, blue, purple, and red) and four types polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyethylene terephthalate (PET) were observed based on their physical characteristics and outcome of the attenuated total reflectance - Fourier transform infrared (ATR-FTIR) spectrometer. Throughout the entire analysis, filter papers were covered properly to prevent contamination from airborne fibers when they were not under microscope.

### **2.4 Microplastic extraction from fish**

Firstly, the morphometric (total body length and body weight) parameters were measured for each fish prior to dissection. Microplastics were extracted following a modified method of [14]. Briefly, fish species were dissected from the anal opening to the upper part near the gills using a dissecting set and their entire gastrointestinal tracts (GIT) were removed using tweezers. The excised GIT were weighed on an electronic balance, recorded and kept in 500 mL clean beaker covered with aluminium foil. Following dissection, digestion was carried

out using 10 mL of 10 % pre-filtered potassium hydroxide (100 g in 1000 mL pre-filtered distilled water) per gram of the GIT sample. The dissection and digestion processes were done in a laminar flow hood to avoid cross contamination. The beaker containing the aqueous solution was sealed with aluminum foil and placed on a mechanical shaker for 1 hr and transfer into an incubator at 50 °C until digestion is completed. After that, 9 mL of 50 % H<sub>2</sub>O<sub>2</sub> was added to remove any organic matter present in the sample. Following the addition of hydrogen peroxide, the samples were incubated for the next 24 hr at 50 °C. Dilution with pre-filtered distilled was made where necessary. Samples were filtered through 0.45 µm membrane filter papers. Filters were stored in Petri dishes and placed in a tight sealed dessicator to avoid contamination prior to identification under the microscope.

### **2.5 FTIR Analysis (chemical identification)**

A total of 96 items (with sizes ranging from 0.5 – 5.0 mm) of microplastics were obtained from the water samples and subjected to attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy analysis to confirm their respective polymer types. The IR spectra were in transmittance mode and ranged from 400 to 4000 cm<sup>-1</sup>. Polymer types were identified by matching the wavelength data with those obtained from literature [16].

### **2.6 Statistical analyses**

All data generated in this study were subjected to statistical analysis using Excel and the software statistical package for social sciences (SPSS version 22) as applicable and a critical  $P = .05$  was selected. Results obtained are presented as mean ± standard deviation. The data were initially tested for normality distributions with Kolmogorov-Smirnov test. Since the data were found to deviate from normality, a non-parametric Kruskal-Wallis ANOVA was used to **analyze** the distribution of microplastics among the sites. Pearson correlation analyses were performed to examine association between the amount of microplastics

ingested by fish species and the morphometric parameters (fish weight, fish length and GIT weight).

### **3.0 RESULTS AND DISCUSSION**

#### **3.1 Microplastics abundance in surface water**

The distribution of MPs found at each location of Owe river water samples is presented in Fig.2. MPs were found in surface water samples collected from all of the sampling sites along Owe River. The research results show that the abundance of MPs in surface water samples ranged from  $203 \pm 50.64$  to  $724.33 \pm 129.89$  items/L with a total of  $1898 \pm 198.34$  items/L. Most of the recovered MPs from river water samples were fragments which are the resultant of degradation of larger plastic objects. High contents of microplastics were observed at Fadama (OWR3) compared to other sites this could be as a result of the presence of local palm oil industries and a quarry site that come with indiscriminate disposal of huge amount of plastic themed wastes. Statistically, the non-parametric Kruskal-Wallis H test revealed that the distribution of microplastics in the water samples was significantly different across the four sampling locations ( $P < 0.05$ ) hence, the null hypothesis is rejected (Fig.3). Pairwise comparison of the microplastics levels showed that the pairs of locations OWR1 and OWR3 differ significantly, with  $P$  value as 0.03 (see Supplementary Information Table S1, and Fig. S1).

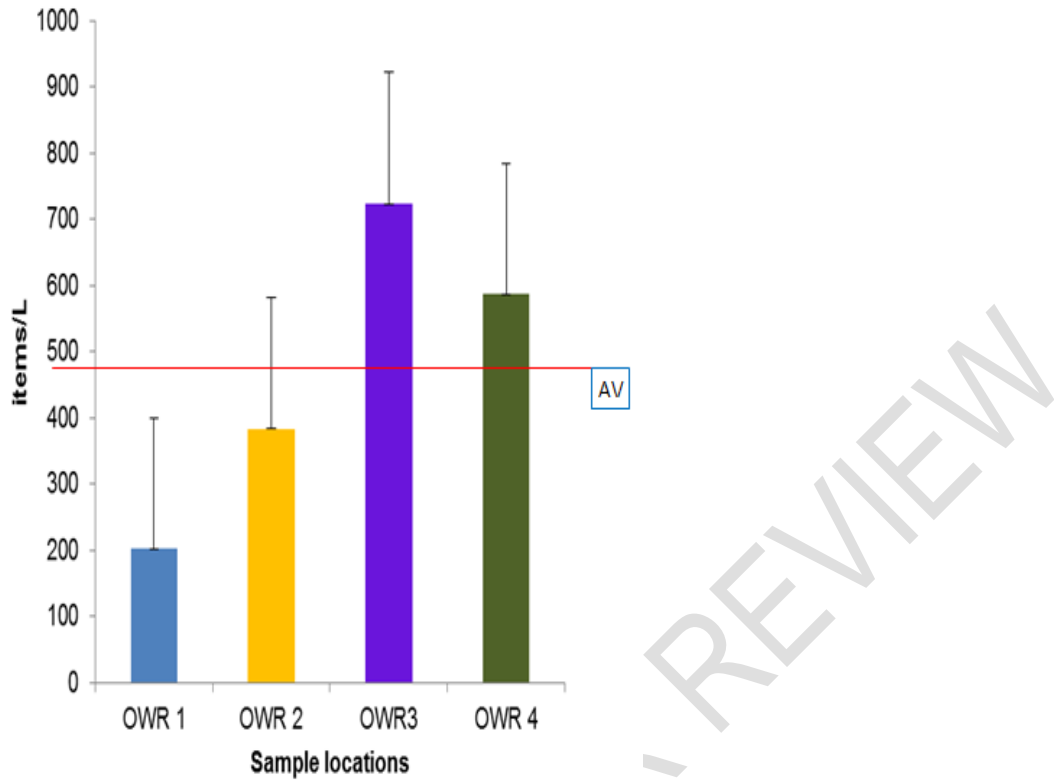


Fig. 2: Total MPs from each Location. OWR 1- Olohunghunmi; OWR 2- Tipper garage; OWR 3- Fadama; OWR 4- Agric village; AV- Average of Microplastic contents

### Independent-Samples Kruskal-Wallis Test

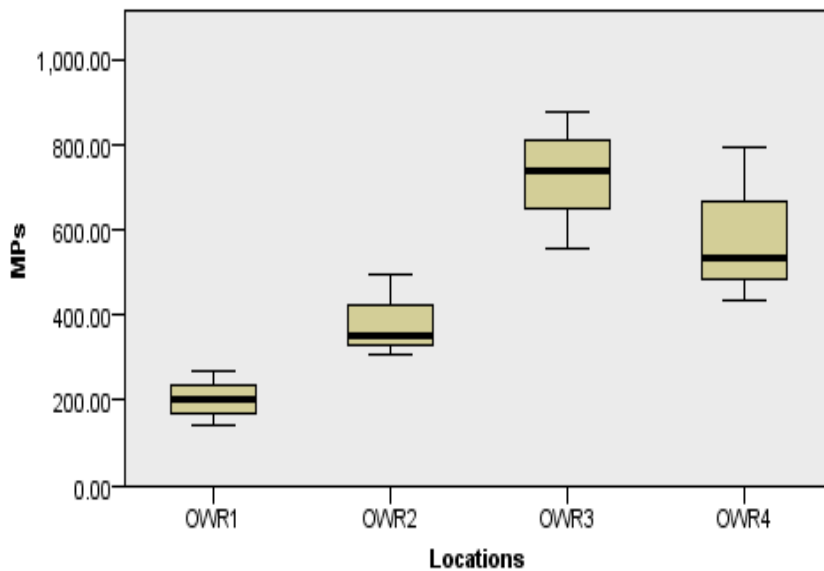


Fig. 3: Kruskal-Wallis independent-samples test comparing total microplastics contents (items/L) in water samples among the locations.

The total microplastic abundance ( $1898 \pm 198.34$  items/L) reported in this study is lower when compared with other flowing river systems studied in Nigeria. [17] recorded the highest abundance of MPs (3791–22,079 items/L) which stands as the highest globally. However, total MPs in Owe river was higher than reported total MPs from studies in other parts of the world (see Table 1).

**Table 1: MPs occurrence for some selected locations around the globe**

Study location	Sample type	Abundance	Reference
Owe River, Ile-Oluji, Nigeria	River water	$1898 \pm 198.34$ items/L	This study
Sürgü Dam Reservoir (Malatya), Turkey	Surface water	141 items/L	[15]
Estuaries of KwaZulu-Natal River system, South Africa	River water	$2.3 \pm 7.2$ items/L	[18]
OxBow Lake Yenagoa, Nigeria	River water	8.37 items/L	[19]
Sakumo II, Ghana	River Water	90 items /L	[20]
South Eastern Coast, Nigeria	River water	440–1556 items /L	[2]
Central Kenya, Kenya	Surface water	0.11 items /L	[21]
Naivasha Lake, Kenya	Surface water	$0.407 \pm 0.135$ items/L	[22]
Guangdong Coastal Areas, South China	Surface water	0.85 to 3.5 items/L	[23].
River Nakdong, South Korea	River water	15.56 items/L	[24]
Lake Ziway, Ethiopia	Fresh water	6.3–115.9 items /L	[12]
Osun River, Oshogbo, Nigeria	River water	3791–22,079 items /L	[17]
West River, South China	River water	2.99–9.87 items/L	[25]
Wei River, China	River water	4.67–12.3 items/L	[4]
Ottawa, Canada	River water	0.05–0.24 items/L	[26]
Los Angeles and San Gabriel rivers, United States	River water	max. 13 items/ L	[27]
Kaduna River, Nigeria	River water	153 items /L	[28]
Owe River, Ileoluji, Nigeria	Fish ( <i>Clarias gariepinus</i> , <i>Oreochromis niloticus</i> )	$4.33 \pm 1.71$ items/individual  $1.44 \pm 0.70$ items/individual	This study

Eastern Central Atlantic Ocean, off the Coast of Ghana	S. maderensis, D. angolensis S. aurita	40.0 ± 3.8 items/Individual 32.0 ± 2.7 items/Individual 25.7 ± 1.6 items/Individual	[1]
Efate, Vanuatu	Marine fish	2.9 ± 4.6 items /Individual	[5]
South Pacific Ocean Vanuatu	Yellow Tuna	Fin 4.3 ± 5.1 items /Individual	[5]
Laucala Bay, vanuaNavakavu and Suava Harbour Fiji	Marine fish	5.5 ± 9.4 items /Individual	[29]
Brazos River Basin, Central Texas, USA	Sunfish bluegill and Longear	10.1 – 13.9 items /Individual	[30]
Sürgü Dam Reservoir (Malatya), Turkey	Fish	0.41 items/individual	[15]

The study of shapes of microplastics is important in understanding their likely sources into a particular environment, as well as their potential impacts on organisms in that environment [5]. The MPs collected from the surface water were classified into four types: fragment, fiber, pellet, and film (Fig. 4). The predominant type of MPs identified was fragment except at Olohunghohunmi station where fiber was significantly higher than the rest of the plastic shape identified. Generally, the level of different shapes of the microplastics among the locations is in order of fragment > fiber > pellet > film. MP fragments and fibers provide strong evidence of secondary MPs as they are typically produced via the breakdown or fragmentation of larger plastic materials indiscriminately disposed into the environment [31]. Fragments are plastic particles which are erratically shaped, this make them physically injurious to aquatic organisms if ingested. In addition, fragment MPs can adsorb and amass other contaminants from the environment due to their relatively larger surface area. This may however subsequently transfer to organisms or humans that ingest them accidentally [17]. Fiber microplastics were the second most abundant shape type in the river water. They account for 36.35 % of the total microplastics found in the river water samples. The occurrence of abundant of fiber at Olohunghohunmi station may be due to the fact that it is a residential area where sewage is discharged through the septic tank, while some inhabitants

discharge their wastes through open drainage. Apart from sewage release, fibers may also emanate from washing of synthetic clothing materials, ropes and threads [32].

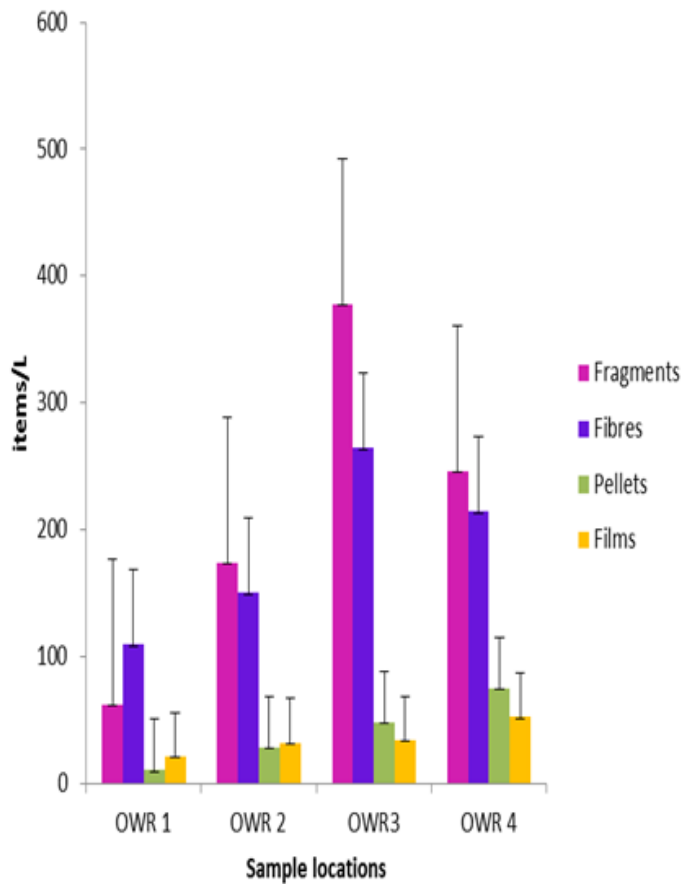


Fig. 4: Concentration of different shapes of MPs in water samples.

There is variation of colours within the locations of the study area. The results showed that MPs comprised of six different colours in the surface water; black, red, blue, white, purple and green (Fig. 5). The blue colour was found to have a significantly higher percentage (37 %) followed by red (27 %), white (12 %), black (12 %), purple (7 %) and green (5 %). Colour identification of MPs is important as it provides insight into their sources and potential impacts on the environment and influences the release, adsorption and degradation of pollutants associated with MPs [4].

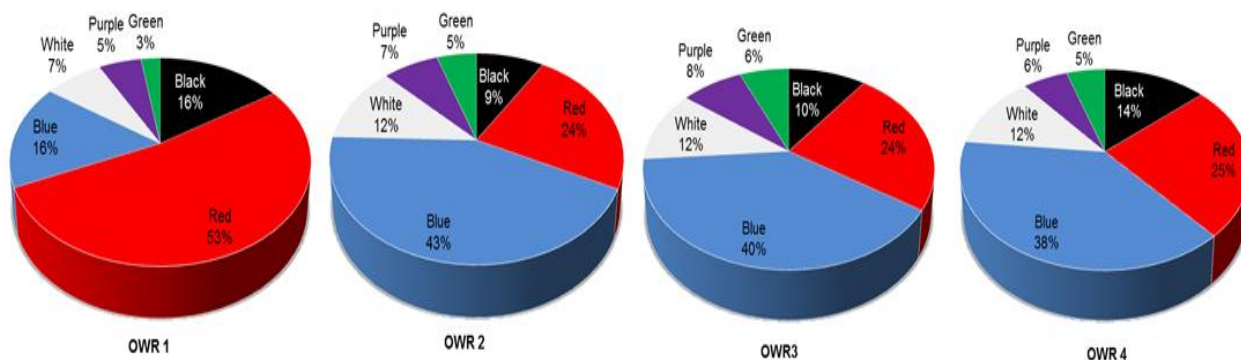


Fig. 5: Composition of coloured MPs sampled in Owe River

### 3.2 Microplastics in Fish Samples

Table 2 shows the morphometric properties of the fish species captured from Owe River. Microplastics were discovered in the gastrointestinal tract (GIT) of all the 36 fish samples under investigation. Previous studies [1][5][6][15] have reported the presence of microplastics in the GIT fish. The total microplastics found in the GIT of African catfish *Clarias gariepinus* and Nile Tilapia *Oreochromis niloticus* fish species in Owe River Ile-Oluji, Ondo State were  $4.33 \pm 1.71$  and  $1.44 \pm 0.70$  items/individual respectively. A total of 104 items with an average of  $52 \pm 26$  items corresponding to an average of  $3.64 \pm 1.13$  items  $g^{-1}$  wet weight of GIT were recorded. A minimum of 1 MP was found in each of the fish species. *C. gariepinus* had the highest count of MPs in the GIT compared to *O. niloticus* that are mostly herbivorous and pelagic. This may be due to the omnivorous nature of *C. gariepinus* and the fact that they are bottom feeders (demersal) which could make them ingest possible MPs from the bottom sediment unlike *O. niloticus*. Similar trend was reported by [12] for the fish species with the highest percentage of individuals with ingested plastics which was *C. gariepinus* and the lowest was *O. niloticus*. Pearson's correlation analyses performed to examine relationships between average numbers of microplastics ingested on one hand, and the morphometric parameters of the fish species, on the other hand, revealed a strong positive correlation between the microplastic content and each of the body weight and GIT weight

parameters. Strong positive correlations were observed between the amount of microplastics ingested and body weight ( $r = 0.155$ ,  $p = 0.540$ ), and between the amount microplastics ingested and GIT weight ( $r = 0.568$ ,  $p = 0.014$ ) of *O. niloticus*. Similarly, strong positive correlations were observed between the amount of microplastics ingested and body weight ( $r = 0.780$ ,  $p = 0.000$ ), and between the amount microplastics ingested and GIT weight ( $r = 0.522$ ,  $p = 0.026$ ) of *C.gariepinus* (Supplementary Information, Table S2). The range values of microplastics ingestion by fish species in this study (ranging from  $1.44 \pm 0.70$  to  $4.33 \pm 1.71$  microplastics/GIT of individual) is slightly above the values that have been reported in other recent studies, both in Nigeria and elsewhere. A study by [13] found an average of  $1.7 \pm 1.05$  microplastics/GIT in brown trout (*Salmo trutta*) from Lake Mahodand in Pakistan. [33] reported an average of  $2.56 \pm 1.42$  microplastics/individual for fish species captured from coastal waters of the Lvisi fishing ground in China. A study of ray-finned fishes from the Salento coastal seas by [34] revealed values between 4.6 and 5.4 MPs per individual in *Boopsboops* and *Sardinapilchardus* species.

Shapes of microplastics identified in the GIT of the two fish species were fragment, fiber, pellet and film. High occurrences of fiber were recorded for both fish species compared to other type of MPs shape found in the fish species (Fig.6). This could be in response to the fact that washing of clothes, effluents from wastewater are the main route of synthetic fibers into the river environment [1]. Similar result was reported by [30] who found fibers to be dominant in the stomach of *Lepomis macrochirus* and *Lepomis megalotis* fishes. Revealing that these fish species may not ingest fragments since plastic particles do not easily clutch into organic food items while fibers plastic particles do [30]. However, [12] revealed fragments as the dominant (57.5%) shape of the plastic particles found in GITs of fishes of Lake Ziway, with 42.5% being fibers. Microplastics can cause a significant effect to biota following exposure with the disruption of normal physiological processes, internal blockages

and disruption of digestion or exposure of organisms to plastic-associated chemicals [6]and the implications of trophic transfer of MPs through the food web [5].

**Table 2. Morphometric properties of the fish species captured from Owe River, IleOluji, Ondo State**

Species	No	Common Name	Length (cm)	Body weight (g)	GIT weight (g)	items/Individual
<i>O.niloticus</i>	18	Nile Tilapia	20.24 ± 2.35	36.93 ± 8.94	3.20 ± 0.94	1.44 ± 0.70
<i>C. gariepinus</i>	18	African catfish	26.04 ± 5.61	45.90 ± 13.00	4.09 ± 1.14	4.33 ± 1.71

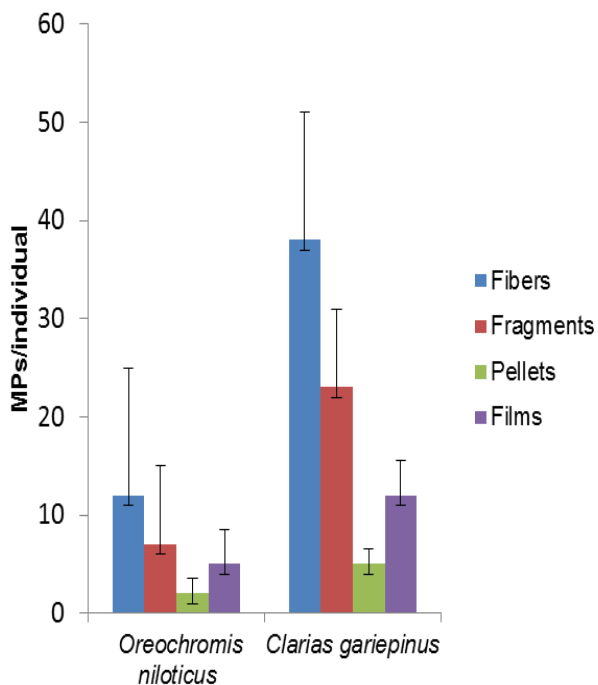


Fig. 6: Concentration of various microplastics shapes in the GIT of fish species

Colour plays an important role in the ingestion of MPs by aquatic organisms. Coloured MPs tend to attract aquatic animals that are visual predators e.g. gobies mistaken MPs with colour for food resembling their prey [12][4]. MPs with blue and black colours were dominant in the GIT of the fishes. Red, green and white coloured MPs were also quantified (Fig.

7).[35] revealed that blue coloured fragments were preferred by plankton fish, because their preys in the natural environment are blue copepods. The images of MPs are shown in Fig. 8, as captured under the digital microscope.

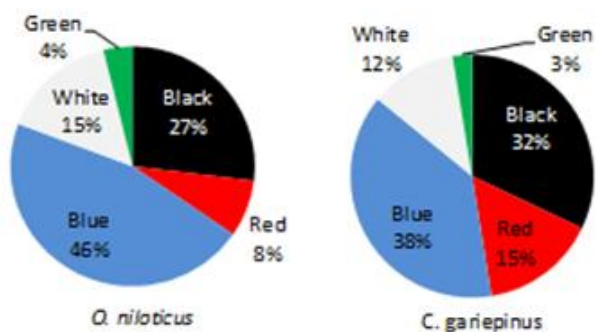


Fig. 7: Composition of different coloured microplastics found in the gastro-intestinal tracts (GITs) of two fish species from Owe River in ile-Oluji, Ondo state

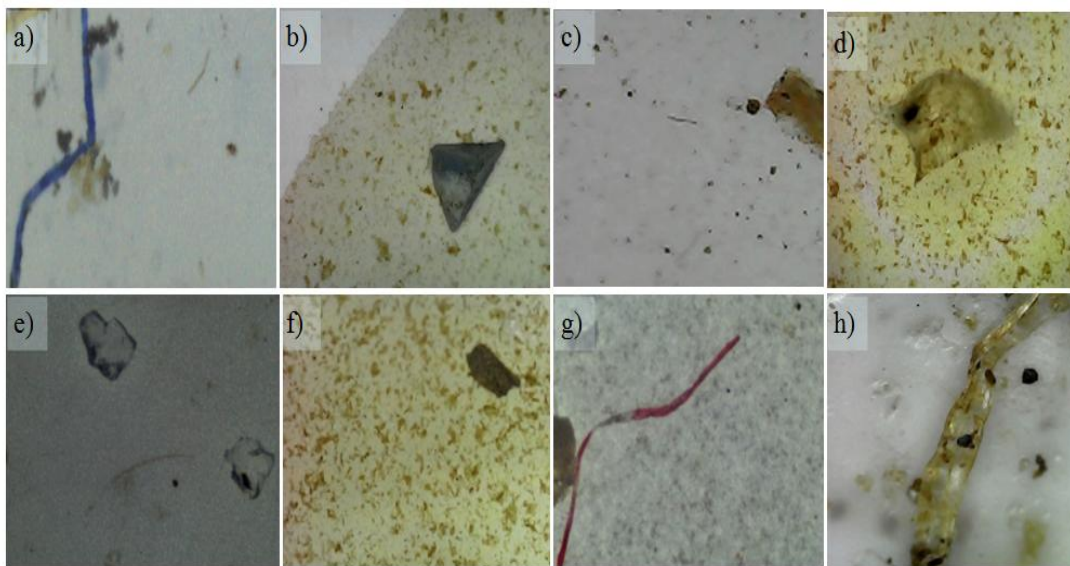


Fig. 8: Typical images of MPs found in water samples; a) fiber, b) fragment, c) pellet, d) film) and images of MPs found in the fish GITs; e) fragment, f) pellet, g) fiber, h) film.

### 3.3 Polymer identification

Following the initial visual identification of polymer in the water samples, a total of 96 particles (> 5 mm) were picked from filter paper with the aid of tweezers and their polymer types were determined by ATR-FTIR. Of these particles 90 (94.00%) were identified as MPs. The remaining particles were not plastic materials. It should be noted that all plastic particles found in fish samples were within the microscopic range hence, they were unable to be selected for FTIR analysis. The results of the ATR-FTIR analysis showed that about 46 % of the total plastic identified was contributed by polypropylene (PP), 36 % by polyethylene (PE), 10 % by Polyethylene terephthalate (PET) and 8 % by polystyrene (PS) (only those spectra that matched the standard database by more than 80% were accepted to ensure the reliability of the results). Absorption bands were used to identify polymers (Jung et al., 2018). See Supplementary Information, Figs. S2 - S5. Polymers with low densities such as PE and PP and expanded PS have the ability to float resulting into prevalent distribution in aqueous systems. They are easily transported via storm, rainwater and amass in rivers and shore lines of ocean [23]. The polymers found during this study may be as a result of migration of wastes into the rivers, or through anthropogenic sources. PE and PP are typically used in packaging applications (bags, bottles, beverage container caps, and drinking straws), fishing-related applications (nets, rope, and tape), making toys and home appliances [32]. PP materials are usually afloat owing to the fact that they have lower densities ( $0.83 - 0.91 \text{ g cm}^{-3}$ ) irrespective of their shapes and sizes. They are readily transported by waters and are abundant in the aquatic environment [36]. Previous studies revealed that PP was mostly detected in the freshwater environments, such as 35.7% of total MPs detected in the Pearl River, China, Manas River (30.6%), Qin River, China (39%), Antuã River, Portugal (29.4%), and Saigon River, Vietnam (4%)[37][38][39][36][40].

### 3.4 CONCLUSION

This research highlights a significant presence of microplastics in River Owe, Ile-Oluji, Ondo State Nigeria and the common fish species (*C. gariepinus* and *O. niloticus*) of the river. As at today indiscriminate disposal of wastes of plastic origin is still a common practice by the locals and the risks this poses may transcend what the present generation alone can bear. We advise that intensive re-orientation programme about plastic wastes disposal be carried out for the people by relevant agencies of the government and effective enforcement of environmental laws will improve the situation. Further research is required to know the extent of damage microplastics presence may directly or indirectly have on individuals who drink water or eat fishes from the river.

### ETHICAL APPROVAL

Animals were handled according to standard ethical protocols and consideration.

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