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OCCURRENCE AND SHAPES OF MICROPLASTICS IN SELECTED SEAFOOD SPECIES, WATER AND SEDIMENTS COLLECTED FROM EPE LAGOON, LAGOS STATE, NIGERIA

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ABSTRACT

Microplastics have become widely distributed in the environment to an extent that their occurrence in human consumables is currently a growing concern globally. In this study, tissues (gill, gut and muscle) of four selected seafood species in Nigeria (*Tilapia zilli*, *Clarias gariepinus, Penaeus notialis* and *Pomacea canaliculata*) were assessed for the occurrence and shapes of microplastics. Samples were collected from three points (Jubilee Chalete, Ojuolokun and Oko orisan) located on Epe lagoon, Lagos State. Collected seafood samples were dissected for tissues collection. The tissues were digested and microplastics were extracted from digested samples using membrane filtration procedure. With the aid of a stereomicroscope, membrane filters (pore size 0.45 um) were examined for visual counting and identification of the shapes of the microplastics. Results showed varying levels of microplastic particles of different shapes in all the accessed tissues of all seafood species examined. While there were no significant differences ($p>0.05$) in the concentrations of microplastics found in the tissues of *Clarias* and *Pomacea*, tissues of *Tilapia* and *Penaeus* showed significant differences ($p<0.05$) in the levels of accumulated microplastic particles. Highest mean concentrations of microplastics in the gill and muscle were recorded from the tissues of *Tilapia* (16.00 and 9.25particles/individual respectively) while the lowest values (7.33 and 6.50 particles/individual respectively) were recorded from the tissues of *Penaeus*. The shapes of microplastics identified, in the order of abundance, include fragment, fibre, filament, pellet/bead, film, and foam. These findings suggest that humans are at risk of ingesting microplastics when they consume seafoods.

Keywords: Microplastics, Occurrence, Shapes, Epe lagoon, Seafood.

INTRODUCTION

While plastic polymers have evolved in roughly seven decades to become indispensable commodities in the human societies, tiny plastic particles, generally referred to as microplastics, have become a source of environmental menace worldwide (Tirkey and Upadhyay, 2021; Yahaya *et al*., 2022). Microplastics are emerging environmental pollutants ranging in size from 0.001 to 5 mm in diameter and introduced into the environment via primary and secondary sources (Yalwaji *et al*., 2022). Primary microplastics are plastic particles that are produced in a size range of less than 1 mm (Anbumani and Kakkar, 2018). They are produced for the production of products such as facial scrubs, scrubs, cleansers, soaps, detergents and plas-

tic fibers used in synthetic textiles, among others (Zhou *et al.*, 2023). On the other hand, secondary microplastics are formed by the breakdown of larger plastics. This process is usually facilitated by factors such as solar ultraviolet radiation, wind, currents and other natural factors (Adeogun *et al.*, 2020).

In recent times, microplastics have become extensively distributed in the environment to an extent that their pollution and potential consequences have raised global concerns (Faleti, 2022). There are evidences of their occurrence in diverse environmental matrices such as surface waters (Abiodun *et al.*, 2019), sediments (Kazmiruk *et al.*, 2018), drinking water (Eerkes-Medrano *et al.*, 2019), soil (He *et al.*, 2018), air (Gasperi *et al.*, 2018), biota (Adeogun *et al.*, 2020), human stool samples (Schwabl *et al.*, 2019) and even in table salt (Zhang *et al.*, 2020). Microplastics occur in different shapes which also influences their physical impacts on biota tissues (Akinhanmi *et al.*, 2023; Hara *et al.*, 2020)

Microplastics currently account for about 80% of all marine debris and marine species are known to ingest and bioaccumulate them (Moruf *et al.*, 2020). Humans also being a part of the aquatic food web can therefore ingest microplastics by consuming aquatic organisms such as contaminated seafood (Moruf *et al.*, 2020). While there is a growing number of studies looking into the potential impacts of microplastics on organisms (Yahaya *et al.*, 2022), concentrations and physical characteristics of microplastics have been identified as influencers of physical micro-injuries in the tissues of organisms. Based on this, establishing the occurrence data and characteristics of microplastics in relevant species has become imperative globally (Hara *et al.*, 2020).

Despite the threats posed by microplastic pollution to biodiversity and food safety, there are research gaps on the occurrence and types of microplastics in commercial seafood species in Nigeria (Yalwaji *et al.*, 2022). In order to fill these gaps, the aim of this study was to investigate the occurrence and shapes of microplastics in four selected seafood species in Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out at the Epe lagoon in Epe Local Government Area, Lagos State, Nigeria. Epe lagoon is one of the ten lagoons found in Lagos State and it lies between latitudes 03°50' – 04°10'N and longitudes 005°30' – 005°40'E (Figure. 1). It has a surface area of more than 243km^2 and is located between two other lagoons, the Lekki lagoon in the east and Lagos lagoon in the west. It is bordered by several towns in the North, East and West, and bordered by the Gulf of Guinea in the South. It is connected to the Gulf of Guinea via the Lagos Harbour.

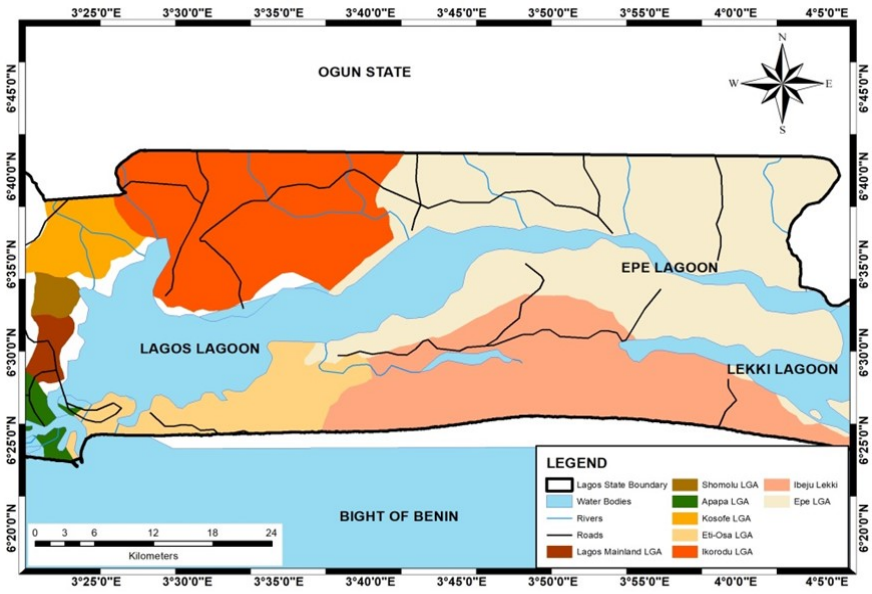


Figure 1: Epe Lagoon with the boundary and surrounding Local Government Areas

Sampling Procedures

Sampling points

Three sampling locations were selected on the lagoon for water and sediment sample collection. These include Jublilee Chalete, Ojuolokun and Oko Orisan (Fig. 2). The coordinates of the sampling points were captured using a mobile GPS. Seafood samples were procured directly from local fishermen at the sampling locations.

Sampling periods

Sampling was done during the rainy season across three months, specifically May, June and July. Water and sediment sampling was done once every month while collection of seafood samples was done in the month of June only.

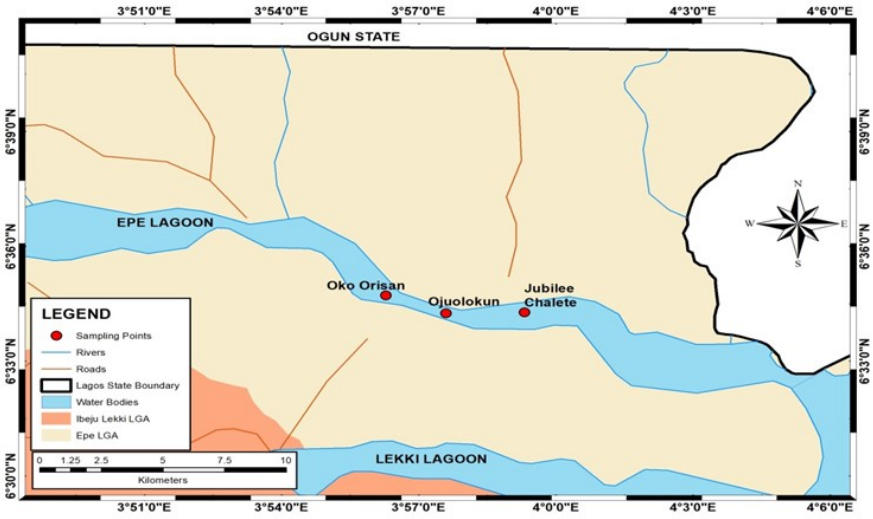


Figure 2: Epe Lagoon showing the sampling points

Collection procedures

Water and sediment sampling

Water and sediment samples were collected from ten different randomly selected spots at each sampling location. These samples were thereafter homogenized to make a whole sample. Analytes were collected for analysis from the homogenized composites. Thirty (30) surface water samples (1litre each) and 30 sediment samples (400 – 500 g each) were collected from each sampling location. Water and sediment sampling operations were carried out on open motorized boat. In order to minimize the lagoon water turbulence during sampling, the boat sailed under 3 knots and was stationed whenever samples were being collected. Water samples were collected directly into precleaned glass jars at approximate depth of 2 - 5cm while sediments were collected with the aid of a grab sampler lowered to the seabed from the stationed boat.

Animal samples collection and size

The selected seafood species (Plate 1) for this study are *Tilapia zilli* (the red belly tilapia), *Clarias gariepinus* (the African catfish), *Penaeus notialis* (the pink shrimp), and *Pomacea canaliculata* (the golden apple snail). The samples were procured directly from fishermen at identified landing spots in each location. Twelve representatives of each species (4 from each sampling location) were collected - Tilapia (average weight 40.90±4.77kg and length 12.58±0.71cm), Catfish (average weight 52.42±2.56kg and length 20.55±1.17cm), Shrimp (average weight 14.27±0.92kg and length 10.35±0.24cm) and Snail (average weight 79.40±8.18kg and aperture 16.08±1.26cm).

Handling of specimens

From sampling locations, water samples were contained in glass jars, sealed and transported in a cooler bag. The same procedure was followed for sediment samples. Furthermore, animal samples were contained in Ziploc bags, loaded with ice packs, and transported in a sealed cooler bag. Pending laboratory analyses, samples were preserved at 4^{o}C.

Measurement of physico-chemical parameters of the lagoon water

Physico-chemical parameters of the lagoon water were determined using a '7 in 1 water tester' C-600 model. Parameters measured include salinity (ppm), pH and Dissolved Oxygen (mg/l).

Laboratory analyses of samples

Laboratory analyses of all collected samples were orderly performed in four phases including (i) dissection of animal samples for tissue collection; (ii) digestion of water, sediment and tissue samples in preparation for microplastics extraction; (iii) ultrafiltration of digested samples for microplastics extraction; and finally (iv) microscopy for visual identification and quantification of extracted microplastics.

A

B

C

D

Digestion of water, sediment and tissue samples

Extraction of microplastics from water and sediment samples was done based on the recommendations of MSFD technical subgroup and NOAA. Water sample was digested with 50ml hydrogen peroxide (H_2O_2) for 12 hours and agitated for 5 days. The digest was transferred into a separating funnel containing an aqueous potassium formate solution. At the end, the lowest water phase in the funnel was filtered.

Sediment sample was weighed, oven-dried at 40°C for 24 hours, weighed again, and digested as done for the water samples. Thereafter, the digest was homogenously mixed with distilled water. Density gradient separation method was then carried out using ZnCl hypersaline solution (1.2 g/cm3), prepared using distilled water. The sample solution was stirred for 10min, allowed to settle for another 10min, and thereafter, the supernatant was decanted into a new 500-mL glass beaker.

Excised tissues were digested using KOH in incubator at 40°C for 3 days.

Extraction of microplastics

Microplastics were extracted from digested samples using membrane filtration procedure. Digest was filtered under vacuum pump through a millipore membrane filter

(0.45 μm). After filtration, the membrane filter was immediately transferred into a glass petri dish. The membrane was thereafter examined under a digital stereomicroscope (AmScope MU300-HS) for microplastics detection.

Microplastics identification and quantification

The water, sediment and tissue membrane filters were examined under a stereomicroscope for visual counting (abundance) and determining the shapes and sizes of the microplastics. The abundance of the microplastics in the water sample was expressed as the number of particles per liter, while that of the sediment and tissue samples was expressed as number of particles per gram. The shapes of the microplastics were classified as fibers, fragments, foam, pellet/bead, and film.

Quality Assurance

In order to prevent cross-contamination of samples with microplastic particles, non-plastic materials were used to contain samples. In addition, all materials and instruments used were washed thoroughly with distilled water. All samples and chemicals contained or stored in glassware were covered with aluminum foil to prevent microplastic cross-contamination. Equally, all laboratory analysis including dissection of animal samples and microplastics analysis were performed inside a fume hood. All glass wares, filters, dissecting sets, and other apparatus were properly covered with aluminum foil and placed in the fume hood, when not in use. Prior to dissection, the exterior of animal samples was rinsed with distilled water.

Data management and statistical analysis

Appropriate templates were created on Microsoft Excel Spreadsheet version 2010 for prompt input and storage of data. IBM SPSS version 26 was used for statistical analyses of data. Values were presented as mean ± standard deviation (SD). Variations in the mean values were evaluated using One-way Analysis Of Variance (ANOVA) while $p \leq 0.05$ was considered statistically significant.

RESULTS

Concentrations of microplastics in tissues

In the tissues of Tilapia and shrimp, values of microplastics concentrations were significantly different. The gills of both tilapia and shrimp jointly had the highest mean values of accumulated microplastics (16.00 and 9.25, respectively) compared to the other species examined. The muscle of tilapia recorded the lowest mean concentration value (7.33). Although there are variations (Figure 3) in the values of microplastics concentrations in the tissues of both Clarias and Apple snail, there were no significant differences in the concentrations (Table 1).

Table 1: Mean concentrations (no of particles/individual) of microplastic particles in assessed tissues

Tissue	Tilapia	Clarias	Shrimp	Snail
Gill	16.00 ± 1.142^{a}	13.08 ± 1.454^{a}	9.25 ± 1.081^{a}	11.75 ± 1.142^{a}
Gut	10.58 ± 1.026^{b}	14.42 ± 0.783^{a}	5.58 ± 0.543^{b}	10.25 ± 0.750^{a}
Muscle	7.33 ± 0.980^{b}	14.50 ± 0.980^{a}	6.50 ± 0.733^{ab}	9.25 ± 1.478^{a}

Mean values with dissimilar superscript along the column are significantly different ($p<0.05$).

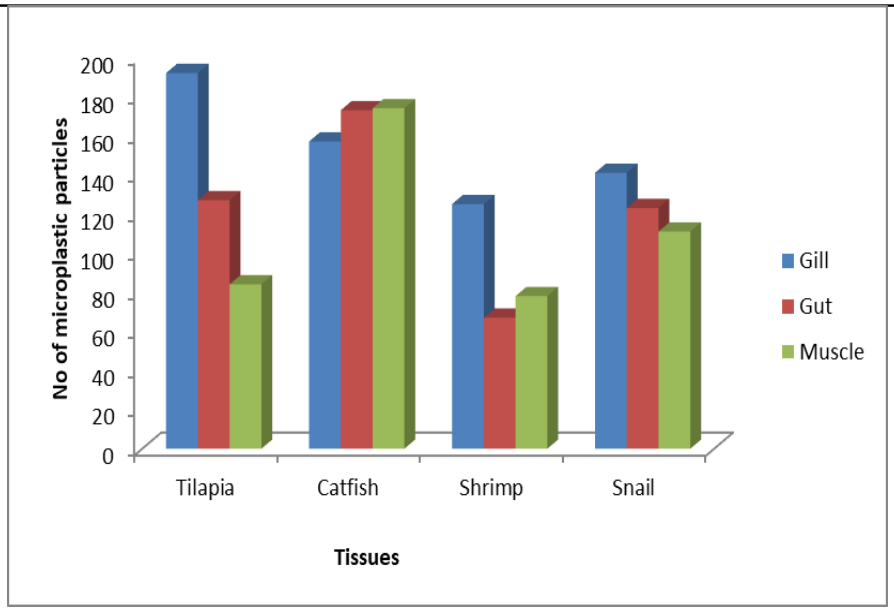
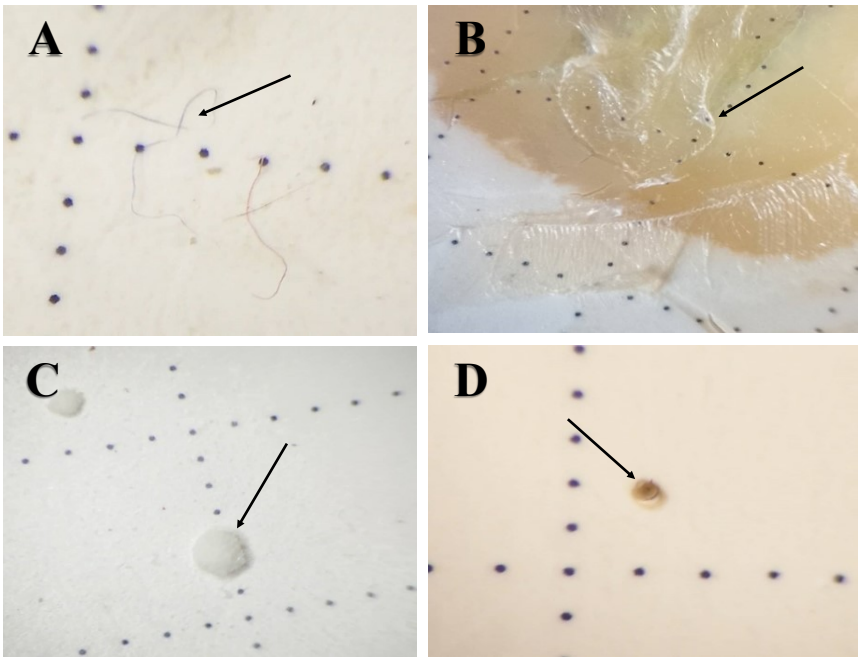


Figure 3: Levels of microplastic particles in assessed tissues

Shapes of extracted microplastics

The fragment type was most abundant in Tilapia, Catfish and Snail but the fibre type in Shrimp (Figure 4). A total number of 1,552 microplastics particles were extracted from assessed tissues. Fragment type recorded the highest abundance, accounting for 29.25% of the total number while foam type had the least abundance, amounting to 3.29% of the total number (Figure 5). Different shapes of microplastics were identified in the study (Plate 2).



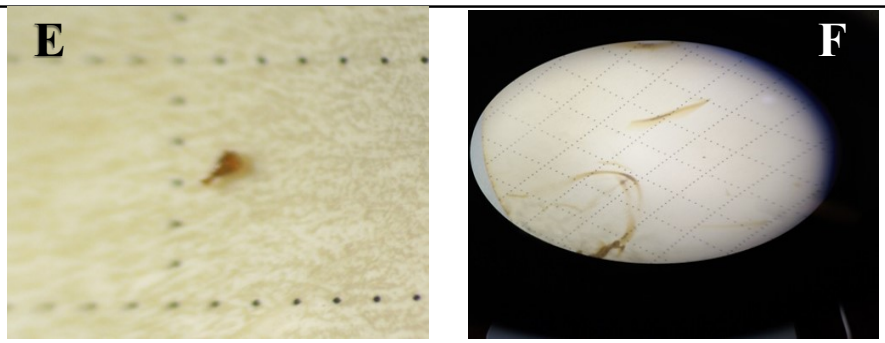


Plate 2: Shapes of microplastics identified in the study (A- Fibre, B- Film, C- Foam, D- Pellet/bead, E- Fragment, F- Filament) MG 10X

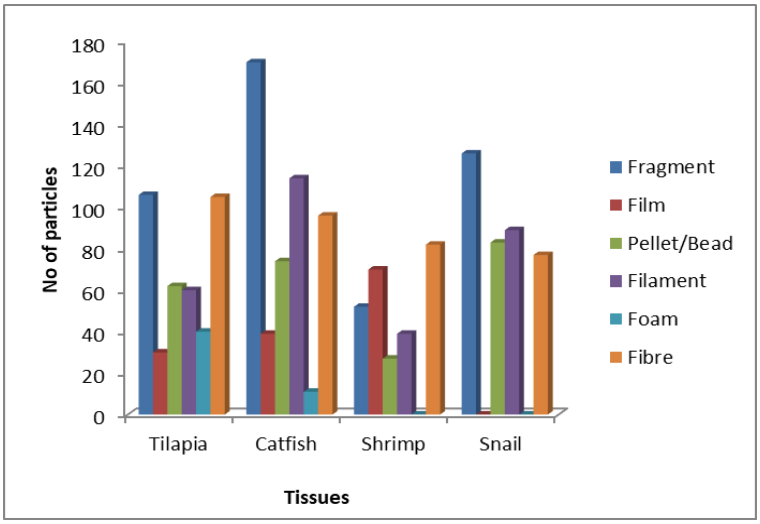


Figure 4: Distribution of microplastic types in assessed tissues

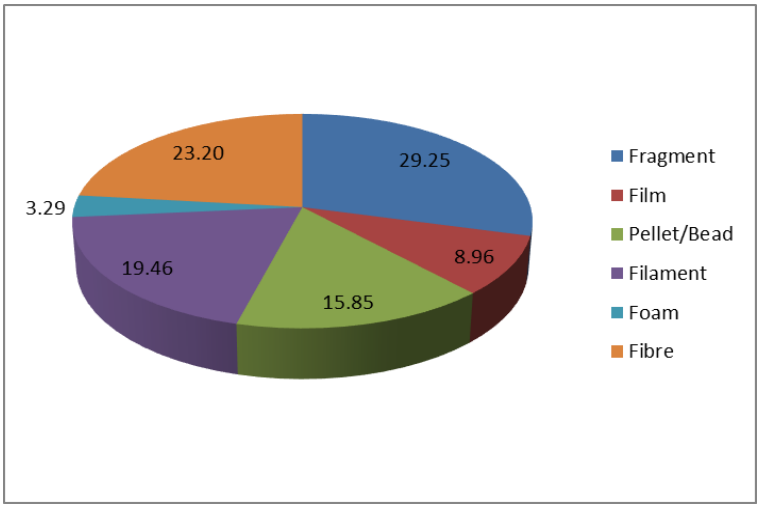


Figure 5: Percentage frequency of types of microplastics extracted across all the species examined

Bio-concentration and Bio-accumulation Factors

Bio-concentration factor of microplastics in all species examined in this study was <1. Bio-accumulation factor was >1 in *Clarias*, approximately 1 (0.99) in Apple snail, and <1 in Tilapia and shrimp (Table 2).

Table 2: Bioconcentration Factor and Bioaccumulation Factor

Animals	MPs in gill	BCF	MPs in muscle	BAF
Tilapia	16.00	0.51	7.33	0.79
Clarias	13.08	0.42	14.5	1.55
Shrimp	9.25	0.30	6.50	0.7
Snail	11.75	0.38	9.25	0.99

MPs concentration in water = 31.33particles/litre; MPs concentration in sediment = 9.33 particles/g;
BCF = MP in gill/MP in water; BAF = MP in muscle/MP in sediment.

DISCUSSION

Occurrence of microplastics in assessed tissues

Microplastics ingestion and bioaccumulation has been reported in several taxa (Adeogun *et al.*, 2020; Bellas *et al.*, 2016). The findings of the present study is a further confirmation that accumulation of microplastics occurs in aquatic animals. Occurrence of microplastics at varying levels was recorded in all species examined. Several studies had earlier reported the occurrence of microplastics in the tissues of different species of animals. For instance, Atamanalp *et al.* (2021) reported the presence of microplastics in gill, brain, muscle and gastrointestinal tract of *Mullus barbatus* and *Alosa immaculata*. A study by Adeogun *et al.* (2020) also detected microplastics particles in the stomach of eight different fish species collected from Lake Eleyele Ibadan, Nigeria. Furthermore, Hara *et al.* (2020) found microplastics in the intestines of *Nephrops norvegicus* collected from Irish prawn grounds.

It was also found in the present study that the levels of microplastics was higher in the gills of 3/4 species examined than in other tissues. This partly agrees with the findings of Atamanalp *et al.* (2021) which showed higher levels of microplastics in the gill than in the muscle. In addition, ingestion of microplastics in aquatic animals may occur via oral, gill and skin exposure while accumulation in different tissues occurs via endocytosis (Lee *et al.*, 2021). In line with this statement, the varying levels of microplastics in the different tissues might be an indication of the dominant routes of entry of microplastics.

The differences in levels of microplastics observed in the four species might be due to differences in their respective ecological niches (Zhang *et al.*, 2020). Tilapia is known to be a pelagic species while Clarias is benthopelagic. According to a study by Dahms *et al.* (2022), *Clarias* being benthopelagic, has the tendency to bioaccumulate more microplastics than pelagic species such as Tilapia. The findings of this study is in line with this

report, showing higher BAF in *Clarias* than in *Tilapia*. A study conducted by Sani *et al.* (2022) also showed a pattern fairly similar to the trend observed in this study. In that study, highest levels of microplastics in *Tilapian zillii* was also recorded in the gut, followed by the gills and lastly muscle. These differences, unlike this present study, were however not significant.

Shapes of microplastics

Microplastics are known to occur in different shapes which also influence their impact in biota tissues (Frias and Nash, 2019). The present study recorded different shapes of microplastics which serves a further confirmation of this statement. Also, shapes of microplastics serve to provide information about their potential sources (Campanale *et al.*, 2020). For instance, potential sources of fragment include bottles; hard sturdy plastics. Foam could be traced to styrofoam; pellet to facial cleansers; film to plastic bags and wrappers; bead to personal care products; fibre to clothing or textiles and so on. Based on this prior knowledge, occurrence of different shapes of microplastics in the tissues of the four seafood species examined in this study suggests diverse land-based origins of microplastics in Epe lagoon.

Furthermore, the results of this study support the findings of (Wang *et al.*, 2020) who reported that aquatic animals are continuously interacting with microplastics present in the ecosystems. Adeogun *et al.* (2020) also reported the presence of microplastics in *Oreochromis niloticus*. However, the results of this study in terms of microplastics shape with the highest abundance, is contrary to the findings of Kazmiruk *et al.* (2018). This study recorded highest abundance in fragment type while the aforementioned recorded the highest abundance in beads. Also Hara *et al.* (2020) reported fibres accounting for 98.1% of microplastics found in their study. This disparity might be due to differences in prominent land-based origins of microplastics in the different geographical areas.

BCF and BAF

BCF of microplastics was <1 in all the four species examined. This indicates a lower concentration of microplastics in the gills of the animals than in the water. In this study, Tilapia has a higher BCF than the other species, suggesting a higher tendency to accumulate more microplastics via gills than the other species. BAF in the muscle of *Clarias* examined was >1. This is an indication of higher concentration of microplastics in the muscle of *Clarias* than in the sediment. Generally, BAF values were higher than BCF values in all the species examined in the present study. This suggests a tendency of accumulating more microplastics from the sediments than from the surface water.

CONCLUSION

In conclusion, the findings of this study serve evidence that accumulation of microplastics occurs in seafood species such as the red belly tilapia, African catfish, pink shrimp and golden apple snail. This suggests that humans are at risk of ingesting microplastics when they consume contaminated seafoods. It is recommended that further studies, using other commercial species, with larger sample size be conducted to appreciate better the problem of microplastic pollution in Nigeria and also help policy makers in making well-informed decisions towards the control of plastic pollution.

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