Nwanna, L. C.; Ajani, E. K.; Omitoyin, B. O.; Magawata, I. Ayoola, S. O.; Orisasona, O.; Kareem, O. K and Abubakar, M. Y. (Eds.) (2004) ***Proceedings of the Fifth Conference of Association of Nigerian Fisheries Scientists.*** **"SUSTAINABLE FISHERIES AND AQUACULTURE PRODUCTION FOR ENHANCING LIVELIHOOD IN NIGERIAN ECONOMIC DEVELOPMENT"** 9TH – 11TH JULY, 2024 In the University of Lagos, Lagos, Nigeria

Bioavailability of Microplastic in Commercially Important Freshwater Fish *Clarias gariepinus* from Eleyele Lake

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ABSTRACT

Aquatic ecosystems are under risk due to global pollution caused by microplastics (MPs). This study investigated the presence of microplastic in the guts of *Clarias gariepinus* from Eleyele Lake. The guts of samples of *C gariepinus* from Eleyele Lake was extracted using density gradient separation approach (hypersaline solution of NaCl) and examined for microplastic using a light microscope. To confirm MPs presence, Fourier Transform Infrared Spectroscopy (FTIR) was conducted.MPs were present with a frequency of 61% and ranged from small fragments to fibres. The IR absorptions (cm^{-1}) observed in the regions of ethers and esters, silicon and silicate, Alkyl chains and Si-O-Si stretching of silicons and silicates were suggestive of microplastic presence in the fish guts. The study reveals that *C. gariepinus* has a high rate of MP intake. This finding may be related to the type, quantity, and degree of plastic pollution present in the Eleyele Lake environment.

Keywords:
Microplastic pollution, Density gradient separation, Aquatic pollution, Fish microplastic contamination

Introduction

Microplastics (MPs) are of increasing concern in aquatic environments due to the ecotoxicological risks they pose. Microplastics are defined as plastic debris between 1 μm and 5mm in diameter. They come in a variety of forms, colours and materials. They are the outcome of larger polymers breaking down as well as the development of commercial products. Polypropylene and polyethylene are the two most widespread forms of Plastic in the aquatic environment (Cole *et al*., 2015). According to Thompson *et al*., (2004), MPs, or minute plastic grains, are frequently found in products made of discarded plastic fragments. Primary and secondary microplastics are the two categories of microplastics. Small plastic granules known as primary microplastics are utilized in air blasting technology, face cleansers and cosmetics, and as drug vectors in medicine. On the other hand, secondary microplastics are remnants that have degraded from the debris of primary microplastics.

Freshwater can be the sources, transferring media and the sink of MPs. Freshwater may accumulate numerous MPs; however, few studies have been done to monitor the MPs in freshwater compared to those in marine water (Li *et al*., 2018). Lakes are the main sinks for microplastics in freshwater ecosystems, as these pollutants can accumulate and remain in sediments for long periods of time. In other countries, microplastics and phthalates have been found in freshwater and saltwater fish (Baini *et*

al., 2017), while recently microplastics were found in the guts of Fish from Lake Eleyele (Adeogun *et al*., 2020) which shows that there is contamination by microplastics in Eleyele lake.
Generally, there is a scarcity of knowledge about the impacts of microplastics (MPs) on the early juvenile stage of freshwater fish (Hamed *et al*., 2019). Hence, this study was conceived to extract and identify the presence microplastic in the guts of *Clarias gariepinus* from Eleyele lake.

Materials and methods

Study Area

This study was conducted in Eleyele Lake (Figure 1), which is situated between longitudes 3° 25´ E and 3° 35´ E and latitudes 7° 23´ N and 7° 31´ N in the northeastern section of Ibadan city, southwest Nigeria. The lake spans 240 metres over the dam and has a catchment area of 323.7 square kilometres. Its impoundment area is 156.2 hectares, and it can store 29.5 million litres of water (Bolaji, 2017). The lake is 6.0 m deep on average and is located 125 m above sea level. The bedrock geology, which has a distinctive dendritic pattern of structurally regulated streams and rivulets, governs the drainage system. Because of the surrounding region's steep terrain, flash overland flow and drainage discharge are typical during the wet season, which runs from May through October (Bolaji, 2017). The sustainability of the fish supply from this lake is threatened by industrialised plastic, metal recycling and solid wastes including plastics dumped from residential areas within the catchment area (Olayinka *et al.*, 2017). Plastic items of different sizes and shapes (plastic bottles and polythene bags of different polymer types) used mainly in household, personal care products, and construction have been observed floating on the surface of the lake with higher aggregation close to the landward portions of the lake (Adeogun *et al*., 2020).

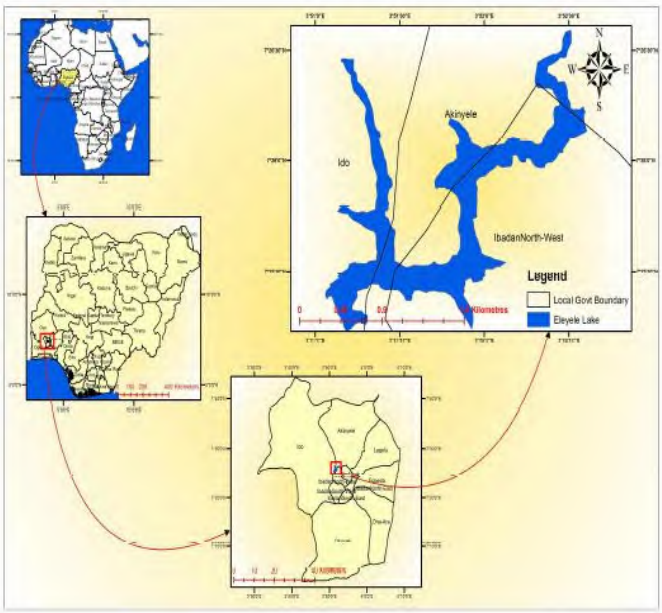


Figure 1: Map of Eleyele Lake
Source: Tope-Ajayi and Adedeji, 2016

Reagents and materials

Sodium chloride (NaCl) and hydrogen peroxide (H_2O_2) were purchased from the Department of Chemistry, University of Ibadan, Nigeria. The reagents used in the study were of the highest commercially available analytical grades.

Sample Collection

A total of 10 samples of *Clarias gariepinus* was collected randomly from the entire length of Eleyele lake with the aid of artisanal fishermen. The fish species was selected based on its feeding pattern as Omnivore. In addition, the fish species is one of the most important wild and cultured freshwater fish in Nigeria. Fish samples were then anesthetized on ice and transported to the laboratory. Fish sampling was planned to target fish species at different trophic levels within the study area.

Fish morphometric data, including body weight (BW) and gut weight (GW), were measured with a digital weighing balance while total length (TL) and standard length (SL) were measured with an absolute digital calipher. Fish condition factor (K) was then calculated as condition factor $(K) = 100 \times W/L^3$, where W = fish body weight and L = total length (Fulton, 1902).

Sampling (Experimental Fish from Eleyele lake)

Fish samples were transported to the laboratory, washed with double-distilled water to remove external debris, labelled, and their length and weight recorded. 10 fish samples were collected.

Extraction of microplastics in the fish samples.

The following procedures was followed for the extraction, detection and quantification of microplastics in the fish samples:

1. The exterior of Fish samples was washed with a deionized water.
2. The stomach was then dissected.

Procedure for dissection of the Stomach

Fish stomach was opened horizontally through horizontal slicing of the stomach. The Stomach content was then emptied into a 500ml glass beaker. In the beaker, 250ml of saturated NaCl solution with distilled water was prepared. The sample solution was stirred for 4 minutes and allowed to settle for 10 minutes. The supernatant was decanted into a new 500ml glass beaker. The extraction procedure on the stomach was repeated. The supernatant was filtered using the vacuum pump using syntact glass. Filtration was done twice to get better performance and higher microplastic recovery. After filtration, the membrane was transferred into a glass petri dish and digested in a 2ml of Hydrogen peroxide solution and then allowed to dry in the oven at 50^0C overnight. The membrane was examined under fluorescent lamp and the microplastic particles counted and recorded. No contact with plastic was allowed throughout the whole procedure (Adeogun *et al*., 2020).

Detection and quantification of microplastics in the fish samples.

Optical microscopy and photography were used to further select the microplastics discovered on filter membranes. The following guidelines were used for microplastic identification and observation. 1) devoid of any cellular or tissue structure; 2) having a single, consistent colour; and 3) that cannot be easily removed with forceps (Peng *et al*., 2017).

The dried filter sheets were inspected using a light digital camera microscope (Digital camera 1600X RoHS China) to separate and count microplastics. Images of the observed microplastic particles were captured, and they were categorised into prominent forms as; fragments, fiber and pellet.

Characterisation of microplastic from the guts of fish samples using Fourier Transform Infrared Spectroscopy

Polymer types of debris were then characterised by using Fourier Transform InfraRed spectroscopy (PerkinsElmer) in ATR (Attenuated Total Reflection) mode. Infrared light was configured at wavelengths ranging from 4000 to 400 cm^{-1}. Debris were put individually on the diamond of the spectroscope and then pressed. Given this handling, only a subsample of 1085 debris of the 2165 visually sorted debris was investigated. To define the subsample, debris of each sample were first examined under a stereomicroscope and pooled if they were strongly similar in shape, colours, curves and borders. Spectra were recorded by the software Perkin Elmer version Spectrum (10.4.3; 2014). The quality of each spectrum was assessed by checking peaks, transmittance range, libraries and recurrence of results to be considered as exploitable.

Quality assurance

All solvents were redistilled and filtered before use to ensure non contamination (Ilechukwu *et al.*, 2019). Glasswares used for sample preparation and extraction was rinsed with double distilled water and dried in the oven before use (Mgbemena *et al.*, 2017). Working surfaces were cleaned with ethanol prior to start of work. Samples were covered when not in use and filters carefully handled to ensure non-contamination by airborne particles. Sample blanks were also extracted alongside the samples to ensure non-contamination.

Statistical analysis

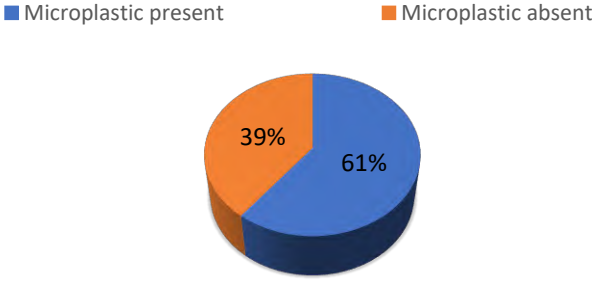
Data obtained were presented as mean ± SD while percentages and frequency of microplastic occurrence in species and entire sampled population were also performed using Prism GraphPad 5. Microsoft 2019 excel package was used for the analysis.

Species	Habitat	TL (cm)	SL (cm)	BW(g)	K	GW(g)	Number of MPs
***Clarias gariepinus* (n=10)**	**Demersal**	**38.17±2.83**	**32.00±2.40**	**416.00±98.33**	**1.27**	**12.00±3.67**	**6.3±0.64**

Results

Table 1: Morphometric data, habitat and Number of MPs of the fish samples captured from Eleyele Lake

TL= Total Length, SL= Standard Length, BW= Body Weight, K= Condition Factor, GW= Gut Weight



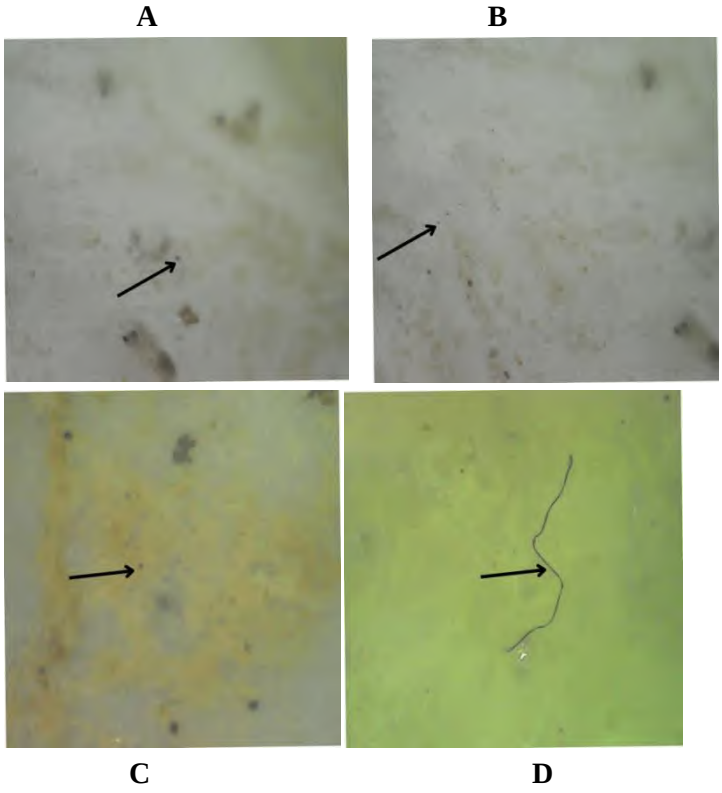


Plate 1*:* Microplastics in the stomach of sampled *Clarias gariepinus* (Magnification: x200)

Physical characteristics of Microplastic found in fish samples

Colour

The predominant colour of microplastic found in the fish species was black. Other colours include brown and white. Aquatic species' ability to absorb MPs is significantly influenced by colour.

Shapes

Shapes of microplastics identified in the GIT of the fish species were fragment, fiber, and pellet. High occurrences of fragments were recorded compared to other types of MP shape found in the fish species.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The presence of microplastics is a concern as the peaks suggest the possible presence of; Polyethylene or Polypropylene (PE/PP) (1029.37 cm^{-1} and 911.5 cm^{-1}), Polyethylene terephthalate (PET) (1630.47 cm^{-1}), Silicones or silicates (possibly from microplastics) (465.35 cm^{-1} and 529.74 cm^{-1}).

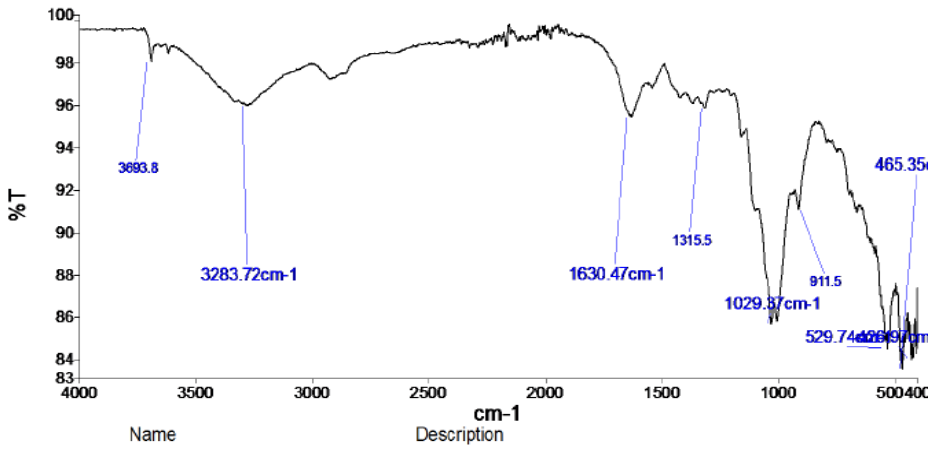


Figure 1: FTIR peaks for the gastrointestinal (GIT) content of *C. gariepinus*

Discussion

Occurrence of MPs in *C. gariepinus*

In the current study, the high number of MPs of 6.3±0.64 in fish stomach of *C. gariepinus* might be because *C. gariepinus* is an omnivore and a bottom feeder (demersal), which could allow them to absorb potential MPs from the bottom sediment. A similar pattern was noted by Merga *et al*., 2020 and Olanipekun *et al*., 2023 for the fish species *C. gariepinus*, which had the highest percentage of individuals ingesting plastics, and *O. niloticus*, had the lowest percentage. Shu *et al*., 2023 reported an average of 2.56±1.42 microplastics/individual for fish species captured from coastal waters of the Lvsi fishing ground in China. A study of ray-finned fishes from the Salento coastal seas by Trani *et al.*, 2023 revealed values between 4.6 and 5.4 MPs per individual in *Boops boops* and *Sardina pilchardus species*.

Prevalence of MPs in *C. gariepinus*

MP prevalence of 60.62% (~61%) found in *Clarias gariepinus* in this study is a potential indicator of the level, kind, and quantity of plastic pollution in the Eleyele Lake environment and could account for the high rate of MP intake by the species under study. These findings are comparable with previous reports on MP prevalence and ingestion in the gastrointestinal tracts of 8 different fish species in Eleyele lake (Adeogun *et al*., 2020) Japanese anchovies from the Tokyo Bay (Tanaka and Takada 2016); fish from the Costa Concordia wreck, at Giglio Island (Avio *et al.*, 2017), fish from Jeløya, Norway (Bour *et al*,. 2018); and sardines from the Northern Ionian sea (Digka *et al.*, 2018).

Characterization of MPs found in the gastrointestinal tracts of *C. gariepinus*

High occurrences of fragment were recorded for the fish species compared to other type of MPs shape found in the fish species. Merga *et al*., 2020 revealed fragments as the dominant shape of the plastic particles found in GITs of fishes of Lake Ziway. Fish consume plastic fragments accidentally, usually mixed with their natural prey or feed (Enyoh *et al*., 2020). Micro and nano plastic particles can be transferred to living cells through the lymphatic or circulatory system. This results in Micro Plastics' dispersion in the whole body and the induction of severe effects such as decreased feeding activity(Campos *et al*., 2021) impeded growth and development (Cormier *et al*., 2021; Naidoo and Glassom, 2019), endocrine disruption (Wang *et al*., 2019) and energy destruction (Barboza *et al*., 2018), immunity and neurotransmission disorders (Kim *et al*., 2021; Barboza *et al*., 2020) and even mortality (Naidoo and Glassom, 2019).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of gastrointestinal contents of the fish species indicate possible presence of Plastics. This is in line with what has been observed before in fish digestive tracts by Lefebvre *et al*., 2019 (1.5 and 2.3%), Zhao *et al*., 2016 (7.7%) and Obbard *et al.*, 2014 (29.2%).

Conclusion

Considering how widely distributed MPs are around the world, it is critical to implement policies that limit these pollutants. Information on the evolution of pollution that is connected with the growth of human activity is better collected through the use of modelling scenarios, life cycle inventory, periodic sampling, and monitoring bioindicators of pollution. Legislation that establishes restrictions on the production of plastic items, oversees the handling of plastic waste, and facilitates the development of strategies to move away from plastic as the primary raw material for products is thus required. Legislation pertaining to the regulation and mitigation of pollutants must also specify the maximum limitations for particulate matter (MPs) by area and/or volume, taking into account the MPs physical-chemical characteristics (size, shape, porosity, and chemical composition). The implementation of strategies that can reduce this kind of pollution include recycling, spending money on waste management infrastructure, running awareness campaigns, and developing more effective, long-term cost-benefit water treatment methods.

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ANIFS 2024
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Proceedings of the Fifth Conference of Association of Nigerian Fisheries Scientists. **"SUSTAINABLE FISHERIES AND AQUACULTURE PRODUCTION FOR ENHANCING LIVELIHOOD IN NIGERIAN ECONOMIC DEVELOPMENT"**
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