



## Correlation analysis of levels of microplastics in fish from river Niger at Onitsha

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### Abstract

This study evaluated the types and distribution of microplastics found in fish samples taken from the River Niger in Onitsha. Fish samples were collected from the [001], [002], [003], [004], and [005] sample sites and examined using FTIR and GC-FID techniques. The concentration (particles L<sup>-1</sup>) of polystyrene (<100 µm), polyester (<100 µm), polypropylene (<100 µm), styrene ethylene butylenes (<100 µm), polyethylene (<100 µm) ranged from 0.00 – 2.67 particles L<sup>-1</sup>, 0.33 – 1.33 particles L<sup>-1</sup>, 0.67 – 2.33 particles L<sup>-1</sup>, 1.00 – 2.33 particles L<sup>-1</sup>, and 0 – 1.66 particles L<sup>-1</sup>, respectively. According to the distribution of plastic types, polystyrene and polyethylene concentrations were found to be highest and lowest, respectively, in all the fish samples from the different sampling locations. The high level of polystyrene found in the fish was attributed to the presence of polycyclic aromatic hydrocarbons (PAHs) and marine debris in the river water.

**Keywords:** water, fish, microplastics, contamination

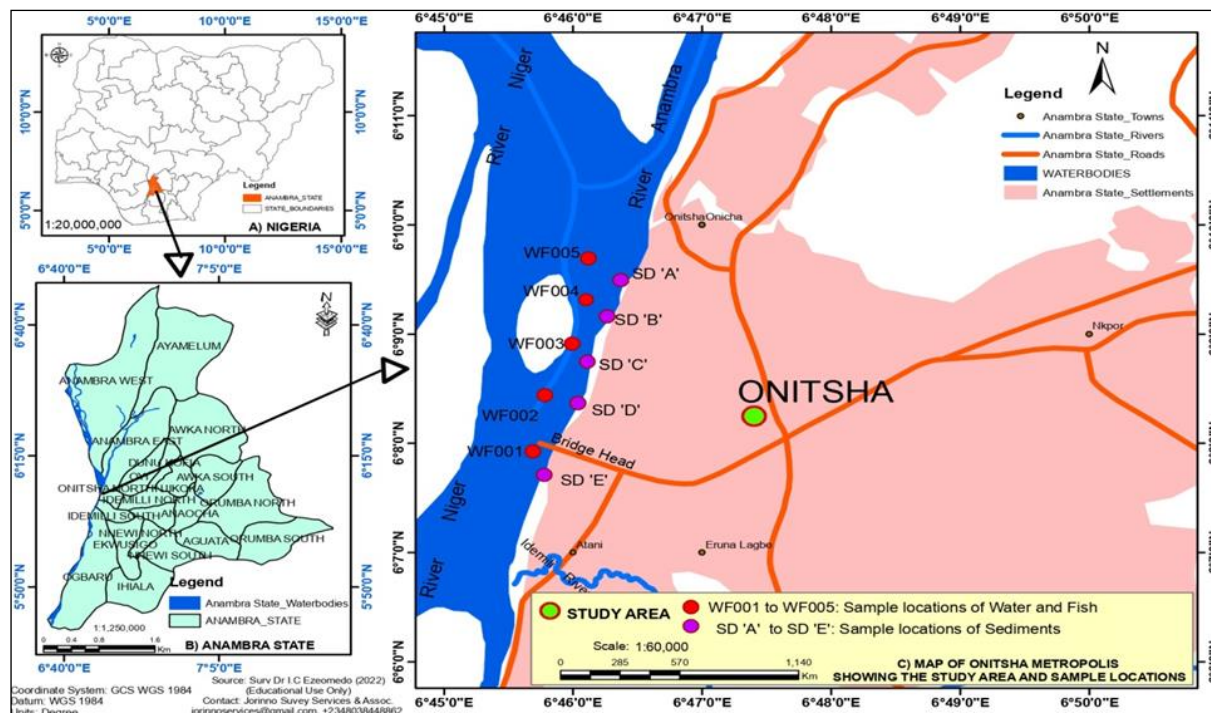
### Introduction

Fish is an important source of protein and energy for people and are infamous for bioaccumulating microplastics because of their feeding habits and continuous exposure to these pollutants in the marine environment (Worm *et al.*, 2017) <sup>[6]</sup>. Microplastic pollution in marine and environmental matrices is a growing global problem. Microplastics were developed with the intention of using them as abrasives in consumer goods and other abrasive processes, such as air blasting. Microplastics (MPs) are tiny pieces of plastic that are 5 mm in size or less. MPs are incredibly common, almost present in every environmental matrix, from the Arctic to the Antarctic seas to sediments, rivers, soil, aquatic species' tissues, and even the air we breathe (Worm *et al.*, 2017) <sup>[6]</sup>. Due to their alarming pace of accumulation in marine environments and their extreme permanence, MPs are a major reason for concern (Wilcox *et al.*, 2015) <sup>[5]</sup>. Research has shown that plastic garbage is a source and a sink for chemical contaminants (Carpenter *et al.*, 1972) <sup>[2]</sup>. In the course of manufacturing, plastics have the potential to release additives into the ocean (Andrady 2011) <sup>[1]</sup>. However, contaminants that are hydrophobic and present in the water may adhere to the plastic particles (Teuten *et al.*, 2007, Andrady, 2011) <sup>[4, 1]</sup>. Fish is a key food source for the local villages who border the River Niger's coast along the Onitsha stretch. Consequently, it is necessary to determine the degree of microplastic contamination in the fish samples that were obtained in the study region.

### Materials and Methods

#### Description of Study Area

The study area is River Niger at Onitsha stretch. The eastern Nigerian state of Anambra contains Onitsha, which is positioned between latitudes 5°22'N and 6°48'N and longitudes 6°32'W and 7°20'W. Onitsha, which is located in the Anambra State, is on the east bank of the River Niger and covers a territory of around 49,000 km<sup>2</sup>. One of the most significant business centres in sub-Saharan Africa, it serves as a vital transportation hub for Nigeria. One million individuals supposedly reside there. Onitsha's workforce, which includes businesses like trading and services, makes up about 75% of the total labor force. 25% of the workforce is made up of manufacturing and industrial jobs.



**Fig 1:** Map of Anambra State showing the sample stations in Rivers Niger at Onitsha stretch

### Sample collection

Five sample stations along the riverbank were used to collect fish samples with the help of local fishermen: [001], [002], [003], [004], and [005]. Before being transported to the lab, fish samples were collected, put in polyethylene bags, and correctly labeled. They were then maintained in the refrigerator at or below 4 °C until analysis.



**Fig 2:** Sampling Site

### FTIR Analysis of Fish Samples

For the analysis, a Buck Scientific M530 USA FTIR was employed. This device had a deuterated triglycine sulfate detector and a potassium bromide beam splitter. The spectra were obtained and altered using the Gram A1's software. About 1.0 g of samples and 0.5 mL of nujol were added, well mixed, and then placed on the salt pellet. FTIR spectra were acquired during the measurement in frequency ranges of 4,000 - 600  $\text{cm}^{-1}$  and co-added at 32 scans and 4  $\text{cm}^{-1}$  resolution. Transmitter values were used to display FTIR spectra.

### Preparation of Samples for GC-FID Analysis MPs (Soxhlet extraction method)

To remove moisture, ten grams (10 g) of the homogenized sample of fish muscles and gills were combined with sixty grams (60 g) of anhydrous sodium sulphate in an agate mortar. The homogenate was put into a 500 mL beaker, and the extraction process was carried out for 24 hrs using 300 mL of n-hexane. Using a rotating vacuum evaporator set to 40 °C, the resultant crude extract was dried off.

### Preparation of sample for GC-FID analysis

A volumetric flask with a capacity of 100 mL was filled with 1 mL of the filtered residue after it had been dissolved in 50 mL of chloroform. At room temperature, the majority of the chloroform was evaporated before adding 1 ml of the reagent (20 vol % benzene and 55 vol % methanol). For 10 minutes, the sealed mixture was heated in a water bath at 40 °C. Hexane and water were used to extract the organic sample after it had been heated, and the final ratio of the two chemicals was 1:1:1. (i.e., 1ml each of hexane and water were added to the reaction mixture). When a stable emulsion had developed after 2 minutes of vigorous hand shaking, centrifugation was used to stop the shaking. A tiny test tube was used to transfer about half of the upper hexane phase for injection. There were precautions made to guarantee that (a) just the organic layer was taken out. (b) Due to the possibility of injecting water, samples were not administered straight from the reaction vial. The GC column might be ruined by water.

### Data and Correlation Analysis

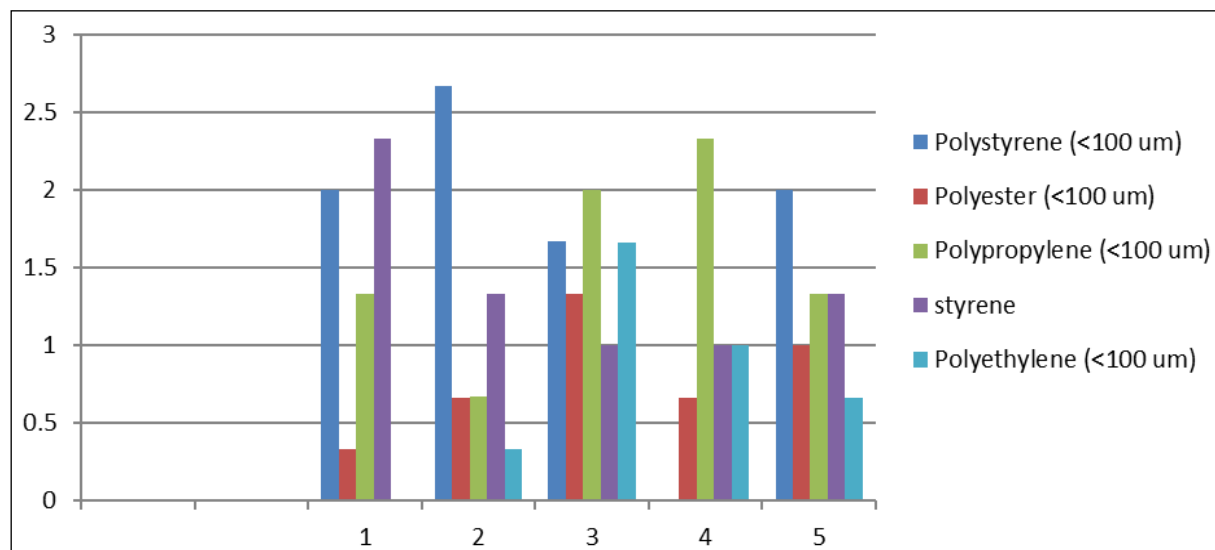
To check the equipment precision, which is the degree of similarity of results of replicate samples or an indication of the reproducibility of results of replicate samples measured under the same condition, each microplastic estimation was performed in triplicates and the results reported as mean standard deviation (David, 2000). The mean values from the triplicate results, the standard deviation, analysis of variance (ANOVA) at a value less than 0.05 ( $P < 0.05$ ) level of significance, and principal component analysis (PCA) based on the Pearson Correlation matrix analysis were all calculated using the SPSS version 20 software package.

### Results

The results of types and distribution of microplastics in fish from River Niger at Onitsha were estimated and tabulated in Table1 below.

**Table 1:** Microplastics in fish samples

Samples	Polystyrene (<100 um)	Polyester (<100 um)	Polypropylene (<100 um)	Styrene ethylene butylenes (<100 um)	Polyethylene (<100 um)
001	2	0.33	1.33	2.33	0
002	2.67	0.66	0.67	1.33	0.33
003	1.67	1.33	2	1	1.66
004	0	0.66	2.33	1	1
005	2	1	1.33	1.33	0.66



**Fig 3:** Microplastics in fish

### Discussion

#### Microplastic distribution

The quantity (particles  $L^{-1}$ ) of polystyrene (<100 um), polyester (<100 um), polypropylene (<100 um), styrene ethylene butylenes (<100 um), polyethylene (<100 um) in the fish samples ranged from 0.00 – 2.67 particles  $L^{-1}$ , 0.33 – 1.33 particles  $L^{-1}$ , 0.67 – 2.33 particles  $L^{-1}$ , 1.00 – 2.33 particles  $L^{-1}$ , and 0 – 1.66 particles  $L^{-1}$ , respectively. Polystyrene and polyethylene concentrations were discovered to be the highest and lowest, respectively, in all the water sampling areas, according to the distribution of plastic types. The presence of PAHs and marine debris in the river water was blamed for the high amount of polystyrene (Xu 2018; Enyoh *et al.*, 2019) [7, 3].

**Table 2:** Pearson's correlation coefficient for Microplastics in Fish

	Polystyrene	Polyester	Polypropylene	styrene ethylene butylene	Polyethylene
Polystyrene	1				
Polyester	0.003054	1			
Polypropylene	-0.89716	0.315311	1		
styrene ethylene butylene	0.403034	-0.71703	-0.43742	1	
Polyethylene	-0.43552	0.861958	0.697936	-0.80552	1

### Pearson's Correlation Analysis for Microplastics in Fish

Microplastics in the fish samples were analyzed for association, and the findings (Table 1) revealed a strong and positive correlation between polyethylene/polyester ( $r=0.861958$  at  $p>0.05$ ), polyethylene/polypropylene ( $r=0.697936$  at  $p>0.05$ ), polyethylene/ styrene ethylene butylene ( $r=0.80552$  at  $p>0.05$ ). However, a strong and negative correlation was observed between polypropylene/polystyrene ( $r= -0.89716$  at  $p>0.05$ ), styrene ethylene butylenes/polyester ( $r= -0.71703$  at  $p>0.05$ ), polyethylene/styrene ethylene butylenes ( $r= -0.80552$  at  $p>0.05$ ). It is important to highlight that the tight link between the various forms of microplastics suggests that their origins are similar. A strong and positive correlation (0.50 – 0.99) between two variables indicates that an increase in one variable will lead to an increase in the other, while a strong and negative/inverse correlation indicates that an increase in one variable will lead to a decrease in the other. A weak and negative correlation between two variables shows that an increase in one variable causes the other to decrease, but in a weaker way. Similarly, a weak and positive correlation (0.10 – 0.49) between two variables shows that while both variables tend to go up in response to one another, the relationship is not strong. A weak and negative correlation between two variables shows that an increase in one variable causes the other to decrease, but in a shaky or unpredictable way. Similarly, a weak and positive correlation (0.10 – 0.49) between two variables shows that while both variables tend to go up in response to one another, the relationship is not strong.

### Conclusion

The extent of the microplastics buildup in the fish in the River Niger at Onitsha, as well as the relationships between the parameters evaluated, has been made public by this study. According to the distribution of plastic types, polystyrene and polyethylene concentrations were found to be highest and lowest, respectively, in all the water sampling areas. The high level of polystyrene was attributed to PAHs and marine debris in the river water. Correlation analysis revealed that most of the microplastics found came from sources that were primarily of comparable origin. Additionally, this study linked the development of microplastic contamination to the unchecked disposal of household and industrial garbage into the water body and its sediment. It is interesting that the microplastic concentrations found in this study are substantially lower than those found in previous studies. In conclusion, this study's findings suggest that statistical approaches can be a powerful tool for gauging the amount of microplastics accumulating in fish and other aquatic matrices as well as for forecasting the threat of future human activity-related water pollution.

### Acknowledgments

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