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ABUNDANCE AND SEASONAL VARIATION OF MICROPLASTICS DETECTED IN EDIBLE FISH SOLD IN LAGOS STATE, NIGERIA

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ABSTRACT: *The global incidence of microplastics pollution is raising safety concerns on the consumption of seafood by humans. This study investigated the seasonal variation of microplastics detected in fish sold for human consumption in Lagos State, Nigeria. A total of 150 fish samples consisting of 3 commercially important fish species, namely: Catfish (Clarias gariepinus) n=25, Red Snapper (Lutjanus campechanus) n=25 and Tilapia (Oreochromis niloticus) n=25 sold for human consumption were purchased from fishermen from 3 sites in Lagos State during the dry season from 22nd January to 7th February 2020 and during the rainy season from 13th to 25th July, 2020. The stomach contents of the fish were analyzed for the presence and characterization of microplastics. The identified microplastics were categorized according to their abundance, sizes, shapes, types, and colours using Dissecting Microscopes and FTIR. Microplastics particles were detected in all samples studied from all locations in both seasons, with the highest level of microplastics abundance detected during the rainy seasons. Highest microplastics abundance was detected in catfish (4.68 microplastics particles per individuals) during the rainy season in Badagry and 5-Cowries Creeks and the least detected in Red Snapper (0.72 microplastics particles per individuals) in Epe and Badagry during the dry season. Majority of the detected microplastics were fiber-shaped, followed by fragments. The least microplastics shape was foam. The most common color of microplastics during both the wet and dry seasons was grey/white (25.0%) while pink (2%) was the least dominant colour. The size of the ingested microplastics ranged from 2.03 mm to 4.86mm during the dry season while the particle sizes found in the species during the rainy season ranged from 1.49mm to 4.95mm. The results of FTIR-ATR analysis indicated that polyethylene (PE) was the most abundant in both seasons. The findings from this study raises concerns on the implication of microplastics on food safety.*

KEYWORDS: Seafood; Microplastics; Health implication; FTIR-ATR; Polyethylene; SEM-EDX.

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INTRODUCTION

Seafood, including fish, has always been consumed as a good source of protein in many diets around the world, especially in coastal areas but due to research linking fish consumption to numerous health benefits, the number of the populace replacing red meat and other protein sources with fish is on the increase. Fish consumption has gradually increased throughout the world as an important part of the human diet and represents 16.7% of the global population's animal protein intake (FAO, 2010).

Fish has a lot of nutritional benefits and is an important source of protein around the world, being essential for people of all ages, children, adults, the elderly and pregnant women. They contain high quality proteins, highly digestible energy, and omega-3 polyunsaturated fatty acids (PUFAs) as well as other essential nutrients needed for the normal functioning of the human body and has been shown to decrease triglyceride levels and the risk of cardiovascular diseases, such as arrhythmias (abnormal heartbeats), hypertension, arteriosclerosis, strokes and other heart-related diseases (World Health Organization, 2000; Zheng *et al.*, 2012). Amiengheme (2005) asserts that nutrient from fish is superior to all terrestrial meats such as beef, mutton, pork, and chicken, being a rich source of high-quality animal protein.

Plastics are produced using resins (organic monomers) as a base (Geyer *et al.*, 2017), followed by the addition of additives, such as plasticizers, ultraviolet (UV) and thermal stabilisers, dyes, colouring agents and flame retardants to improve the durability and aesthetics of the plastic (Geyer *et al.*, 2017; Law, 2017) or to reduce cost (Ikpe *et al*., 2017). The malleable nature of plastics allows them to be molded into different shapes and sizes, resulting in their different applications (Law, 2017; Geyer *et al.*, 2017). The global increase in the production of plastic is reflected in its wide use (Law, 2017; GESAMP, 2015).

Microplastics are associated with a cocktail of hazardous pollutants (Lithner *et al.*, 2011), such as toxic metals, styrene, phthalates, bisphenol A (BPA), polychlorinated biphenyls (PCB), and polycyclic aromatic hydrocarbons (PAHs) (Ashton *et al.*, 2010; Holmes *et al.*, 2012; Oliveira *et al.*, 2013; Rochman *et al.*, 2014; Massos & Turner, 2017; Barboza *et al.*, 2018) which may transfer to animals upon ingestion (Browne *et al.*, 2013; Chua *et al.*, 2014).

Plastics accumulate in the environment through many sources, the major source being from land, particularly in the form of packaging materials (Derraik, 2002). Plastics are partially degraded in the marine environment by the combined forces of biotic and abiotic factors to give rise to microplastics. These microplastics are more prone to pollution due to their small surface areas. The National Oceanic and Atmospheric Administration (NOAA) defined microplastics as particles less than 5mm in size (Wright *et al*., 2013; Hidalgo-Ruz *et al.*, 2012). Chemical additives are added during the production of plastics and they adsorb or absorb persistent, bioaccumulative and toxic contaminants (PBTs) from the environment and can be leached to the surrounding aquatic environment. The ingestion and bioaccumulation of PBTS from microplastics by fish is a global concern to food safety and human health.

There has been serious global environmental concern on the widespread occurrence of microplastics in oceans, seawater, and biota (Woodall *et al.*, 2014; Su *et al.*, 2016). The detection of microplastics in nearly every matrix of the environment, coupled with the toxicity of its associated contaminants, has led to an increasing concern of its impact on human health (Seltenrich, 2015), through the consumption of microplastics-contaminated fish.

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MATERIALS AND METHODS

Study Area

The study was carried out in selected water-bodies in the coastal areas of Lagos State, Nigeria where fish are harvested and sold for human consumption. Lagos State is a cosmopolitan city in the Southwestern part of Nigeria with an area of 3,577 sq. km landmass and about 22% of it occupied by lagoons and creeks (Enaikele, 2007). The sampling locations selected randomly from each of the 3 senatorial districts of the state include Badagry Creek, Epe Lagoon, and 5-Cowries Creek.

Sample Collection

A total of 150 seafood samples, comprising 3 species, namely: catfish (*Clarias gariepinus*) n=25, tilapia (*Oreochromis niloticus) n=25* and red snapper (*Lutjanus campechanus*) n=25 sold for human consumption, were purchased from the fishermen in the fishing ports in Lagos State between 22nd January – 7th February, 2020 (dry season sampling) and 13th – 25th July, 2020 (rainy season sampling) to measure the seasonal variation in the abundance of microplastics in them. The sampling gears used by these fishermen for catching the fishes include baited long lines, fishing baskets and gill nets. The biota samples were counted per family, washed with deionised water to remove any trace of microplastics that could adhere to the body, and wrapped individually in aluminum foil. Each sample was then labeled using indelible ink, preserved in ice-packed coolers, and immediately transferred to the laboratory where they were frozen. They were later thawed, and the total length of each species was measured in centimeter from the snout tip to the extended caudal fin. The species identifications of each species were provided by the fisherman and confirmed using the Food and Agricultural Organisation species identification sheet for fishery purposes (FAO, 1990).

Microplastics Extraction, Detection, and Characterization

The preserved samples were brought out from the freezer in the laboratory and allowed to thaw in preparation for microplastics analysis. The fishes were dissected, and the gastrointestinal tract removed and pooled together by species. They were weighed in a weighing scale (Ohaus) and transferred into conical flasks covered with aluminum foil. All the equipment used for the dissection was carefully cleaned with deionised water every time a fish was dissected to avoid possible contamination. Microplastics were isolated from the sample matrix by digestion using 10% KOH according to the digestion protocol by Foekema *et al.* (2013). A concentrated saline solution (1.2 g/mL, NaCl) was used to separate the microplastics and then filtered using a vacuum filtration apparatus system onto Whatman glass fiber filters (~1μm pore size). Distilled water was added to enhance filtration performance. The filters were stored in covered Petri dishes individually and dried in an oven at 40ºC for 24hrs. The microplastic content of each sample was examined under an Olympus stereomicroscope (x40 magnification) on which an Olympus UC30 digital camera is attached, for photographing (Hidalgo-Ruz *et al.*, 2012; Masura *et al.,* 2015). Microplastic particles were identified by abundance (particles individual^{-1}) and categorized by shape (film, fiber, fragment, foam, pellet), color (transparent, blue, red, black, green, orange, yellow, and purple), and size. The identifiable microplastics from the samples under observation were removed using tweezers. All the microplastics in the sample were removed in this way and counted.

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The microplastics particles identified were later placed into labeled 4 ml screw cap glass vials using thin forceps according to their sizes and then sealed and stored for preservation for further verification using FTIR and SEM-EDX.

Identification and FTIR and SEM Verification of Samples

Polymer identification of the microplastics particles was carried out using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) with an Agilent 5500a ATR-FTIR instrument by comparing the spectrum obtained with a self-generated polymer library in the Agilent **MicroLab** software.

The ATR takes advantage of the physical properties of light when encountering two materials with differences in the index of refraction. Spectra were collected in absorbance mode in the range 4000–700 nm and Polymer identification was verified on a factor threshold of 0.70 against polymer libraries (Synthetic polymers ATR-library).

Analysis of polyethylene (PE) and polystyrene (PS) reference materials before each batch was done as a quality control procedure.

Quality Assurance/Control

Precautions taken to reduce potential external microplastics contamination of the sample includes thorough washing of glass wares, filters, dissecting sets and all equipment used in the analysis, and rinsing them with distilled water. Cotton laboratory coats and cleaned latex gloves were worn during the analysis. All the surfaces were cleaned, and plastic materials were completely avoided. All the reagents used were analytical grades and a wet filter paper was placed near the working table throughout the analysis and then observed for external microplastics contamination. Laboratory blanks composed of cleaned petri dishes filled with filtered deionised water were used in each laboratory step for every sample batch to detect procedural contamination.

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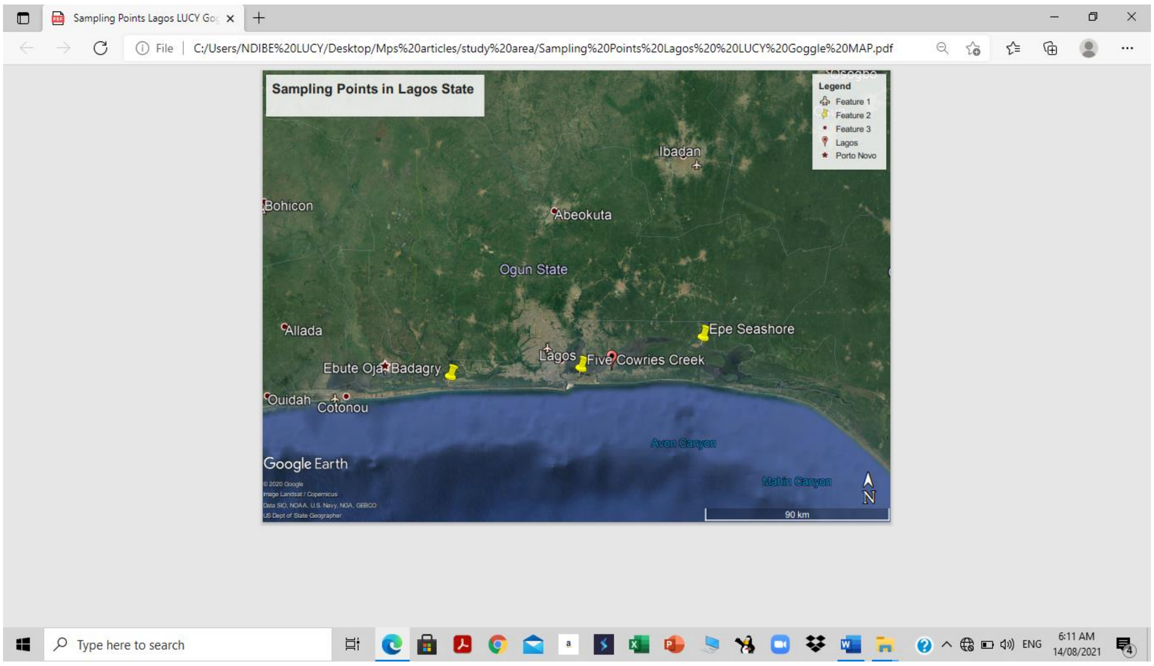

Figure 1: Sampling Locations

Statistical Analysis

All data analysis were done using SPSS 28.0. with 0.05 significance level. All the data in the graph and table were displayed using descriptive statistical analysis. The Shapiro-Wilk test was used to test data for Normality and all showed non-normal distribution except Badagry Creek Dry season. Non-parametric test was used for further analysis. The relationship between the wet and dry season microplastics abundance for each site was assessed using linear regression.

RESULT AND DISCUSSION

Microplastics Abundance

A total of 150 commercial fishes belonging to 3 species were sampled during the wet and dry seasons to determine the seasonal variation in the microplastics ingested by them. Microplastics abundance was determined per individual of the sample. Microplastics were detected in all the fish species sampled in the two seasons. The highest microplastics occurrence was however observed in the wet season with the highest occurrence recorded in catfish during the rainy season (4.68 microplastics particles per individual) in both Badagry Creek and 5-Cowries Creek. This was followed by red snapper (2.8 microplastics per individual) in Badagry Creek and the least was recorded in tilapia (0.64 microplastics particles per individual) in Epe Lagoon. In the dry season, the highest microplastics contaminated species was same catfish (3.48 microplastics particles per individual) in 5-Cowries Creek, followed by tilapia (0.72 microplastics particles per individual) in Epe Lagoon, and the least contaminated species during the dry season was red-snapper (0.72 microplastics particles per individual) as recorded in Epe Lagoon and Badagry Creek.

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The mean abundance of microplastics particles during the rainy seasons has a significant effect on the abundance of microplastics in dry seasons. The mean microplastics abundance of Lagos Badagry Creek, Epe Creek and 5-Cowries Creek during the rainy season has 59%, 96% and 94% significant effect respectively on the abundance of microplastics in the wet season. During both seasons, the abundance of microplastics particles were highest in Badagry Creek, and least in Epe Lagoon for the two seasons. This could be attributed to the economic activities going on in these two locations.

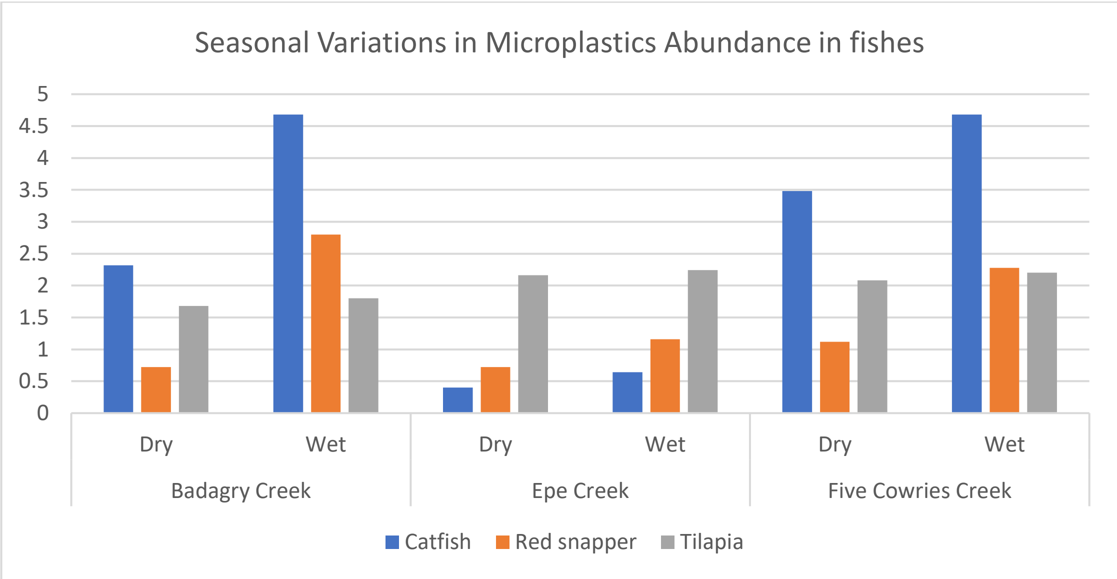

Figure 2: Microplastic Abundance in the Fish Species

The detection of higher microplastics abundance in the rainy season could be attributed to run offs after rainfall of plastics and plastic debris from floods through drains and municipal waste dumps in Lagos metropolis. Andrady (2011) reported that the runoff after rainfall on land, where most plastic productions are used, could deliver these microplastics into the lake. This report is consistent with former reports that storm water is a considerable source of microplastics (Moore *et al.*, 2002; Dikareva & Simon, 2019).

Microplastic Shapes Detected in Examined Species

Fibers, fragments, sheets, beads, foams, and pellets were recognized as distinct shapes of microplastics (Hidalgo-Ruz *et al.*, 2012). The most abundant microplastics type recorded in all 3 fish species for all locations and both seasons was fiber. This is consistent with the results of previous studies (Bellas *et al.*, 2016; Guven *et al.*, 2017; Baalkhuyur *et al.*, 2018). The exception was in the Epe dry season where beads were recorded as the most abundant shape while the least abundant shape recorded was foam.

This highest recorded shape of fibers occurred mainly in catfish as seen in Badagry Creek (18.00±1.00 dry season and 27.33±1.52 rainy season), and 5-Cowries Creek (28.00±2.00 dry season and 37.00±1.00 rainy season). Fragments followed as the second dominant shapes as seen in Badagry Creek (12.33±0.57 dry season and 24.00±1.00 rainy season), Epe Lagoon

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(10.00±1.00 both dry and rainy seasons), 5-Cowries Creek (18.00±1.00 dry season and 24.00±1.00 rainy season). This was consistent with previous studies carried out by Compa *et al*. (2018). The type of ingested microplastics was found to be related to the feeding behaviors of examined fish. Omnivorous fishes often ingested fibers, while fragments were mostly ingested by benthic organisms (Markic *et al.*, 2018). The least microplastics shape was recorded in pellets (O.66±0.57) in tilapia during the rainy season while no foam was recorded in red snapper in the dry season.

Microplastics Type Distribution in the Investigated Species

Analysis of polymer types found in the microplastics particles using FTIR-ATR revealed the presence of PET, PC, PE, PP, ABS, PMMA, Nylon (PA), PU PVC, PFTE, and others. The identified polymers are the ones which make up the common plastics commonly in use in different parts of Lagos and in other parts of the world (Browne *et al.*, 2011). PE was the most abundant polymer in the assayed fish samples. The FTIR-ATR shows OH-stretch which represents compounds such as -OH which are free hydroxyl groups of alcohol and also -COOH free carboxyl groups, indicating the presence of PP, ABS, PETE, HDPE, LDPE, PMMA, etc. with the lower wavenumbers (from 2922.1) indicating absorption bands attributed to C-H aliphatic or aldehyde stretching modes, showing the existence of alkyl and aldehyde which are regular polymer chains found in Nylon and PU, LATEX which are common components of plastics polymers. The C-H stretching vibrations observed at ν=2922.2 and 2855.1.5cm^{-1} are characteristics of PP, HDPE, LDPE PP and ABS. The absorption band at 1740.7 and 1162.9,1032.5 and 1092.1cm^{-1} may be attributed to C-O stretch of esters characteristics of microplastics like PETE, EVA, Carboxylic acid, Nylon, PC, PMMA, PP, PU, PC. All these attest to the presence of diverse polymers in the microplastics ingested by the biota species.

Microplastics Distribution by Colour in the Fish Species

The most abundant colours detected across all the species in all location for the rainy and dry season were white/grey colours as seen in Badagry Creek (20% dry season and 18% rainy season), Epe Lagoon (21% dry season and 20% rainy season) and 5-Cowries Creek (25% dry season and 21% rainy season) with occurrence more in the dry season than the rainy season. Colourless colours followed with almost the same quantity (Badagry Creek 16% both dry and rainy season), Epe Lagoon (14% each for the dry and rainy season) and 5-Cowries Creek (13% dry season and 19% rainy season). Black was also significant in the colour distribution of the microplastics in all the species for all locations and seasons as seen in Badagry Creek (13% dry season, 14% for the rainy season), Epe Lagoon (15% dry season and 14% rainy season), and 5-Cowries Creek (18% dry season and 19% rainy season). Pink colour appears to be the least distributed among the microplastics detected in all species at all locations for the two seasons as seen in Badagry Creek (3% dry season and 5% rainy season), Epe Lagoon (0% for both dry and reason seasons), and 5-Cowries Creek (2% dry season and 4% rainy season).

Similar colours were observed in other previous studies (Alomar *et al*., 2017; Digka *et al.*, 2018; Compa *et al.*, 2018). The colours of white, transparent, and blue are likened to the colours of plankton (Boerger *et al.,* 2010). The colour of microplastics particles in the environment increases the potential of ingestion because of the similarity to natural prey. According to Ory *et al.* (2018b), blue microplastics were similar to colour of copepods in the South Pacific Ocean while Boerger *et al.* (2010) and Wright *et al*. (2013) reported that

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microplastics with white, blue, and clear colours resemble the colour of planktons as fishes potentially consume them.

Size Distribution of Ingested Microplastics

The size distributions of the ingested microplastics detected in all samples were significantly different among different species in both the dry and wet season. The size of the ingested microplastics ranges from 2.03 mm to 4.86 mm during the dry season while the particle sizes found in the species during the rainy season range from 1.49 mm to 4.95 mm as can be seen in the table below. Red-Snapper seems to have a preference for large sized microplastic particles and Tilapia prefers smaller-sized microplastic particles while catfish is in-between the two.

Sampling Location	Species	Dry Season Size Range	Rainy Season Size Range
Badagry Creek	Catfish	2.67-3.19	1.49-4.22
	Red-Snapper	3.46-4.58	3.88-4.02
	Tilapia	2.76-3.45	2.78-4.95
Epe Lagoon	Catfish	3.29-4.86	3.46-4.65
	Red-Snapper	3.46-4.59	3.66-4.26
	Tilapia	3.45-4.22	3.25-4.10
5-Cowries Creek	Catfish	3.26-4.86	3.50-4.34
	Red-Snapper	3.46-4.45	3.88-4.02
	Tilapia	2.03-2.45	2.55-2.65

CONCLUSION

The contamination of seafood by microplastics is an increasing environmental concern due to the ability of microplastics to act as vectors of pathogens and other environmental contaminants of concern. This is in addition to the potential leaching of monomers and additives from them on degradation. These contaminants can bioaccumulate and biomagnify along the food chain posing great risks to humans on their consumption. This research work demonstrated the occurrence of microplastics in commercial fish in Lagos State, Nigeria. The results showed high microplastics abundance of the 3 species of fish under investigation, with greater abundance during the rainy season.

This result of this study is a clarion call to all stakeholders in plastic waste management of the potential effect it will have on edible fish if not properly monitored. There is a greater need for microplastics monitoring by regulatory bodies to prevent microplastics from coming back to haunt us in our plates of food. It calls for a change in the management of plastic waste as an increasing number of fish are being consumed globally due to numerous studies linking its consumption to the overall well-being of the human body.

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