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Assessment of microplastic-contaminated liver through gene expression profiling of four commercial fish species in the Lagos Lagoon, Nigeria

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ABSTRACT

Microplastic (MP) occurrence in the Lagos Lagoon has been on a progressive increase in recent years. The potential impacts and hazards of MPs on commercially available fish species from the Lagoon have been understudied. This study aims to assess molecular damage due to microplastic accumulation in the liver of four commercial fish species (*Oreochromis niloticus, Chrysichthys nigrodigitatus, Clarias gariepinus,* and *Gymnarchus niloticus*) sourced directly from the Lagoon. The results revealed that MP load in the liver of the fish species ranged between 7.2 ± 1.9 and 9.5 ± 4.4 particles/individual, with *G. niloticus* recording the maximum concentration. The observed extracted MPs are predominantly black and blue fibres while the Fourier Transform Infrared spectroscopy revealed polyethylene dominance in the plastic polymers. Relative gene expression results revealed statistically significant downregulation of the CYP 1a gene and upregulation of the HSP 70 gene in all the microplastic-laden liver samples except in *G. niloticus*. The findings from this study suggest that microplastic contamination in fish tissues may exacerbate cellular toxicity in commercially available biota in the Lagos Lagoon, thereby calling for urgent measures to curb microplastic and ultimately plastic pollution. However, there's a need for in-depth research on the effects of environmentally relevant microplastics on edible fish tissues to further elucidate their molecular impact.

Introduction

Global plastic production has been on a steady rise from 370.5 million tonnes in 2018 to 400.3 million tonnes in 2022 [1], 9% of which originating from Africa. In Nigeria, the estimated annual generation and release of marine plastic pollution has consequently increased from 2.5 million tonnes in 2019 to 3.2 million tonnes in 2022 [2], placing the country 9th as regards contribution to global plastic pollution. Low circular economy of post-consumption plastics at less than 9% [1] as well as rarely enforced environmental protection laws are contributing factors to increased accumulation of plastic waste in the soil and eventually drained into the aquatic systems. Plastic waste is a progressive source of concern in the aquatic as well as terrestrial environments due to improper waste disposal, hence its management requires great efforts to document its accumulation in the environment and biota, while highlighting potential harm to human health [3]. More particularly, there has been a dearth of information on plastic waste accumulation and

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Fig. 1. Map of the Lagos Lagoon showing the sample sites.

Fig. 2. Photographs showing the Lagos Lagoon and the sampling sites.

subsequent consequences in regions such as West Africa. These plastic debris are continuously degraded into smaller particles termed as 'microplastics', by either physicochemical or biological processes in the environment [4] (Omoregie, Osagie and Oritseweyinmi, 2023). Microplastics (MPs) are tiny plastic particles that range between 1 – 1000 μm in size and are discharged into the atmospheric, terrestrial, and aquatic environments either directly or indirectly [5]. MPs can be released directly into the environment through industrial and manufacturing processes that utilize microspheres and microbeads for manufacturing personal care products, or indirectly as a result of abrasion, weathering, and fragmentation of environmental macro-plastic debris [6]. Beyond the ubiquitous environmental presence of MPs are hazards related to their size, composition, and latent ability to adsorb and desorb chemicals, therefore serving as contaminant-vectors for persistent organic pollutants, endocrine-disrupting chemicals and consortiums of other environmental toxicants in aquatic systems [7]. Hence, the presence and potential effects of MPs on the ecosystem raise concerns for food safety, environmental and public health.

Increasing reports on the ingestion of MPs in different soil biota, plants from contaminated soil and aquatic organisms in their natural environments have raised global scientific concerns regarding their latent ecological impacts [8]. Terrestrial biota such as snail, soil collembolans and earthworms, as well as aquatic biota such as sea turtles, mussels, crustaceans and fish have documented reports of MP ingestion [9,10]. More daunting is the reported presence of MPs in the human placenta and meconium in Italy [11]. Although physiological impacts have been documented upon laboratory exposure of MPs to several aquatic biota, reports of their effects on wildly sourced biota remains sparse. For instance oxidative stress and neurotoxicity in *Clarias gariepinus* [12], oxidative stress in *Tubifex tubifex* [13], oxidative stress and growth alterations in *Neomysis awatschensis* [14], equilibrium loss, abnormal swimming and growth in *Oreochromis niloticus* [15], disruption of biochemical homeostasis and altered gene expression in *Cyprinus carpio* [16], and effects on hematological parameters and alterations in antioxidant enzymes in *Pseudobagrus fulvidraco* [17], have been documented. In addition to the directs effects of MPs is their capacity to leach inherent and environmentally-sorbed toxicants as well as serving as vectors for biofilms and trace metals in the food chain [3]. Thus, the concentration and potential impacts of MPs on edible fish species should be assessed to protect wild aquatic species and the public.

The presence and load of MPs have been reported in African aquatic systems and biota. MP concentrations and risk assessment in the water, sediment and aquatic insects of the Nile River have been highlighted by Khedre et al. (2024) with their findings suggesting inter-trophic level MP transmission and possible harm to different aquatic organisms. Microplastic abundance have been reported in the surface water and sediment of Lagos Lagoon and Osun river [2,18,19] as well as in fish species [2,20]. However, studies highlighting subsequent potential impacts on commercially sourced biota from the Lagoon are lacking. Although the impacts of MP ingestion on oxidative stress biomarkers of wild fish species have been previously reported, potential effects on the level of molecular damage and cellular stress are yet to be understood. Furthermore, the adverse effects of MP ingestion transcends impacts on fish species but poses health risks due to human exposure through consumption of microplastic-laden edible fish species [21,22].

Lagos Lagoon is a coastal wetland that borders Southern Nigeria's most populated and largest metropolitan agglomeration. The city of Lagos has had significant economic expansion in addition to a quickly expanding population, which has contributed to a rise in both residential garbage creation and consumption [23]. Poor waste management techniques exacerbate this issue by allowing plastic waste and other debris to enter the aquatic environment of the metropolis. Lagos Lagoon is a socio-economically significant body of water that offers various amenities to some Lagos residents, including source of livelihood and recreation, residential and industrial waste dump, as well as a natural buffer to balance forces within the ecosystem [23]. The Lagos Lagoon is home to important commercial centers for the retail of a variety of seafood and aquatic species which are intentionally harvested for retail and direct consumption purposes. These centers serve as some of the state's most important retail locations for aquatic animal species, catering to the state's population of nearly 20 million. As MPs along with inherent chemicals and environmental toxicants pose prospective threats to biota, ecological and human health, there's a growing need to assess MP pollution in the Lagos Lagoon biota and assess potential effects, moreso at molecular level. Hence, the aim of this study was to assess the effects of MPs presence in the liver of four commercial fish species (*Oreochromis niloticus, Chrysichthys nigrodigitatus, Clarias gariepinus* and *Gymnarchus niloticus*) sourced from the Lagos Lagoon through their relative gene expression profile using two target genes: cytochrome p450 and heat shock protein 70 with β-actin serving as the housekeeping gene. However, this study is apparently the first extensive investigation of the assessment and potential impact of MPs on commercially available fish species in the Lagos Lagoon. Consequently, findings from this study will help policymakers and regulators implement strategies to enforce plastic-use regulation and plastic waste minimization in the Lagos Lagoon.

Materials and methods

Study site

The study site is the Lagos Lagoon, a stretch of coastal wetland within the South-western region of Nigeria, on the skirt of the Atlantic Ocean. The main body of the lagoon complex which serve as the study site is located between longitude 3̊ 24'01.49″E and 6̊ 23'49.69″N (Fig. 1). The Lagos Lagoon complex is in the hub of Lagos, Nigeria's major commercial center, that stretched from Cotonou in the Republic of Benin and extends to the fringes of the Niger Delta along its 257 km course. At over 50 km in length and 3 – 13 km width, the Lagos Lagoon has several inland water systems and the Badagry creek with Benin connected in the west and the Lekki Lagoon in the east. It is an expanse of shallow water which in most areas is between 0.3 – 3 m with a maximum of about 5 m in the main lagoon and 25 m in some dredged parts of the Lagos harbor. The Lagos Lagoon is an economically important water system that serves as a transport system, source of livelihood (including fishery resource) and recreation for residing communities (Fig. 2). Four of the largest fishing and retailing communities along the Lagoon were sampled from Badagry, Sagbo Koji, Makoko and Epe (LBS, 2017). Two of the sampling stations are located around the center of the Lagoon, while the remaining two stations lie to the east and west.

Sample collection and preparation

The present study investigated a total of 32 specimens of the Nile tilapia (*Oreochromis niloticus*), the silver catfish (*Chrysichthys nigrodigitatus*), the African catfish (*Clarias gariepinus*), and the African knifefish (*Gymnarchus niloticus*) were procured from local fishermen on the Lagos Lagoon, Nigeria, between June and November 2022. Eight samples of each species were preserved live and transported to the laboratory. Upon reaching the laboratory, the specimens were pithed, and the total body length (cm) and weight (g) of each sample were determined. Subsequently, the liver tissues were excised and preserved in DNA/RNA shield solution (Zymo Research, USA). These commercial fish species were selected based on their appreciation as relished food by humans and availability all year round. The mean ± standard deviation (SD) of fish total body weight and length were, respectively: 244.1 ± 48.2 g and 25.3 ± 1.6 cm for *O. niloticus*; 423.4 ± 91.8 g and 35.1 ± 5 cm for *C. nigrodigitatus*; 477.9 ± 35 g and 38.4 ± 8.4 cm for *C. gariepinus*; and 337.4 ± 79.4 g and 45.9 cm for *G. niloticus*. Subsequently, microplastics were isolated from the liver and gene expression was determined.

Microplastic isolation, visual characterization, and identification

Approximately three times the volume of 10% KOH (potassium hydroxide) for each tissue was added to each liver sample meant for tissue digestion in conical flasks. They were placed in an incubator, set at 40 °C for 72 h [24] for organic material digestion without compromising the integrity of the plastic polymer. After incubation, the resultant liquid digestate was vacuum strained through cellulose filter membranes (pore size 0.45 μm, Millipore, USA). Sealed in glass Petri dishes, the filters were oven-dried at 30 °C for 24 h (Drying oven Genlab, UK). Subsequently, the dried membrane filters were analyzed and photographed in a stereomicroscope microscope (Olympus SZ61, USA) with a digital camera, giving up to 4,000X magnification. Microplastic identification was carried out based on procedural guideline recommendation by Mariano et al. [25]. All the tentative plastic items recovered from the samples were sorted and quantified by color (black, blue, transparent, red, and green) and shape (fibres and fragments). The number of tentative microplastics in the liver was expressed as the number of microplastic particles per fish (MP particles/fish).

Polymer identification was performed on all the microplastics extracted from the liver of the fish using a Fourier Transform Infrared spectrometer (Agilent Technologies, Germany) linked with a universal attenuated total reflectance accessory (ATR-FTIR). Each query spectrum was collected in 46 scans with a resolution of 8 cm^{-1}, in the wavenumber range of 4000 – 650 cm^{-1}. The obtained query spectra are presented without corrections or any further alterations. Spectra were analyzed using OpenSpecy software, and compared with a database of accepted references with a correlation at $r \geq 0.7$ [26].

Quality control

The liver tissues were processed and analyzed in an environment with limited access and previously decontaminated to avoid microplastic cross-contamination. Pre-washed cotton laboratory garments and disposable gloves were worn throughout the analysis. All working surfaces and dissecting equipment were thoroughly cleaned with 70% ethanol before and between sample-use to avoid cross-contamination. The external sections of the fish were thoroughly washed with distilled water and ethanol to remove any potentially adhered plastic particles. In all procedures, glass and stainless-steel wares were utilized to avoid contamination from the equipment. Procedural blanks were utilized and analyzed for atmospheric microplastic contamination that might have occurred despite taking careful precautions.

Gene expression analysis

The liver of the selected commercial fish species was analyzed for the expression of selected genes to perform real-time quantitative polymerase chain reaction (RT-qPCR). The tissue samples were preserved in RNA shield solution (Zymo Research, USA), kept at room temperature according to manufacturer's protocol and then temporarily stored at −80 °C until RNA extraction. Procedures for gene expression analysis and selected primers was according to Nataraj et al. [27]. The primers (Integrated DNA technologies, USA) used include β-actin-F: 5'-ATGGATGAGGAAATCGCTGCC-3': β-actin-R: 5'-CTCCCTGATGTCTGGGTCGTC-3'; HSP70-F: 5'- GCACAA-GAAGGACATCAGTCAGA −3': HSP70-R: 5'-CGATGCCCTCGTACAGAGAGT-3'; CYP1a-F: 5'-GCAGGGAAAGAGTCCCAAATA-3' and CYP1a-R: 5'-GCTTCCATCGGCTTTCATAAC-3'.

Total RNA was extracted using the TRIzol reagent (RNA Extraction Kit, Invitrogen, USA). The quality and quantity of the RNA were measured using a NanoDrop spectrometer (NanoDrop One, USA). Real-time PCR was carried out in real-time instrument (Applied Biosystems, USA) with SYBR Premix qPCR master mix (Luna Universal, NEB, UK) according to the primer manufacturer's protocols. The relative expression of target genes was normalized against the mRNA of reference gene β-actin using the 2-ΔΔCT method [28].

Statistical analysis of data

Data from this study were statistically analysed using the GraphPad prism analysis package (version 8.0) and the significance level was 0.05. The data were presented as means ± standard deviation (SD). For the relative gene expression in each fish species, one-way ANOVA and Tukey's multiple comparison test was used to compare the expression in the liver of all the microplastic-free and microplastic-contaminated fish species.

Results and discussion

Microplastics in fish liver

Microplastic accumulation was observed in the liver of the sampled four fish species (Fig. 3) sourced in the Lagos Lagoon and procured from artisanal fishermen at retail points to the populace. The diverse morphotypes of isolated tentative microplastics are presented in Fig. 4, while Fig. 5 shows the observed colors of tentative plastic particles accumulated in fish liver. Of the 32 fish liver examined, microplastics were found in 13 (41%). The African knifefish, *G. niloticus* had the highest concentration of plastic particles in the liver (9.5 ± 4.2 particles/fish). Average microplastic accumulation in the liver of the fish species was of the order: *G. niloticus* > *C. gariepinus* > *C. nigrodigitatus* > *O. niloticus* (Fig. 3). This result suggests that the individual nature of the fish species– habitat and feeding habit – plays a key role in the amounts of microplastics accumulated in the liver. For instance, *G. niloticus* are demersal and potamodromous fish species which are typically omnivorous and carnivorous on insects, fish and crustaceans[29] (Oluwale, Ugwumba and Ugwumba, 2019). Hence, the high amounts of accumulated microplastics. In addition, microplastic accumulation in the liver of *C. gariepinus* could be attributed to their benthopelagic opportunistic feeding nature [30], thus prone to high microplastic ingestion and subsequent tissue accumulation. Microplastic accumulation in fish liver is an indication of microplastic ingestion and bioavailability in fish tissues, which are overtime translocated to the visceral tissues.

High prevalence of fiber microplastics in the fish liver could be attributed to the predominant presence of fiber morphotypes in the surface water and sediment of the Lagos Lagoon [18,19]. Significant amounts of environmental microplastic fiber in the fish tissue may be caused by sewage overflows, domestic wastewater streams, in-situ washing of synthetic garments, terrestrial runoffs, and tidal hydrological interaction with the adjacent Atlantic Ocean. Five major colors of microplastics were observed in the analyzed liver tissues (Fig. 5), 48% of which are black, 22% is blue, 20% is transparent, 13% is red and 2% green. Observed microplastic colors could be due to sorption of heavy metals, other pollutants as well as direct colors of disintegrated plastic debris or an information on the weathering process and origin of isolated microplastics. For instance, black colored MPs are often associated with polyethylene polymers, usually used as single-use plastic bags while blue polymers may arise from textile industry waste [31].

All the isolated microplastics were analyzed with ATR-FTIR (Fig. 6) and the predominantly identified plastic polymers were polyethylene (PE), polystyrene (PS) and polychloroprene (PCR). The predominance of polyethylene and polystyrene polymers is an indication of indiscriminate plastic waste disposal in the Lagos Lagoon as they are commonly used in single-use plastic packaging in the Lagos metropolis.

Relative gene expression

The relative expression of the cytochrome P450 (CYP 1a) gene in all the fish species was significantly downregulated in the microplastic-laden groups compared to the microplastic-free liver samples ($F = 4.567$, $p \leq 0.05$). However, the lowest regulation was

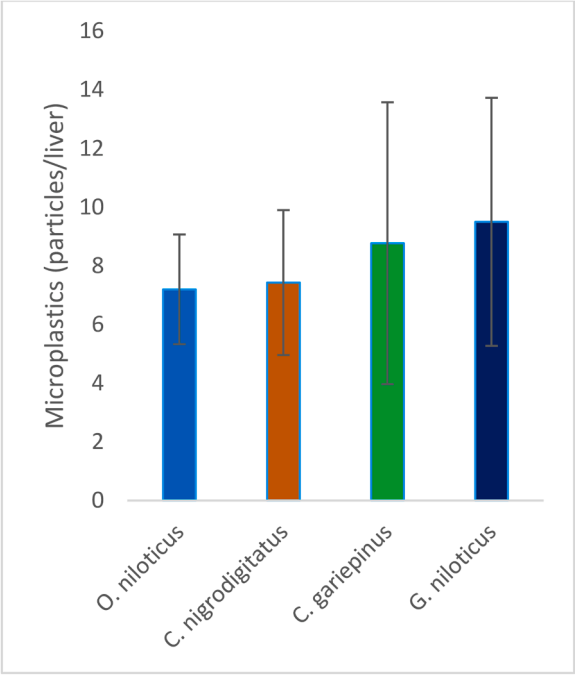

Fig. 3. Abundance of microplastics in the liver of fish species from the Lagos Lagoon.

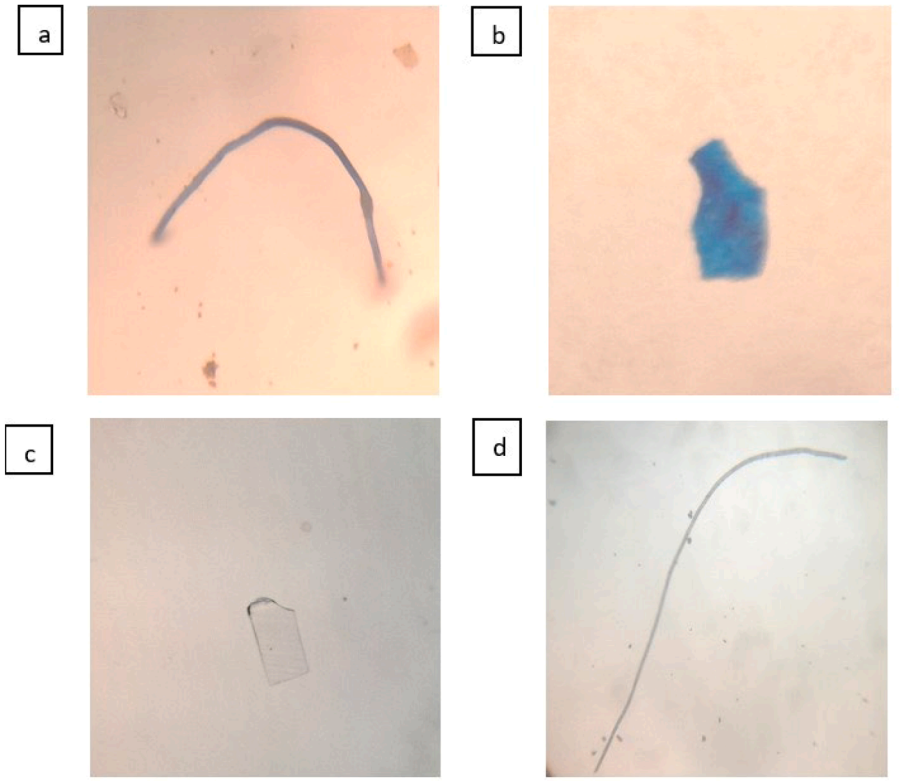

Fig. 4. Varied types of microplastic particles isolated from the liver of the sampled fish species from the Lagos Lagoon (a & d – fiber, b – blue fragment, c – transparent fragment) (x 4000).

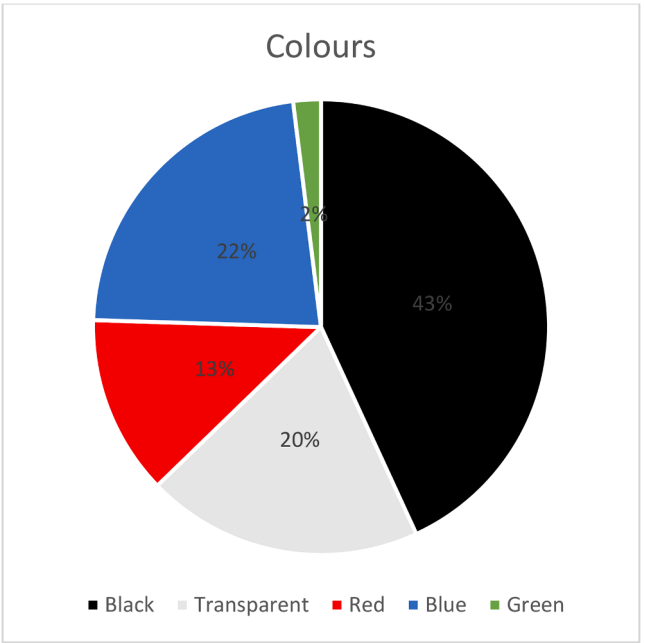

Fig. 5. Colors of observed microplastic particles.

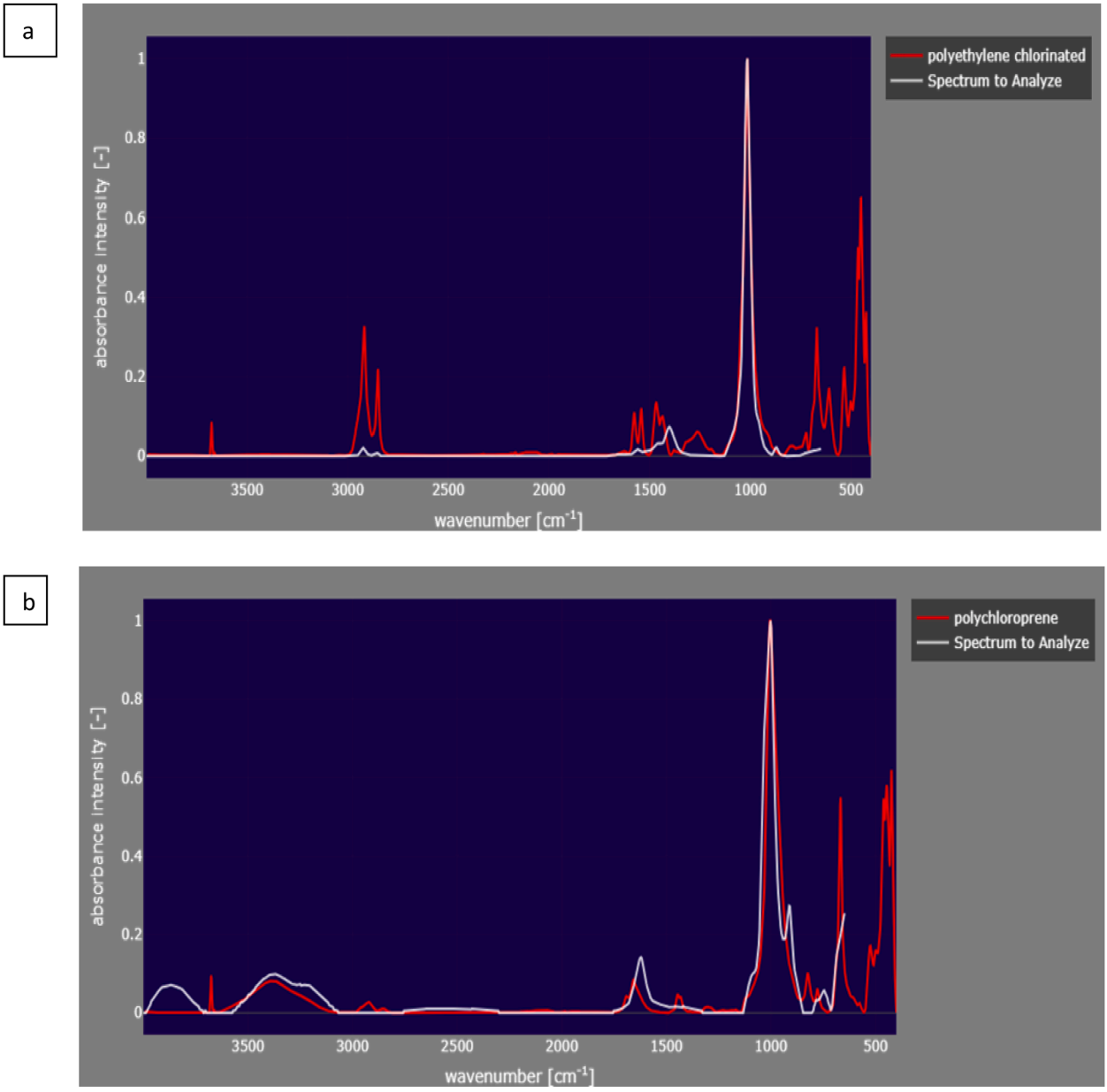

Fig. 6. FTIR spectra of plastic polymers (a) Polyethylene (b) Polychloroprene.

observed in *G. niloticus* and *C. gariepinus* which are the species with the highest microplastic accumulation (Fig. 7a). This result suggests that microplastic accumulation in the liver of fish could influence the downregulated expression of the CYP1a gene. Cytochrome P450 are a family of proteins that are responsible for the metabolism of endogenous as well as exogenous compounds and are mostly studied as biomarkers of environmental exposure to xenobiotics [32] (Okoro and Tawari-Fufeyin, 2024). According to Liu et al. [33], the downward regulation of cytochrome p450 due to microplastic exposure arises from overt toxicity effects. Similarly, the results from this study supports the findings of Wu et al. [34] in the *In vitro* studies of microplastic effects on insect cells, which revealed that MPs may invade the locus of the endoplasmic reticulum and thereby inhibit the catalytic activity of the CYP enzyme. In this study, the accumulation of microplastics in the fish liver suggests enhanced toxicity as well as a shift in metabolic equilibrium, hence resulting in the downregulation of the CYP1a gene [35]. However, contrasting observations was reported in a study by Okoro and Tawari-Fufeyin (2024), where the expression of CYP 1a gene was induced in wild fish population exposed to xenobiotics. The upregulation was attributed to coping mechanism and adaptation strategy of the fish species to environmental stress.

Fig. 7b shows the expression of the heat shock protein 70 (HSP70) gene in the liver of fish species sourced from the Lagos Lagoon. The relative expression of the HSP70 gene was upregulated in the MP-laden fish liver when compared to the microplastic-free samples in *O. niloticus, C. nigrodigitatus,* and *C. gariepinus*, although not statistically significant ($F = 1.182$, $p \leq 0.05$). *G. niloticus*, on the other

hand, revealed a downward trend in the relative expression of HSP70 gene. Heat shock proteins (HSP) are a highly conservative protein family and are generalized stress response genes in several organisms [36]. This study reveals that the regulation of stress response genes may be induced because of microplastic accumulation in fish tissues, hence suggesting cellular damage. High HSP70 expression reiterates the role and importance of this gene as an adaptive mechanism to stress experienced by wild fish populations in the environment. Corroborating our result is the reported upregulation of HSP70 genes in *Chironomus riparius* in response to tire rubber microparticle exposure [37]. Furthermore, Tenji et al. [38] documented the up-regulation of HSP70 gene in wild *Abramas brama* population exposed to chemicals in River Danube, Serbia. It is pertinent to note that the downregulation of HSP70 in microplastic-contaminated *G. niloticus* contrasts the fact that HSP70 genes are usually lowly expressed in non-stressed cells and induction is eventually increased because of their vulnerability to stressors [39]. The downregulation of these stress biomarkers indicate that fish species become adapted to the long-term dietary exposure and ingestion of microplastics or that there was a recovery process of environmental stress biomarker activation [32]. Further studies are necessary to evaluate the molecular impact of microplastics, inherent chemicals and environmental co-contaminants on edible aquatic biota. While this study assesses the potential impact of microplastics on the gene expression of wild fish populations, further laboratory investigation is necessary to ascertain the cause-effect relationship between microplastics and relative expression profiles of genes of high physiological importance in fish tissues. Overall, the mechanism of physiological disturbance in wild fish populations with microplastic accumulation remains unclear and still needs to be investigated by further studies (Figs. 6 and 7).

Conclusion

The findings reported by this study highlight microplastic presence in the liver of commercially available *O. niloticus, C. nigrodigitatus, C. gariepinus*, and *G. niloticus*. These fish species are important economic species that are retailed within the urban center of Lagos city. Hence, the presence of microplastics in visceral tissues like the liver precludes calls for concerns about microplastic accumulation in visceral tissues as well as the consequent potential impact on public health. Subsequently, varied expressions of CYP 1a and HSP 70 genes in microplastic-laden fish samples reveal the potential overt toxicity of MPs to fish tissue. These findings reiterate the urgent need to curb plastic and microplastic pollution, as the environmental occurrence influences bioavailability and bioaccumulation in wild commercially available fish population in the Lagos Lagoon, Nigeria, thereby posing potential threats to the populace. Furthermore, these reports contribute to available data on microplastic occurrence in edible biota and elucidate the understanding of microplastic pollution in wild conditions. Hence, forming the basis for an in-depth investigation into the mechanism of molecular impacts of environmentally relevant microplastics *in vivo* to further understand the pathways involved in microplastic translocation in visceral tissues and subsequent effects in biota at molecular and cellular levels.

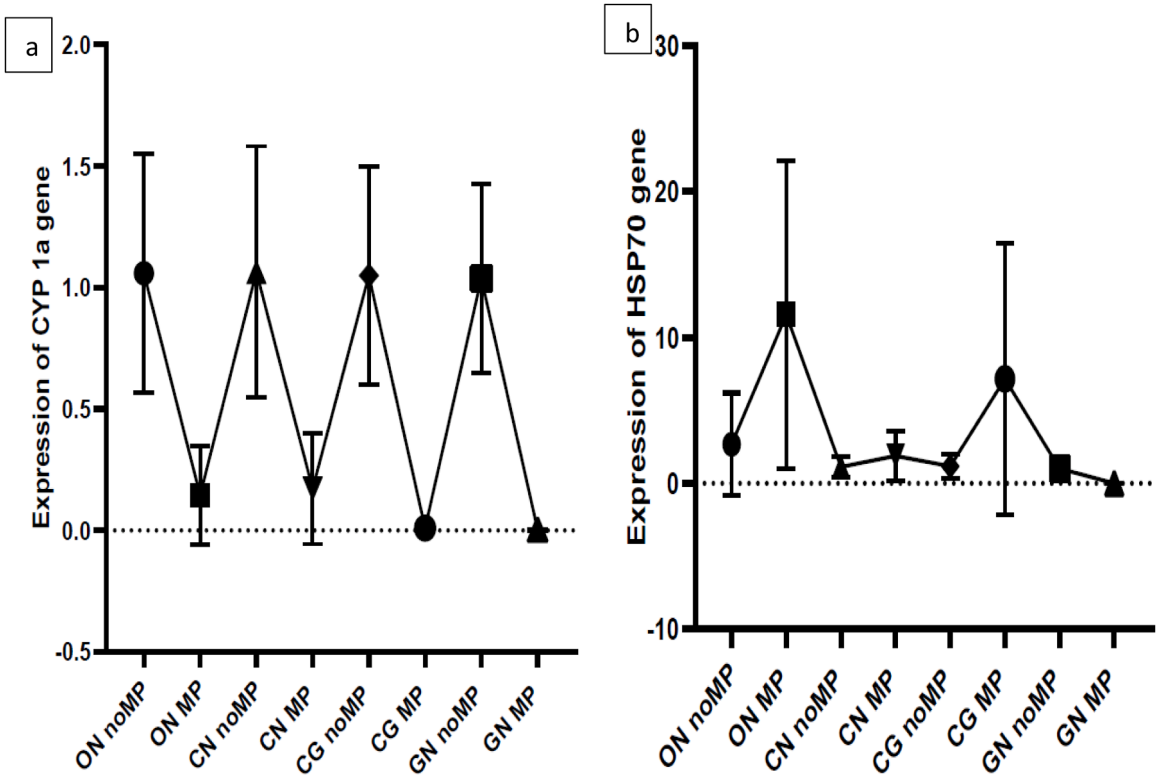

Fig. 7. Relative expression of cytochrome p450 and heat shock protein 70 genes in the liver of fish species in the Lagos Lagoon.

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CRediT authorship contribution statement

Fadekemi O. Akinhanmi: Conceptualization, Writing – original draft. **Opeyemi I. Ayanda:** Writing – review & editing, Validation, Supervision. **Gabriel A. Dedeke:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest for this publication.

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