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Research Article

Impact of Microplastics on the Biological Health of Tilapia (*Oreochromis Mossambicus*): A Multilevel Toxicological Study

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Abstract: Microplastics (MPs) are widespread environmental contaminants known to exert adverse effects on aquatic organisms. This study evaluates the impact of laboratory-prepared MPs on the health of Tilapia (*Oreochromis mossambicus*) using a multilevel toxicological approach. MPs were characterized by FTIR spectroscopy to confirm polymer identity and examine interactions with tissue homogenates. Exposed groups were compared with controls under controlled conditions. Histological studies revealed tissue-level alterations. Protein expression changes were examined via SDS-PAGE. MP exposure led to significant disruptions, histological damage, and changes in protein profiles. These results demonstrate the potential of MPs to impair organisml level and emphasize the value of integrating physicochemical and biological assessments in evaluating microplastic toxicity.

Keywords: FTIR Spectroscopy, Histology, Microplastics, *Oreochromis mossambicus*, Proteomic profiling.

Introduction

Microplastics (MPs), typically defined as plastic particles less than 5 mm in size, have emerged as a significant class of environmental contaminants in aquatic ecosystems [1,2]. Their persistence, small size, and widespread distribution enable easy ingestion by aquatic organisms, raising concerns regarding their potential to induce physiological and biochemical disturbances. Recent studies have demonstrated that MPs can act not only as physical stressors but also as vectors for chemical additives and adsorbed pollutants, thereby exacerbating their toxicological impact at multiple biological levels [3,4,5].

Fish, particularly freshwater species such as Oreochromis mossambicus, serve as important model organisms for evaluating environmental stress due to their ecological relevance and sensitivity to pollutants [6]. Exposure to MPs in fish has been associated with alterations in feeding behaviour, oxidative stress, inflammatory responses, and tissue damage [7,8,9]. These disruptions may ultimately compromise overall biological health, affecting growth, survival, and fitness.

Despite increasing reports on MP toxicity, there remains a need for integrated, multilevel assessments that correlate physicochemical characterization of MPs with their biological effects. Techniques such as Fourier Transform Infrared (FTIR) spectroscopy provide reliable identification of polymer composition, while bioassays including histological examination and protein expression analysis (SDS-PAGE) offer insights into systemic, tissue-level, cellular, and molecular responses, respectively [10,11].

Therefore, the present study aims to investigate the impact of laboratory-grade microplastics on the biological health of *Oreochromis mossambicus* using a comprehensive toxicological approach.

Materials and Methods

1. Preparation of Test Material and Measurement of Prepared Microplastic Particle Size Using Digital Vernier Calliper

The test material used in the present study consisted of microplastics, specifically low-density polyethylene (LDPE) plastic beads procured from HiMedia (polystyrene grade). These beads were ground using a laboratory mixer to obtain microplastic particles with a size of less than 5 µm. The size of microplastic was measured using Vernier Caliper [1].

2. Procurement and acclimatization of fingerlings

Tilapia (*Oreochromis mossambicus*) were obtained from Malhar Fish Farm, Shirwal. The fingerlings were acclimatized in a fish tank filled with 25 litres of borewell water under controlled conditions. All experimental procedures were conducted in accordance with the ethical guidelines prescribed by Savitribai Phule Pune University [6].

3. Exposure of Fingerlings to MPs

Fingerlings were divided into three tanks first was control group second and third was experimental (Microplastic) group. 30 fish were kept in each tank; they were provided with 10gms of feed regularly. And were observed for 30 days, 60days, 90days, 120 days [7,8].

Table a: Mortality rate of the Fingerlings.

Time of exposure	Sample size	Concentration of MP	Survival		Mortality (%)	
			C	MP	C	MP
30 days	30	0.25g/L	24	23	20%	23%
60 days	30	0.25g/L	20	17	16%	26%
90 days	30	0.25g/L	19	14	5%	17%
120 days	30	0.25g/L	19	14	0%	0%

4. Physicochemical Analysis of Water

Water quality analysis was carried out using a Water Analyzer 371 at the Department of Botany. The analysis was performed to assess various physicochemical parameters, including pH, temperature, salinity, conductivity, total dissolved solids (TDS), dissolved oxygen (DO), and turbidity. Water samples were analyzed following standard operating procedures, and all measurements were recorded under identical conditions to ensure accuracy and comparability between samples [5].

5. Fourier Transform Infrared (FTIR) Analysis of water sample

Fourier Transform Infrared (FTIR) spectroscopy was performed to carry out a comparative analysis between microplastic-treated water sample and control water sample in order to study the effect of microplastic exposure. The samples were subjected to FTIR analysis to identify and compare the functional groups and characteristic absorption peaks. The obtained spectra were analyzed to assess any chemical changes or differences between the microplastic-treated and control water samples [10,11].

6. Fourier Transform Infrared (FTIR) Analysis of Fish Tissue Homogenate

Fish tissue samples were analyzed using Fourier Transform Infrared (FTIR) spectroscopy to evaluate biochemical composition and detect possible molecular alterations in the tissues. Freshly collected fish tissues were first homogenized to obtain a uniform sample suitable for spectroscopic analysis. For homogenization, the tissue was mixed with phosphate-buffered saline (PBS, pH 7.0) in a 1:10 (w/v) ratio, where 1 g of tissue was suspended in 10 mL of PBS. The mixture was thoroughly homogenized using a tissue homogenizer to ensure complete disruption of the tissue and uniform distribution of cellular components within the buffer solution [10,11].

7. Histological Slide Preparation of Fish Tissues (Paraffin Method)

i. Collection of Samples

Live fish were sacrificed and muscle was collected for histological examination.

ii. Fixation of Samples

Immediately after dissection, the tissues were fixed to preserve cellular structure and prevent degradation. Small tissue pieces of approximately 3–5 mm thickness were prepared to ensure rapid penetration of the fixative. The tissues were placed in labelled plastic vials containing fixative, indicating the sample name and collection date. Each vial was filled with approximately two-thirds of the fixative, maintaining a tissue to fixative ratio of 1:20. The tissues were kept in the fixative for the required duration depending on the type of fixative used.

iii. Dehydration of Tissues

Table b: Fixation Conditions.

Fixative	Fixation Time
Formalin	10–12 hours
Alcohol–Formalin	7–10 hours
Bouin's solution	4–6 hours

After fixation, the tissues were dehydrated to remove water from the tissue blocks. Dehydration was carried out through a graded alcohol series, with each step lasting approximately 1 hour.

Table c: Dehydration Using Graded Alcohol.

Alcohol Concentration	Duration
50% Ethanol	1 hour
70% Ethanol	1 hour
85% Ethanol	1 hour
95% Ethanol	1 hour
100% Ethanol	1 hour

iv. Clearing

Following dehydration, the tissues were cleared to remove alcohol before paraffin embedding. Clearing agents were used that were miscible with both alcohol and paraffin wax.

Table d: Clearing Agents Used in Histology.

Clearing Agent	Clearing Time
Chloroform	Overnight

v. Paraffin Impregnation

After clearing, the tissues were impregnated with molten paraffin wax. Paraffin was melted in a hot air oven at 50–60°C, depending on the melting point of the wax. Care was taken not to exceed 5°C above the melting point to avoid tissue shrinkage or hardening. The tissues were transferred to molten paraffin, ensuring that the wax volume was 25–30 times greater than the tissue volume. Impregnation was carried out with 2–3 changes of molten wax to completely remove the clearing agent.

vi. Embedding

Embedding involved orienting the tissue in molten paraffin so that it could later be sectioned using a microtome. Molten paraffin wax heated 2–3°C above its melting point was poured into embedding moulds to a sufficient depth to cover the tissue block. As the wax in contact with the mould began to solidify, the tissue was placed into the semi-solid wax using pre-warmed forceps to prevent sticking. The tissue was gently pressed to ensure proper orientation. The mould was then submerged in cold water (~20°C) to allow rapid solidification of the paraffin. Ice water was avoided as it could crack the paraffin blocks. After solidification, the blocks were removed from the mould and the surface facing the base of the mould was designated as the cutting surface.

vii. Trimming and Sectioning

The paraffin block was fixed in the block holder of the microtome in such a way that it remained clear of the knife during operation. The microtome knife was inserted into the knife holder and securely tightened. The clearance angle was adjusted to 3–4°, while the slope angle was maintained at 90°.

Initially, trimming of the block was carried out by setting the section thickness to 15 µm to remove excess wax and expose the tissue surface. Once the tissue was exposed, thin sections were cut at a thickness of 5µm, which is suitable for routine histological examination.

During sectioning, the microtome was operated rhythmically with the right hand while the left hand guided the sections away from the knife. Continuous ribbons of sections were formed due to the slight heat generated during cutting, which caused the edges of successive sections to adhere together.

viii. Mounting of Tissue Sections on Slides

After trimming of the paraffin block and obtaining suitable ribbons of tissue sections using the microtome, the sections were mounted onto clean glass slides for subsequent staining. Prior to mounting, the slides were coated with an adhesive solution prepared using glycerine and egg albumin in a 1:1 ratio.

This adhesive layer was applied evenly across the surface of the slide to enhance the adherence of the paraffin sections during subsequent staining procedures.

Following the application of the adhesive, a few drops of distilled water were added onto the coated surface of the slide. The water facilitated the spreading and proper positioning of the tissue sections. The ribbon of paraffin sections obtained from the microtome was then carefully cut into the required size using forceps. Individual sections or small groups of sections were gently transferred and placed onto the prepared slides using fine forceps, ensuring that the sections remained intact and properly oriented.

Once placed on the slide, the sections were carefully spread to remove folds or wrinkles and to achieve a smooth and even attachment to the slide surface. The slides containing the mounted sections were then placed on a heated plate for approximately 5–10 minutes. The gentle heating allowed the paraffin sections to soften slightly, enabling the sections to adhere firmly to the adhesive-coated slide surface and improving the contact between the tissue and the slide.

After heating, the slides were removed from the heated plate and allowed to air dry completely. Proper drying ensured strong attachment of the sections to the slide and minimized the risk of section detachment during the subsequent staining steps. Once the slides were fully dried, they were subjected to the Haematoxylin–Eosin staining procedure for histological examination.

Haematoxylin-Eosin (H&E) Staining

Histological preparations were photographed under a compound microscope at 10× and 40× magnifications to observe tissue architecture and cellular morphology. Photomicrographs were captured for documentation and comparative analysis of control and treated samples following standard histological examination procedures [12,13].

Table e: Haematoxylin–Eosin (H&E) Staining.

Reagent / Solution	Process	Duration
Xylene	Deparaffinization	5 minutes
100% Ethanol	Hydration	10 minutes
90% Ethanol	Hydration	10 minutes
70% Ethanol	Hydration	10 minutes
70% Ethanol	Hydration	10 minutes
50% Ethanol	Hydration	7 minutes
30% Ethanol	Hydration	7 minutes
Distilled Water	Washing / Hydration	5 minutes
Haematoxylin	Nuclear staining	3–4 minutes
Tap Water	Washing	30 seconds
30% Ethanol	Dehydration	10 minutes
30% Ethanol	Dehydration	10 minutes
50% Ethanol	Dehydration	10 minutes
70% Ethanol	Dehydration	5 minutes
Eosin	Counterstaining	4–5 minutes
90% Ethanol	Dehydration	Dip and pull out
100% Ethanol	Dehydration	Dip and pull out
Xylene	Clearing	Dip and pull out

Proteomic Analysis: SDS PAGE

Muscle protein profiles were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Muscle proteins were isolated using a shift method in which minced muscle tissue was homogenized in ice-cold distilled water (1:9 w/v) for 2 minutes at high speed. The homogenate pH was adjusted to 11 with 1M NaOH, stirred for 18 minutes and centrifuged at 10,000 × g at 4°C. The supernatant was collected and precipitated by adjusting pH to 5.5 with 1M HCl followed by centrifugation at 4°C for 15 minutes to collect the protein pellet. The pellet was resuspended in cold distilled water and neutralized to pH 7.0 with dilute NaOH or HCl. For gel preparation, a 10% resolving gel was prepared by combining acrylamide-bisacrylamide solution, Tris-HCl buffer (pH 8.8), SDS, ammonium persulphate (APS) and TEMED. The resolving gel was allowed to polymerize completely at room temperature. A 4% stacking gel was subsequently prepared using acrylamide-bisacrylamide solution, Tris-HCl buffer (pH 6.8), SDS, APS and TEMED and poured over the polymerized resolving gel.

A comb was inserted immediately to form sample wells and the stacking gel was allowed to polymerize completely before sample loading. Protein samples were prepared by mixing with SDS loading buffer and heated at 95°C for 5 minutes to ensure complete denaturation. Samples were loaded into wells and electrophoresis was performed at 80V through the stacking gel and 120V through the resolving gel until the bromophenol blue dye front reached the bottom of the gel. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 for overnight and destained using methanol acetic acid-water solution with multiple changes until clear protein bands were visible against a clear background [14,15].

Results

1. Vernier Calliper

Figure 1: Vernier Calliper.

The size of the prepared microplastic particle was measured using a digital vernier calliper. The measured length of the particle was 1.14 ± 0.03 mm (mean ± SD). This confirms that the prepared particles fall within the microplastic size range (<5 mm) and were suitable for use in the experimental study.

2. Physicochemical Analysis

The physicochemical parameters of the experimental water were measured before and after microplastic (MP) exposure to ensure suitable conditions for the study. The pH of the water slightly increased from 7.45 to 7.5, remaining within the acceptable range for aquatic organisms. The temperature showed a minor decrease from 27.1°C to 26.5°C, indicating stable environmental conditions during the experiment.

The salinity increased from 3.28 ppt to 4.12 ppt, while conductivity showed a slight rise from 253 µS to 256 µS, suggesting a minor increase in dissolved ions after microplastic addition. Similarly, total dissolved solids (TDS) increased from 0.129 ppt to 0.223 ppt.

Dissolved oxygen (DO) levels remained within the optimal range for fish survival, increasing from 6.6 ppm to 7.3 ppm. In contrast, turbidity decreased from 4.8 NTU to 0.96 NTU, indicating improved water clarity during the exposure period. Overall, all measured parameters remained within acceptable limits, confirming that the experimental conditions were suitable for assessing the effects of microplastic exposure.

Table 1: Observed values of water quality analysis.

Parameters	Standard Range	Control	Experimental
pH	7-8.5	7.45	7.5
Temperature	20-30 °C	27.1 °C	26.5 °C
Salinity	>2ppt	3.28ppt	4.12ppt
Conductivity	Up to 2000 µS	253 µS	256 µS
TDS	< 0.5 ppt	0.129 ppt	0.223 ppt
D.O	>5ppm	6.6 ppm	7.3 ppm
Turbidity	5 NTU	4.8 NTU	0.96 NTU

3. FTIR Spectral Analysis of Water Samples (Control and Microplastic)

The FTIR spectrum of the control water sample showed a broad peak at ~3295 cm^{-1}, which is typical of O–H stretching and simply reflects the natural structure and hydration of water. Another peak at ~1636 cm^{-1} falls within the protein region (1580–1700 cm^{-1}), but in this case it is due to bending of water molecules (1630–1650 cm^{-1}) and not actual protein content. Importantly, no peaks were seen in the carbohydrate region (960–1130 cm^{-1}) or in the lipid region (1710–1765 cm^{-1}). This clearly shows that the sample does not contain proteins, carbohydrates, or lipids, and therefore acts as a nutritionally neutral medium.

The FTIR spectrum of the treated water sample showed a similar broad peak at ~3278 cm^{-1}, indicating that the basic water structure and hydration remain unchanged. The peak at ~1636 cm^{-1} again appears in the protein region but represents water bending vibrations rather than true protein presence. A peak at ~1319 cm^{-1} was observed, which lies outside the defined carbohydrate region (960–1130 cm^{-1}), suggesting the presence of some carbon-based functional groups but not actual carbohydrates. As with the control, there were no peaks in the carbohydrate region or the lipid region (1710–1765 cm^{-1}. Overall, the treated water remains largely free from nutritional biomolecules, indicating that it does not provide proteins, lipids, or carbohydrates, even though minor chemical changes are present.

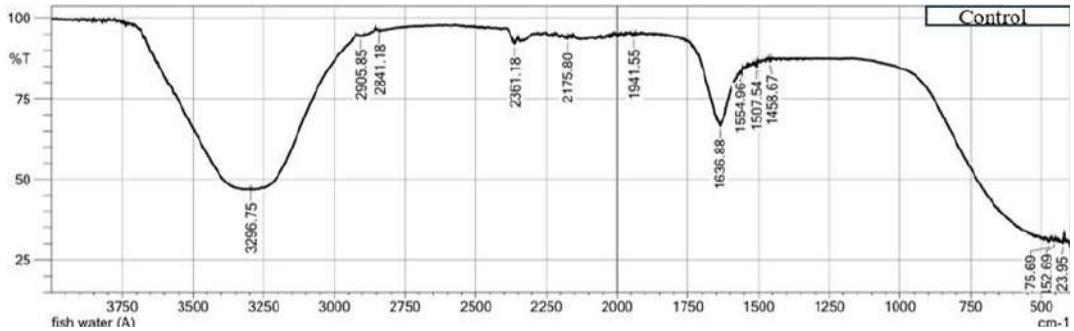

Figure 2a: FTIR analysis of control water sample.

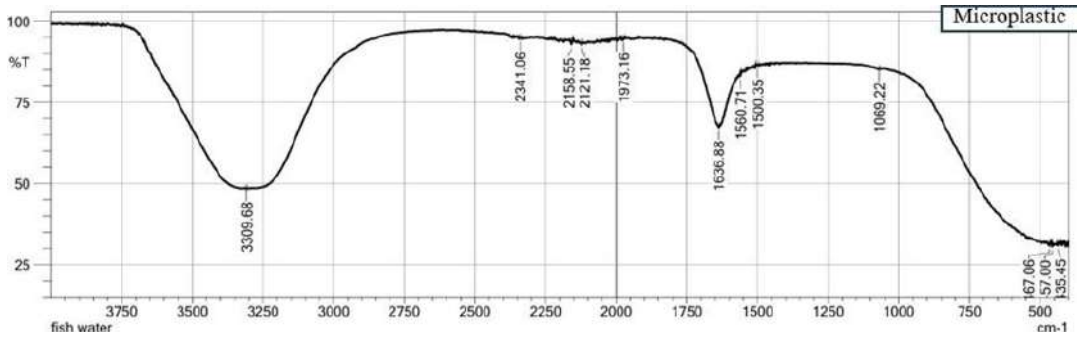

Figure 2b: FTIR analysis of water sample with Microplastics.

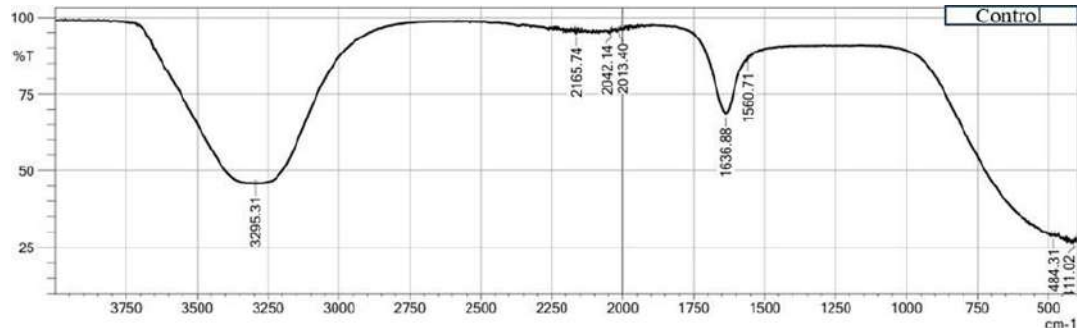

Figure 2c: FTIR analysis of control water sample.

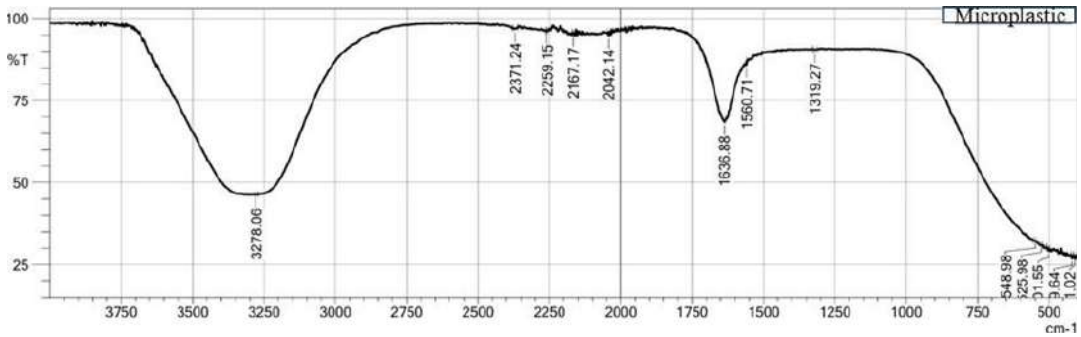

Figure 2d: FTIR analysis water samples with Microplastics.

4. FTIR Spectral Analysis of Control and Microplastic-Exposed Fish Tissues

The FTIR spectrum of the control muscle homogenate showed a broad peak at ~3289 cm^{-1}, which reflects good hydration along with strong protein presence in the tissue. A clear peak at ~1636 cm^{-1} in the protein region (1580–1700 cm^{-1}) indicates a well-maintained protein structure, suggesting that the muscle contains intact and stable proteins. The peak at ~1559 cm^{-1} further supports normal protein interactions and integrity.

In addition, strong peaks at ~2923 cm^{-1} and ~2854 cm^{-1} show the presence of lipids, which are important for energy storage and maintaining cell membranes. No peaks were seen in the carbohydrate region (960–1130 cm^{-1}), indicating that carbohydrates are not a major component. Overall, this pattern shows a healthy muscle tissue with a balanced nutritional profile consisted of proteins and supported by lipids.

The FTIR spectrum of the microplastic exposed muscle homogenate showed a broad peak at ~3296 cm^{-1}, confirming that hydration and protein presence are still maintained, but with slight changes in intensity that suggest a shift in the tissue environment. The peak at ~1636 cm^{-1} confirms that proteins are still the main nutritional component, while the more noticeable peak at ~1559 cm^{-1} indicates changes in protein interactions and structure. Compared to the control, the lipid-related peaks around ~2923–2854 cm^{-1} are less prominent, suggesting a reduction in lipid content and energy reserves. The peak at ~1457 cm^{-1} still shows that some structural lipids are present, but their contribution appears altered. As in the control, no peaks were observed in the carbohydrate region (960–1130 cm^{-1}). Altogether, the results suggest that although the muscle remains rich in proteins, there is a noticeable change in protein along with a decrease in lipid-related nutritional components, indicating a shift in the overall nutritional quality of the tissue.

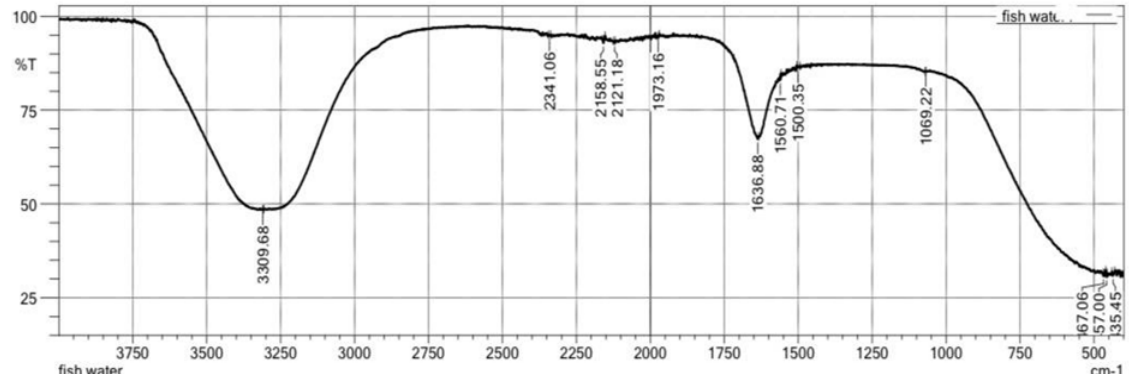

Figure 3a: FTIR analysis of control Muscle homogenate.

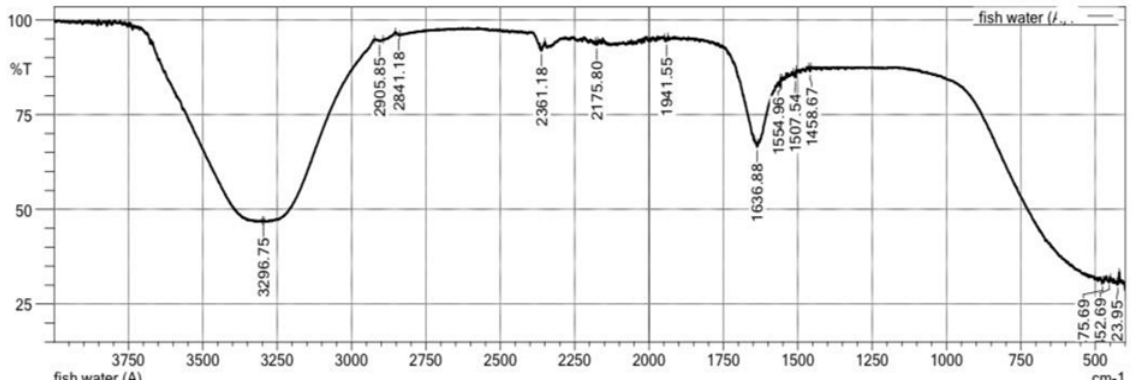

Figure 3b: FTIR analysis of Microplastic exposed muscle homogenate.

5. Histology: Double Staining

Histological examination of muscle tissue of *Oreochromis mossambicus* revealed clear structural and pathological differences between control and microplastic MP exposed groups, indicating tissue-level toxicity and its implications on biological health. In control samples, muscle tissue exhibited normal histoarchitecture, with compact, parallelly arranged fibers showing distinct striations, reflecting intact contractile proteins and normal physiological function.

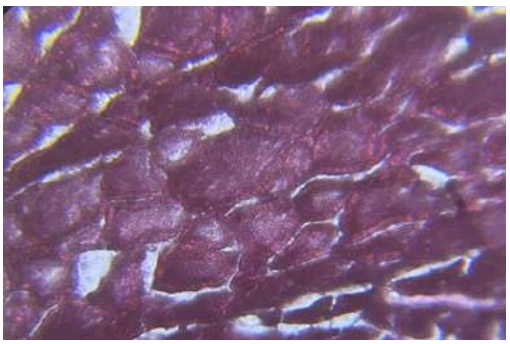

Figure 4a: Control Muscle.

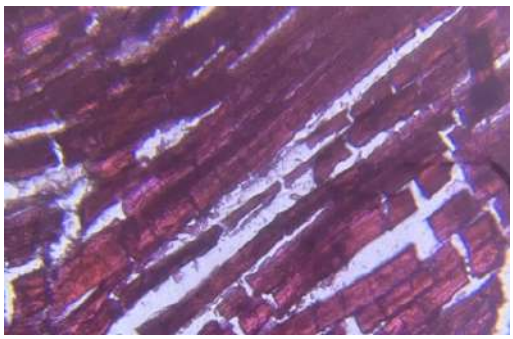

Figure 4b: Microplastic exposed muscle.

In contrast, MP exposed muscle tissue demonstrated significant histopathological alterations, including disrupted and disorganized fibers, loss of striations, and increased gaps between fibers. These changes suggest degradation of structural proteins such as actin and myosin, ultimately affecting muscle integrity and locomotor function. These structural abnormalities indicate that microplastic exposure induces notable tissue damage in muscle, disrupting normal physiological processes related to movement. Such impairments reflect a decline in overall biological health, as the integrity of muscle tissue is essential for maintaining proper locomotion and organismal function. Therefore, the histological findings provide strong evidence that microplastics exert significant toxic effects at the tissue level, contributing to overall health deterioration in tilapia.

6. Proteomic Analysis: SDS PAGE

SDS-PAGE analysis (10% gel, Coomassie Brilliant Blue staining) of muscle proteins in Oreochromis mossambicus showed clear alterations in the LDPE microplastic exposed group compared to control. Major protein bands were observed at ~220 kDa (Myosin Heavy Chain), 70 kDa (HSP70), and 42 kDa (Actin). Densitometric analysis revealed a significant increase in HSP70 band intensity in the exposed group (165239 AU) compared to control (128723 AU), indicating upregulation of stress-response proteins. In contrast, Myosin Heavy Chain intensity decreased in the exposed group (302071 AU) compared to control (326735 AU), suggesting structural protein degradation.

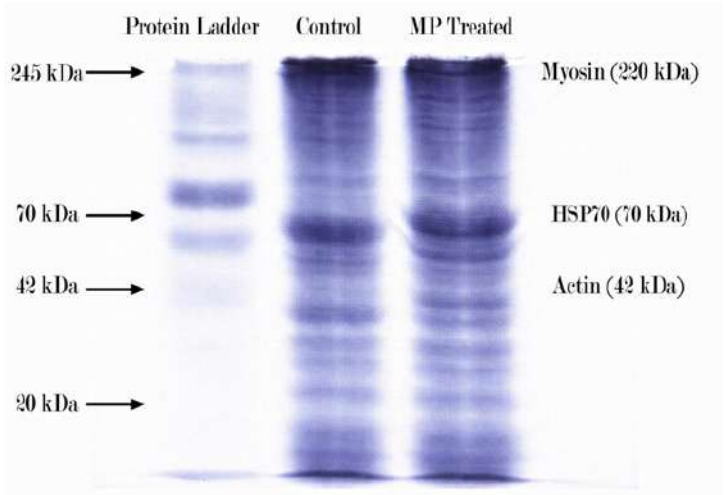

Figure 5: SDS PAGEprofile of muscle tissue proteins of control and LDPE microplastic exposed Oreochromis mossambicus.

Discussion

The present study provides a comprehensive, multi-level evaluation of microplastic (MP)-induced toxicity in Oreochromis mossambicus, integrating physicochemical, cellular, tissue, and molecular responses. The findings collectively demonstrate that microplastic exposure exerts significant adverse effects on fish health, even under controlled environmental conditions, thereby supporting previous reports on the biological impacts of microplastics in aquatic organisms [16,17,18].

The physicochemical analysis confirmed that all water quality parameters remained within permissible limits throughout the experimental period, indicating that the observed biological effects were primarily due to microplastic exposure rather than environmental fluctuations. However, slight increases in salinity, conductivity, and total dissolved solids suggest that microplastics may influence the physicochemical properties of water and act as carriers of dissolved contaminants, enhancing their bioavailability [2,5,19]. FTIR analysis of water samples further supported this by revealing subtle spectral variations in treated samples, indicating potential interactions between MPs and aquatic media [1,17].

At the molecular level, FTIR analysis of fish tissues revealed significant biochemical alterations, including changes in protein-associated peaks and shifts in functional group regions. Proteins were primarily identified in the amide region (1700–1600 cm^{-1}), lipids in the fatty acid region (3000–2800 cm^{-1}), and carbohydrates in the region of 1000–1100 cm^{-1}, consistent with standard FTIR spectral assignments [10,11]. The observed variations in protein-associated peaks suggest alterations in protein structure and interactions, while changes in lipid-associated bands indicate modifications in lipid composition and membrane stability. Such biochemical disruptions are widely reported in microplastic-exposed organisms and are often associated with oxidative stress and membrane damage [7,9,20].

Histological analysis provided direct evidence of tissue-level damage, linking molecular and cellular alterations to functional consequences.

Structural abnormalities such as disorganization of muscle fibers indicate compromised locomotion and physiological stress. Similar histopathological changes have been reported in fish exposed to microplastics, including epithelial damage, necrosis, and tissue degeneration [8,9,21].

Proteomic analysis using SDS-PAGE revealed alterations in protein expression patterns, including increased intensity of stress-related proteins such as HSP70 and decreased structural proteins like myosin. These findings suggest activation of stress response pathways and degradation of essential proteins, which are indicative of cellular stress and toxicity [14,15].

Conclusion

The present study provides a comprehensive evaluation of the toxicological impact of microplastics (MPs) on Oreochromis mossambicus using an integrated multi-assay approach, including FTIR spectroscopy, histology, and SDS-PAGE proteomic profiling. The convergence of results from these assays clearly demonstrates that microplastic exposure induces significant biological disturbances at molecular, cellular, tissue, and systemic levels.

FTIR analysis confirmed the interaction of MPs with both the aquatic environment and fish tissues, revealing biochemical alterations such as protein denaturation, disrupted hydrogen bonding, and lipid peroxidation. Histological examination provided direct evidence of tissue damage, including muscle fiber disorganization. Proteomic alterations observed through SDS-PAGE further confirmed stress-induced changes in protein expression and possible degradation of structural and functional proteins.

Collectively, these findings establish that microplastic exposure compromises the overall biological health of fish by impairing essential physiological functions such as respiration, digestion, metabolism, and cellular integrity. Importantly, the observed damage to muscle tissue and protein profiles has direct implications for fish quality as a food source. Structural protein degradation, and biochemical imbalances can lead to diminished nutritional value, particularly in terms of protein content, texture, and overall flesh quality. This raises serious concerns regarding food safety and the long-term sustainability of fish as a reliable source of nutrition.

From an economic and societal perspective, these findings are highly significant. Aquaculture is widely recognized as the second largest industry after agriculture, contributing substantially to global food security, employment, and economic development. The presence and accumulation of microplastics in aquaculture systems therefore pose a dual threat affecting both fish health and the quality of produce reaching consumers. Chronic exposure to MPs could lead to reduced productivity, increased disease susceptibility, and compromised market value of fish stocks.

In conclusion, this study highlights microplastics as a critical emerging contaminant with the capacity to adversely affect aquatic organisms at multiple biological levels. The demonstrated decline in fish health and potential reduction in nutritional quality underscore the urgent need for monitoring, regulation, and mitigation strategies to control microplastic pollution in aquatic environments, particularly within aquaculture systems.

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