***Original Research Article***

Histopathological Alterations in Gill, Liver, and Kidney of Goldfish (Carassius auratus) Exposed to Polystyrene Microplastics

**Abstract**

The contamination of aquatic ecosystems by plastic waste, particularly microplastics, poses a serious environmental threat globally. Microplastics are defined as plastic particles ranging from 1 µm to 5mm in size and are known to accumulate in various aquatic organisms. This study investigates histopathological alterations in goldfish liver, gills, and kidneys (Carassius auratus) following chronic exposure to 1 µm polystyrene microplastics (PS-MPs) at a concentration of 1.53 mg/L over 28 days. In the gills, significant pathological changes included lamellar aneurysm, lamellar deviation, epithelial lifting, and the coalescence of secondary lamellae. The liver of the treated group exhibited signs of hepatocellular vacuolation, sinusoidal dilation and congestion, focal necrosis, and nuclear degeneration. In the kidney, notable alterations involved tubular degeneration, glomerular shrinkage, hemorrhage, and necrosis. These physiological findings suggest that PS-MPs induce multi-organ toxicity and compromise vital physiological functions in goldfish. The results highlight the risk of microplastic pollution to freshwater species and reinforce the need for stricter environmental regulations.

**Keywords: -** Carassius auratus, Histopathology, Polystyrene Microplastics, Gill, Liver, Kidney

# 1. Introduction

Plastic pollution has emerged as a critical environmental issue, with the accumulation of plastic waste in aquatic ecosystems posing significant risks to biodiversity and ecosystem health (Mohammed et al., 2021). Plastics are synthetic polymers that persist in the environment due to their non-biodegradable nature. Based on particle size, plastic debris is categorized into nanoplastic, typically smaller than 1 µm, and microplastics, ranging from 1 µm to 5 mm in size, mesoplastics, and macroplastics (Hammer et al., 2012). Among these, microplastics (MPs) are of particular concern due to their widespread distribution and bioavailability to various aquatic organisms (Hasan Anik et al., 2021).

Several pieces of evidence have accumulated in recent years indicating the harm microplastics cause to the aquatic ecosystem (Avio et al., 2015). Since plastics began being produced on an industrial level in the 1950s, their global production has risen dramatically. https://doi.org/10.3390/eng6010017 This surge has resulted in substantial plastic accumulation in both freshwater (Bordós et al., 2019) and marine environments (Barnes et al., 2009; Mihai et al., 2022).

Microplastics are primarily formed through the breakdown of larger plastic materials or are manufactured directly as small particles, such as microbeads used in cosmetics and industrial applications. Due to their small size, buoyant properties, and resistance to degradation, MPs easily infiltrate aquatic systems through urban runoff, sewage discharge, and industrial effluents (Mihai et al., 2022; Rani, 2022; Thompson et al., 2005).

Polystyrene (PS), a synthetic aromatic polymer derived from styrene monomers, is one of the environment's most common forms of plastic. It is extensively used to produce packaging materials, disposable cutlery, insulation foams, and biomedical products. Polystyrene has multiple forms, including rigid plastic, expanded foam (EPS), and thin films. Despite its utility, polystyrene is a major contributor to environmental pollution due to its persistence, low recyclability, and potential to release toxic monomers such as styrene and styrene oxide. These compounds have been associated with various toxicological effects, including genotoxicity, endocrine disruption, and bioaccumulation across trophic levels (Hwang et al., 2020).

Recent studies have documented the ingestion and aggregation of polystyrene microplastics (PS-MPs) in several aquatic species. These particles can translocate across biological membranes and accumulate in vital organs, potentially leading to oxidative stress, inflammation, and tissue damage. Histopathological examination of organs is a valuable approach to understanding the sublethal effects of toxicants, offering insights into cellular and tissue-level damage that may compromise physiological function (Nugnes et al., 2022).

Potential toxicity of polystyrene microplastic particles, Evaluation of size-dependent uptake, transport and cytotoxicity of polystyrene microplastic in a blood-brain barrier (BBB) model | Nano Convergence | Full Text

Although numerous studies have investigated the effects of MPs on aquatic organisms, there remains a need for detailed organ-specific toxicity assessments, especially under chronic exposure scenarios, in freshwater fish. The gills, liver, and kidneys are critical organs involved in respiration, detoxification, and osmoregulation, respectively, and are particularly susceptible to environmental pollutants.

Therefore, the present study aims to assess the histopathological alterations in goldfish's gills, kidney, and liver (Carassius auratus) after prolonged contact with polystyrene microplastics. This investigation provides crucial insights into the organ-specific toxicological impacts of PS-MPs in freshwater fish and underscores the ecological risks posed by microplastic pollution.

# 2. Materials and Methods:

The freshwater gold fish, Carassius auratus, was procured from a local aquarium, Darbhanga. The healthy fish had an average length of 7.4±0.5 cm (measured using a digital vernier caliper) and a weight of 20±0.5g (measured using a digital weighing balance). They were brought to the laboratory and washed with KMnO4 (0.1%) to avoid external infection. The fish were acclimatized for 15 days under laboratory conditions before the experiment. The fish were fed commercial floating feed (Tetra Goldfish Gold Growth) once daily at 3% of their body weight. Throughout the experiment, the aquatic environment was maintained according to APHA guidelines, with a temperature range of 14-22 °C, dissolved oxygen concentration between 6.62 and 6.76 mg/L, alkalinity from 62 to 68 mg/L, and a pH level spanning 6.5 to 8.5, and Free CO2 remained absent (American Public Health Association, 2005).

The stock of 1 μm PS-MPs (product no. 89904) was purchased from Merck/Sigma Aldrich Pvt. Ltd. and stored at 4ºC in a laboratory refrigerator (Haier HYC-260) to ensure stability and prevent degradation. The beads were supplied as an aqueous solution with 10% solid concentration and a 1.05g/cm3 density. Before each use, the stock was sonicated. The fish were subjected to PS-MPs (1μm) at a concentration of 1.53 mg/L for 28 days. Simultaneously, control fish were maintained without PS-MPs in parallel with the treatment group. The fish were sacrificed on the 29th day, after completing the 28-day exposure period. 3-4 mm-thick tissue samples from vital organs of fish, such as the gills, liver, and kidney, were taken from both the treated and control groups. These samples were fixed in 10% Neutral Buffered Formalin for some time (18-24 hours). The fixed tissue samples were processed and embedded in paraffin blocks following standard histological procedures. Using a rotary microtome, 5–7 µm-thick serial sections were obtained. For routine histological staining, stains like Ehrlich’s Haematoxyline (H.) and alcoholic Eosin (E.) were prepared and applied according to Luna (Luna, 1968).

Photomicrographs were taken to document the most characteristic histopathological lesions in the stained tissue sections.

# 3. Experimental Design:

Prior literature was reviewed to formulate the experimental design, ensuring relevance and validity. The study involved goldfish (Carassius auratus), which were divided into two groups: a control group and a treated group exposed to 1.53 mg/L Polystyrene microplastics with a particle size of 1μm. The concentration of polystyrene microplastics (PS-MPs, 1 μm) used in this study was a sublethal dose derived from the LC50 value experimentally determined by the author in preliminary bioassays conducted on Carassius auratus. This concentration enabled a reliable evaluation of histopathological alterations in exposed fish. Each group was maintained in triplicate, requiring a total of six tanks.

The experiment was conducted in 70 L aquaria, each containing 50 L of water. Five fish (n=5) were housed in each tank to maintain consistent stocking density. The PS-MPs were prepared from a stock solution and administered to the treated groups, while the control groups were maintained without exposure. Optimization of exposure duration and doses was conducted to reflect realistic environmental conditions.

# 4. Results:

The goldfish (Carassius auratus) exposed to sublethal concentrations of polystyrene microplastics (PS-MPs, 1 μm) exhibited significant tissue-level abnormalities in the gills, liver, and kidneys.

4.1. Histopathology: The histopathological alterations were detected in the gills, liver, and kidney of *C. auratus* exposed to PS-MPs.

4.2. Gill: The gills of goldfish exhibit a typical teleostean plan. They comprise primary and secondary gill lamellae, supported by a cartilaginous skeletal framework, multilayered epithelium, and a well-developed vascular system. The primary gill lamellae appear as flat, leaf-like structures arranged in double rows, with alternately arranged secondary lamellae extending laterally. These secondary lamellae are lined with squamous epithelium and supported by pillar cells (Figure 1).

Significant histopathological alterations were observed in the gills of the treated group, including lamellar aneurysm, protrusion of the secondary lamellar epithelium, lamellar deviation, and secondary lamellar fusion (Figure 2). Statistical analysis revealed that gill lamellar fusion was significantly higher in the treated group compared to the control group Confirming the severity of microplastic-induced damage. These structural changes indicate impaired respiratory function, which could lead to reduced oxygen uptake and increased physiological stress in fish. Similar gill pathologies have been reported in other fish species exposed to polystyrene microplastics, reinforcing the concern that PS-MPs contribute to respiratory dysfunction in aquatic organisms (Abarghouei et al., 2021; R. Kumari, 2021).

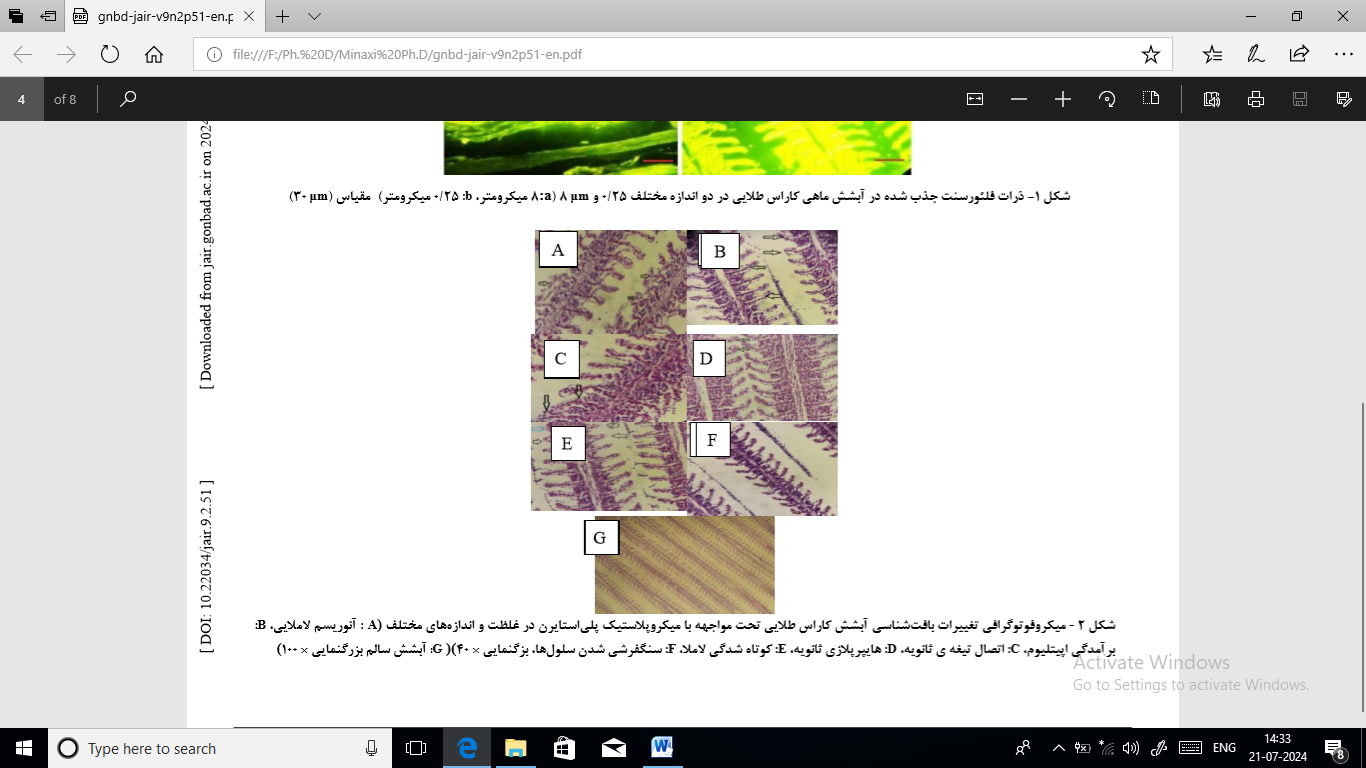


Figure 1: Micrograph of gill tissue of normal Gold Carp (Carassius auratus) (H&E 100x).

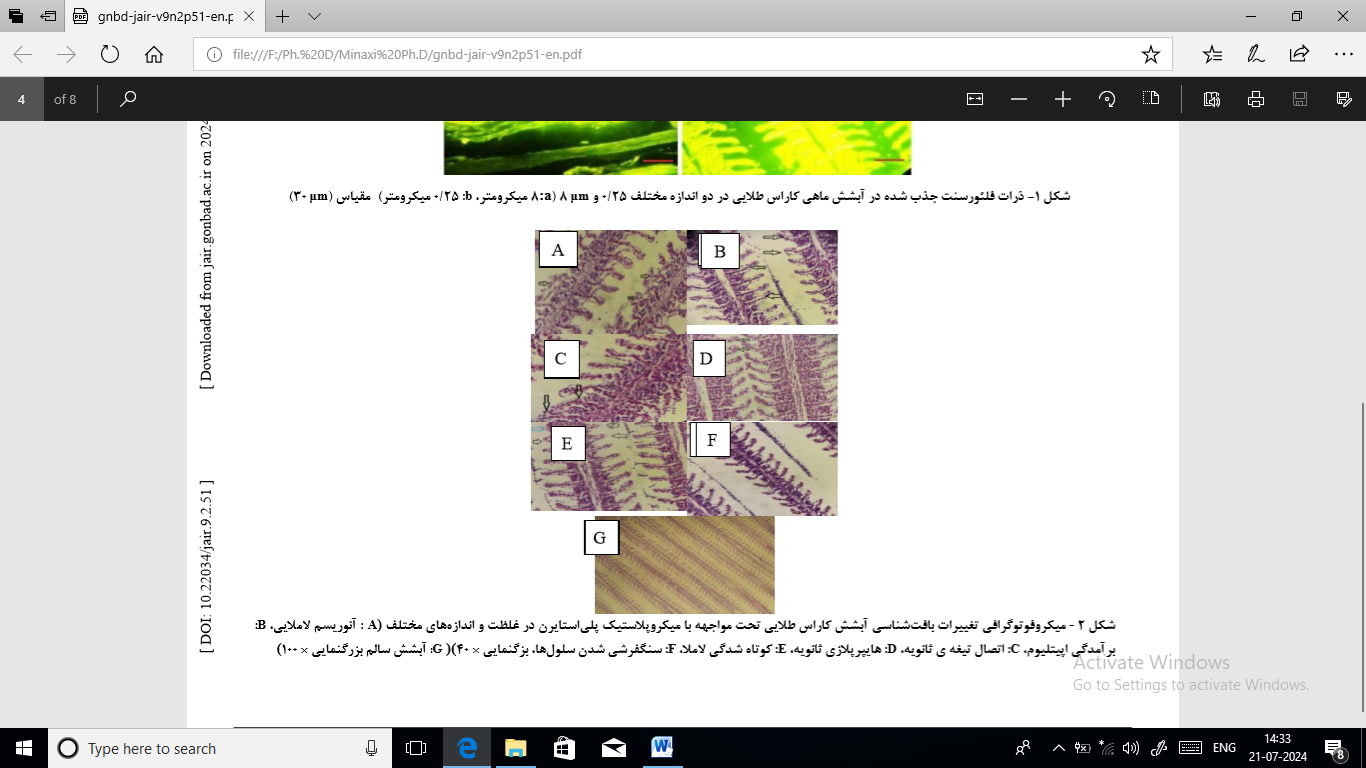


Figure 2: Micrograph of gill tissue of treated Carassius auratus, exposed to PS-MPs (1 μm) at a concentration of 1.53 mg/L for 28 days, showing lamellar aneurysm, secondary lamellar fusion (H&E 150x).

4.3. Liver:The liver tissue of the control Gold carp (Carassius auratus) exhibited the typical teleost hepatic structure. Hepatocytes appeared polygonal with centrally located nuclei, arranged in irregular cords around blood sinusoids. The hepatic parenchyma lacked a defined lobular architecture, and bile ducts were dispersed randomly, without a distinct portal triad, consistent with the diffuse plan of teleost livers. The cytoplasm of hepatocytes was homogenous, with occasional vacuolations likely associated with regular metabolic activity. Scattered melano-macrophage centers were also observed, indicating regular immune surveillance in hepatic tissue (Figure 3).

In contrast, liver tissues of gold carp exposed to polystyrene microplastics showed distinct pathological alterations, including vacuolation of hepatocytes, pyknotic nuclei, hepatocyte degeneration, and sinusoidal dilatation. Some sections revealed necrosis and congestion in blood vessels (Figure 4). Quantitative analysis revealed that 75% of treated fish showed moderate to severe hepatic vacuolation, compared to only 15% in controls, . These changes point to impaired liver function, potentially due to oxidative stress and accumulation of microplastics, consistent with findings in microplastic-exposed zebrafish and common carp reported in previous studies (Abarghouei et al., 2021; Hamed et al., 2021; Lu et al., 2016).

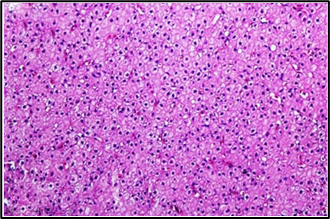


Figure 3: Photomicrographs of representative liver sections stained with hematoxylin and eosin (H&E; original magnification 40x) of goldfish.

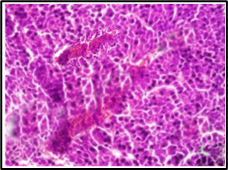


Figure 4: Photomicrograph of the transverse section (T.S.) of the intestine of treated fish, Gold Carp (Carassius auratus), exposed to PS-MPs at a concentration of 10 mg/L for 28 days (H&E, 50x).

4.4. Kidney:The renal tissue of the control gold carp showed a well-organized structure, with clearly defined renal tubules, glomeruli, and hematopoietic tissue. The renal tubules were lined with cuboidal epithelial cells, and glomeruli were compact and surrounded by Bowman’s capsules. Hematopoietic regions were prominent and intact, indicating healthy renal function (Figure 5).

However, kidney sections from fish exposed to polystyrene microplastics displayed pronounced histological alterations, including tubular degeneration, glomerular shrinkage, vacuolization, and necrotic cells. Disruption of tubular epithelial cells and detachment from the basement membrane were also evident (Figure 6). Statistical evaluation showed renal tubular degeneration in 68% of treated individuals compared to 12% in controls . These pathological changes reflect compromised excretory and osmoregulatory functions, paralleling toxic effects observed in microplastic-exposed zebrafish, Nile tilapia, and guppy fish (Bakieva et al., 2024; Elshaer et al., 2013; Hamed et al., 2021).

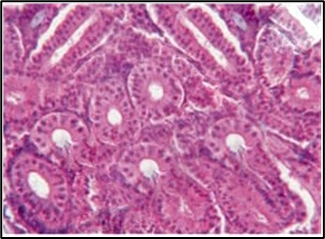


Figure 5: Photomicrograph of the control kidney of Carassius auratus (goldfish) showing normal histoarchitecture at 400× magnification. Structures appear well-organized with no signs of degeneration or pathological alterations.

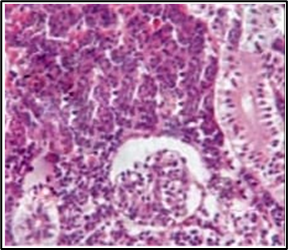


Figure 6: Photomicrograph of kidney tissue of Carassius auratus exposed to polystyrene microplastics (400x), showing marked histopathological alterations including tubular degeneration, glomerular shrinkage, and necrotic regions.

5. Discussion: The present study provides clear evidence of histopathological alterations in the vital organs- gill, liver, and kidney- of the freshwater fish Carassius auratus under chronic exposure to polystyrene microplastics (PS-MPs) at a sublethal concentration of 1.53 mg/L. The observed structural alterations in these tissues provide substantial evidence of PS-MP-induced toxicity, aligning with and extending findings from earlier studies on microplastic exposure in aquatic organisms.

The gill, the primary site of gas exchange and direct contact with the external environment, exhibited prominent pathological changes in the PS MP-exposed group. These included epithelial lifting, lamellar aneurysm, lamellar deviation, necrosis, and the coalescence of secondary lamellae. Such alterations impair respiratory efficiency and ion regulation, vital for fish survival in aquatic habitats. The findings are consistent with previous research, which reported similar gill damage in zebrafish and other fish following microplastic exposure (Bakieva et al., 2024).

The epithelial lifting may be a defensive mechanism to reduce contact surface area with toxicants, while lamellar fusion and necrosis point towards irreversible damage due to prolonged exposure.

The liver, a vital organ for metabolism and detoxification, showed significant histological damage, including hepatocyte vacuolation, focal necrosis, pyknosis, sinusoidal dilation and congestion, possibly due to the bioaccumulation of PS-MPs or associated chemical leachates like styrene monomers. Liver vacuolization, often linked to lipid accumulation or mitochondrial damage, reflects cellular stress or altered metabolic activity. Similar hepatic damage has been reported in zebrafish and other model organisms exposed to micro- and nanoplastics (Ali et al., 2023; Hamed et al., 2021; A. P. D. K. Kumari, 2023; Tian et al., 2024). The oxidative stress generated by internalized particles may impair cellular antioxidant defenses and initiate apoptotic pathways, contributing to the observed histopathological lesions.

The kidney, essential for osmoregulation and excretion, showed signs of histological damage, including tubular degeneration, glomerular shrinkage, and congestion. These changes may compromise renal function, affecting filtration, excretion of metabolic wastes, and water balance. The observed nephrotoxicity may be attributed to direct accumulation of microplastics or associated inflammatory responses. Previous studies have similarly reported tubular deformities and glomerular abnormalities in fish exposed to microplastics, reinforcing our findings(Hamed et al., 2021; Liu et al., 2023; Usman et al., 2021).

The histopathological effects across multiple organs reflect the systemic toxicity of polystyrene microplastics, even at sublethal concentrations. Mechanistically, these changes may result from physical irritation, particle accumulation, oxidative stress, inflammatory responses, and chemical toxicity. Due to their small size, PS-MPs can closely interact with biological membranes and, under certain conditions, may even penetrate cells, triggering mitochondrial damage, lysosomal dysfunction, and immune activation—this dual mode of toxicity, both mechanical and chemical, is increasingly recognized as a significant concern in plastic pollution research. PS-MPs generate reactive species (ROS), compromise membrane integrity, and disrupt enzymatic activity, ultimately leading to cellular apoptosis and necrosis.

Furthermore, the organ-specific responses observed highlight tissue vulnerability based on physiological roles and exposure routes. Gills are directly exposed to the aquatic environment, the liver functions in detoxification and metabolism, and the kidneys play a key role in osmoregulation and excretion, making all three organs vital biomarkers in microplastic toxicology studies. The histopathological changes observed, even at relatively low concentrations during chronic exposure, indicate that PS-MPs can significantly compromise the physiological health of freshwater fish. This may adversely impact key biological functions such as growth, reproduction, and survival. As Carassius auratus serves as a representative model for freshwater ecosystems, these findings hold broader ecological implications, potentially affecting biodiversity and trophic interactions within freshwater food webs.

While the current study focused on tissue-level alterations, further research is warranted to investigate the molecular and biochemical pathways involved in PS-MP-induced toxicity. In particular, studies exploring gene expression related to oxidative stress, inflammation, and apoptosis could provide deeper insights into damage mechanisms. Moreover, using environmentally relevant mixtures of plastic types and associated chemical additives would offer a more realistic assessment of ecological risks posed by microplastic pollution.

# 6. Conclusion:

This study demonstrates that exposure to polystyrene microplastic (PS-MPs) of 1 μm size, even at sublethal concentrations, can induce significant histopathological alterations in vital organs of goldfish (Carassius auratus). The gills, liver, and kidney organs, critical for respiration, detoxification, and excretion, showed distinct and measurable tissue damage after chronic exposure. Observed changes, including epithelial lifting, lamellar fusion, hepatocytic vacuolization, necrosis, and renal tubular degeneration, highlight the vulnerability of freshwater fish to microplastic-induced toxicity.

These findings emphasize the systemic nature of PS-MP toxicity, likely mediated by physical interference, oxidative stress, and inflammatory responses. Given the increasing presence of microplastics in aquatic environments, such sublethal impacts can impair fish health, potentially affecting their survival, reproduction, and ecological roles.

This research contributes valuable evidence to the growing concern about microplastic pollution in freshwater ecosystems. The use of histopathological biomarkers in this study offers a sensitive and practical approach to evaluating the ecological risks of microplastics. Further investigations incorporating molecular, biochemical, and behavioural endpoints are essential to fully understand the long-term consequences of microplastic exposure on aquatic organisms and ecosystem health.

# Data Availability Statement:

The manuscript incorporates all datasets produced or examined throughout this research study.

# Ethical Statement:

This study did not use human subjects. However, animal models were used, and the authors declare that all experiments were performed according to the guidelines and regulations of the Institutional Animal Ethics Committee (IAEC). Fish handling and maintenance followed humane treatment protocols to minimize stress and discomfort.

# Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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