***Original Research Article***

Histopathological Alterations in Gills, Liver, and Kidneys of Goldfish (*Carassius auratus*) exposed to Polystyrene Microplastics

**Abstract**

Microplastics pollution presents a major ecological risk to globe. The present study investigates the histopathological effects of chronic exposure to 1 µm polystyrene microplastics (PS-MPs) at 1.53 mg/L in goldfish (*Carassius auratus*) over 28 days. Histopathological analysis showed the considerable alterations in the gills, kidneys, and lever of it. Gills showed the epithelial lifting, fusion of secondary lamellae and lamellar aneurysms. Liver tissues exhibited the hepatocellular vacuolation, sinusoidal congestion, necrosis, and nuclear degeneration. The kidneys displayed tubular degeneration, glomerular shrinkage, haemorrhage, and necrosis. These results highlight the potential risks that microplastics pose to freshwater species by clearly demonstrating PS-MP-induced organ-specific toxicity. In this way the present study highlights the urgent need for regulatory measures to mitigate the microplastic pollution in aquatic environments.

**Keywords: -** *Carassius auratus*, Histopathology, Polystyrene Microplastics, Gills, Liver, Kidneys

# 1. Introduction

Plastic pollution has become a major global environmental concern because plastic waste accumulates in the water ecosystems. This makes it harmful for biodiversity and ecosystem health. Plastic pollution has become a major environmental problem because plastic waste builds up in water ecosystems, which is harmful for biodiversity and ecosystem health (Mohammed et al., 2021). Plastics are synthetic polymers and are non-biodegradable in nature. So, they persist in the environment. Plastic wastes are majorly classified into four types based on the size of the particles, namely, nanoplastics, microplastics, mesoplastics, and macroplastics (Hammer et al., 2012). Nanoplastics are less than 1 µm in size and microplastics are from 1 µm to 5 mm in size. Microplastics (MPs) are of special concern because of their bioavailability and spread to different aquatic organisms (Hasan Anik et al., 2021).

A number of research in recent years has showed the impacts of microplastics on aquatic ecosystems (Avio et al., 2015). Since the industrial production of plastic bags began in the 1950s, global output has increased significantly (Stanley et al., 2025). This rise has led to considerable plastic buildup in both freshwater ecosystem (Bordós et al., 2019) and marine ecosystem (Barnes et al., 2009; Mihai et al., 2022).

Microplastics are either produced as small particles, such as microbeads for industrial or cosmetic use, or formed primarily through the degradation of larger plastic materials. Because of their small size, buoyant properties, and resistance to degradation, MPs readily enter aquatic systems through and industrial effluents, sewage discharge and urban runoff (Mihai et al., 2022; Rani, 2022; Thompson et al., 2005).

Polystyrene (PS), is a synthetic aromatic polymer derived from styrene monomers. It is among the most common types of plastic found in the environment. Polystyrene is commonly used in producing biomedical products, insulation foams, disposable cutlery, and packaging materials. Polystyrene exists in various forms, such as rigid plastic, expanded foam, and thin films. Despite of its variety of use, it contributes to the environmental pollution since its persistence, and potential to release toxic monomers like styrene and styrene oxide. Also, its recyclability is very low. These compounds are associated with various toxicological effects. For example, bioaccumulation across trophic levels, endocrine disruption, genotoxicity, and bioaccumulation across trophic levels (Hwang et al., 2020).

Although numerous studies have shown the impacts of microplastics (MPs) on aquatic organisms, a detailed organ-specific toxicity assessments are still required, especially for freshwater fish under chronic exposure conditions. The kidneys, liver, and gills, which are crucial for respiration, detoxification, and osmoregulation, are highly susceptible to environmental pollutants (Nugnes et al., 2022).

This study seeks to evaluate the histopathological changes in the kidneys, liver, and gills of goldfish (*Carassius auratus*) following extended exposure to polystyrene microplastics. It highlights the ecological risks of microplastic pollution. It also provides significant insights about the organ specific toxicological impacts of PS-MPs on freshwater fish.

# 2. Materials and Methods:

Healthy goldfish (*Carassius auratus*) specimens, with an average length of 7.5 ± 0.5 cm and weight of 20 ± 0.5 g, were procured from a local aquarium shop in Laheriasarai, Darbhanga, Bihar for use in this study. Once the goldfish reached to the lab, they were treated with a 0.1% Potassium permanganate (KMnO₄) solution so that external infections could be minimized. The goldfish were then acclimatized for 15 days in the lab to get them settled into their new environment. During this time period, the fish were fed one time daily with commercial floating pellets (Tetra Goldfish Gold Growth) at a rate of 3% of their body weight. This ensures the adequate nutritional intake and growth support. The water quality was managed in compliance with the American Public Health Association (APHA, 2005) guidelines. The temperature was maintained between 14 and 22 °C. The dissolved oxygen levels ranging from 6.62 to 6.76 mg/L. The pH values were kept within 6.5 to 8.5. Also, alkalinity ranged from 62 to 68 mg/L. No detectable free CO₂ was present. Merck/Sigma Aldrich Pvt. Ltd. supplied the 1 μm-sized polystyrene microplastics (PS-MPs) (Product No. 89904). These were supplied as an aqueous stock suspension containing 10% solids, with a density of 1.05 g/cm³. The stock solution was maintained at 4 °C and subjected to sonication before each use to ensure uniform particle dispersion. This confirms the consistency in experimental applications. A sublethal exposure concentration of 1.53 mg/L was determined based on a preliminary LC₅₀ assessment conducted in our laboratory. This ensures the biologically relevant toxicity evaluation. A total of 30 goldfish were randomly divided into two groups: a control group and a treatment group exposed to PS-MPs. Both groups were maintained in triplicate using six 70 L aquaria, each filled with 50 L of dechlorinated water. Five fish were stocked in each aquarium to ensure consistent density across tanks. The treatment group was continuously exposed to PS-MPs for 28 days, while the control group was kept under identical conditions without exposure.

For tissue collection, the fish were humanely euthanized, at the end of the exposure period, on the 29th day. Samples were taken from the gills, liver, and kidneys of both control and treated groups. Each sample was cut into sections 3–4 mm thick. The tissues were then fixed in 10% neutral buffered formalin. Fixation lasted for 18 to 24 hours. This was then dehydrated in ascending ethanol series and embedded in paraffin wax following standard histological techniques. Thin sections of 5–7 µm thickness were prepared using a rotary microtome. The sections were stained using Ehrlich’s haematoxylin and alcoholic eosin (H&E), as described by (Luna, 1968). This was examined under a light microscope. Photomicrographs were captured to record and assess the histopathological changes resulting from PS-MP exposure.

# 3. Experimental Design:

The experimental design was developed based on a comprehensive review of existing literature to maintain both relevance and scientific accuracy. This study used goldfish (*Carassius auratus*) as the test organisms, which were randomly divided into two groups: a control group and a treatment group. The treatment group was exposed to PS-MPs at a concentration of 1.53 mg/L with a particle size of 1 µm. The reason behind selecting this concentration as a sublethal dose was based on the existing LC50 obtained via existing bioassay tests conducted on goldfish in our laboratory and effectively assess potential histopathological alterations in the exposed test organisms. A total of 30 goldfish were randomly distributed across six tanks, with both control and treatment group maintained in triplicate. The setup comprised 70 L glass aquaria, each filled with 50 L of properly dechlorinated water. A total of five goldfish were taken in each aquarium (n = 5), which ensures uniform solution density among all experimental units. To maintain standard water quality parameters, all tanks were continuously aerated. Using PS-MPs stock solution, the treatment tanks were dosed. While the control tanks were maintained under identical conditions but without any PS-MPs concentrations. The microplastic concentration and the exposure period were optimized to mimic realistic conditions for accurate toxicological assessment.

# 4. Results:

Goldfish (*Carassius auratus*) exposed to sublethal concentrations of polystyrene microplastics (PS-MPs, 1 µm) displayed notable tissue-level abnormalities in the gills, liver, and kidneys.

4.1. Histopathology: The gills, liver, and kidneys of *Carassius auratus* exposed to PS-MPs exhibited notable histopathological alterations.

4.2. Gill: The gill structure of goldfish adheres to the standard anatomical configuration seen in teleost fish. The structure comprises primary and secondary lamellae, supported by a cartilaginous skeletal framework. These lamellae are enveloped by a stratified epithelial layer and accompanied by a dense vascular network. They facilitate efficient gas exchange and physiological functions. The primary lamellae are flat, leaf-like projections arranged in double rows. From these, the secondary lamellae extend laterally in an alternating fashion. These secondary lamellae are covered by thin squamous epithelial cells and are supported internally by pillar cells (Figure 1).

Notable histopathological changes were detected in the gill tissues of goldfish exposed to PS-MPs. These include lamellar aneurysms, protrusion/bulging of the secondary lamellar epithelium, deviation of lamellar structure, and fusion of secondary lamellae (Figure 2). Statistical evaluation showed a significantly higher incidence of lamellar fusion in the treated group when comparing to the control group This highlights the extent of damage caused by PS-MP exposure. These morphological changes in the body suggest compromised respiration. As a result, the body may struggle to take in enough oxygen. Essentially, these changes could make breathing harder and cause more strain on the system.

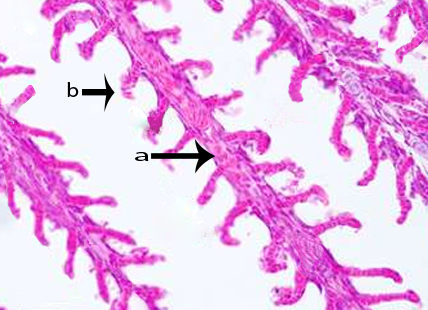


Figure 1: Light micrograph of gill tissue of goldfish (*Carassius auratus*) showing normal architecture, including primary gill lamellae (a) and secondary gill lamellae (b), using H&E stain at 100x magnification.

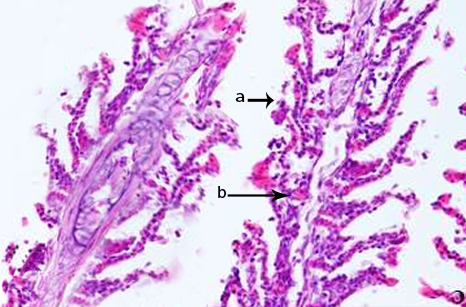


Figure 2: Light micrograph of gill tissue from *Carassius auratus*, exposed to 1 μm polystyrene microplastics (1.53 mg/L) for 28 days, exhibiting fusion of secondary lamellae (a), and lamellar aneurysm (b). Stained with Hematoxylin and Eosin (H&E), observed at 100x magnification.

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 (Hamed et al., 2021; Lu et al., 2016).

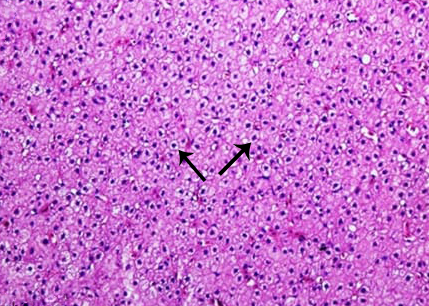


Figure 3: Light micrograph of a representative liver section from the control group of goldfish (*Carassius auratus*), showing normal histoarchitecture. Hepatocytes are indicated by arrowheads. Tissue stained with Hematoxylin and Eosin (H&E) and viewed at 40x magnification.

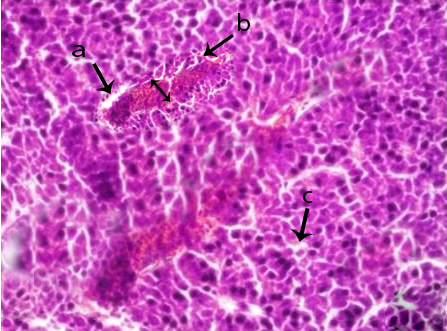


Figure 4: Light micrograph of liver section from treated group of goldfish (*Carassius auratus)* exposed to polystyrene microplastics (1 µm; 1.53 mg/L), showing marked histopathological alterations. Double arrowhead indicates sinusoidal dilation and congestion. Pyknosis (a), hepatocyte necrosis (b), and vacuolization (c) are also observed. Tissue stained with Hematoxylin and Eosin (H&E) and observed at 100x magnification.

4.4. Kidney:The renal tissue of the control goldfish 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. Similar toxic effects observed in microplastic-exposed zebrafish, Nile tilapia, and guppy fish (Bakieva et al., 2024; Elshaer et al., 2013; Hamed et al., 2021).

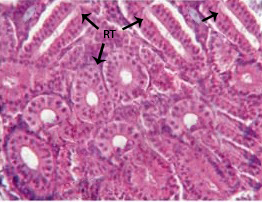


Figure 5: Photomicrograph of the control kidney of *Carassius auratus* (goldfish) showing normal histoarchitecture at 400× magnification. Renal tubules (RT) appear well-organized with no signs of degeneration or pathological alterations.

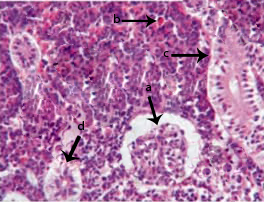


Figure 6: Photomicrograph of kidney tissue of *Carassius auratus* exposed to polystyrene microplastics (400x), showing marked histopathological alterations. Glomerular shrinkage (a), hemorrhage (b), necrosis (c), and degeneration of tubular epithelium (d) are observed. including tubular degeneration, 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 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. These changes hinder respiratory efficiency and disrupt ion regulation, both of which are vital for the survival of fish in aquatic environments. These findings are consistent with previous studies that have reported similar or comparable gill damage in zebrafish and other fish species following exposure to microplastics (Bakieva et al., 2024; R. Kumari, 2021). Epithelial lifting could serve as a protective response. This minimizes the contact surface area with toxicants. Due to prolonged exposure, irreversible tissue damage is indicated by lamellar fusion and necrosis.

The liver which is an important organ for metabolism and detoxification, exhibited notable histological damage, like congestion, focal necrosis, hepatocyte vacuolation, pyknosis and, sinusoidal dilation. These alterations may be attributed to the bioaccumulation of PS-MPs or the presence of associated chemical leachates such as styrene monomers. Liver vacuolization, commonly associated with lipid buildup or mitochondrial dysfunction. This signifies cellular stress and disrupted metabolic processes. Similar liver damage has been seen in zebrafish and other model organisms upon exposure to microplastics and nanoplastics (Hamed et al., 2021; A. P. D. K. Kumari, 2023; Tian et al., 2024). The Oxidative stress induced by the internalized particles may compromise cellular antioxidant defences, which can trigger apopotic pathways and thereby contributing to the observed histopathological lesions.

Similarly, the kidney, which is also an important organ for osmoregulation and excretory function, exhibited histopathological alterations, including tubular degeneration, glomerular atrophy, and vascular congestion, which may compromise renal function. In these ways they affect filtration, excretion of metabolism wastes, and water balance. The noted nephrotoxicity may be due to direct renal accumulation of microplastics or secondary inflammatory pathways triggered by their presence. The existing studies also supports the evidences of tubular deformities and glomerular abnormalities in fish exposed to microplastics (Hamed et al., 2021; Liu et al., 2023; Usman et al., 2021).

The histopathological alterations were observed across multiple organs even at sublethal exposure levels, indicating the widespread toxicity of PS-MPs. This means these are toxic even at low concentrations. These alterations may arise from a combination of chemical toxicity, inflammatory responses, oxidative stress, particle accumulation, and physical irritation. The PS-MPs can closely interact with biological members because of their small sizes. They can penetrate cells, leading to immune activation, lysosomal dysfunction, and mitochondrial damage. This mode of toxicity is increasing day by day, is a major concern in plastic pollution research.

PS-MPs have been shown to generate the reactive oxygen species (ROS), which compromise the membrane integrity, and disrupt the enzymatic activity. As a result, cellular apoptosis and necrosis were observed, which highlight the potential of PS-MPs to induce severe biological damage. Additionally, the observed organ-specific responses highlights tissue susceptibility, which differs according to exposure routes and physiological functions. Gills are the primary sites of microplastic exposure as they are in the direct contact with the aquatic environment.

The liver plays an important role in processing contaminants as it is responsible in detoxification and metabolism. Also, the kidneys aid in eliminating the harmful substances. In these ways, the three organs, Gills, Kidneys, and liver act as vital toxicological markers in MP-toxicity studies. Such responses indicate the widespread physiological effects of environment pollutants. The histopathological alterations observed, even at chronic sublethal concentrations indicate that PS-MPs can effectively impair the physiological health of freshwater organisms. Such types of disruptions could adversely affect the vital biological functions including growth, reproduction, and overall survival. Since, *Carassius auratus* serves as a suitable model organism for freshwater habitats, these findings carry broader ecological relevance, impacting biodiversity and trophic interactions inside freshwater food chains. Therefore, continued investigations into toxic effects to PS-MPs on freshwater organism under sublethal exposure is important for understanding long term ecological threats.

Although the present study mainly addressed histopathological alterations, additional research is warranted to elucidate the molecular and biochemical pathways central to polystyrene microplastic toxicity. Investigating gene expressions related inflammation, oxidative stress, and apoptosis may provide a deeper understanding of the mechanisms that cause tissue damage. Additionally, using environmentally realistic mixtures of plastic polymers and their chemical additives might offer a more thorough and accurate assessment of the ecological risks due to microplastic pollution. This will help bridge the gap between laboratory findings and real-world ecological outcomes.

# 6. Conclusion:

The study highlights the harmful effects of polystyrene microplastics (PS-MPs) on the vital organs of freshwater goldfish (*Carassius auratus*) following chronic sublethal exposure. Pronounced histopathological changes were identified in the gills, liver, and kidneys, highlighting distinct organ-specific toxic effects. Structural damage observed in the gills included lamellar aneurysm, lamellar fusion, and epithelial lifting. These indicate compromised respiratory process. The liver exhibited hepatocellular vacuolation, necrosis, pyknosis, sinusoidal dilation, and congestion, indicating impaired metabolic and detoxification functions. Histopathological analysis of the kidneys revealed glomerular shrinkage, necrosis, and tubular degeneration. This also suggests impaired renal function and compromised physiological processes.

The data clearly demonstrate that even low-level, prolonged exposure to PS-MPs can cause significant organ-specific damage, likely driven by mechanisms including oxidative stress, inflammation, and mechanical interference. The application of histopathological biomarkers, as demonstrated in this study, serves as a sensitive and reliable approach for identifying early toxicological effects of microplastic contamination in aquatic organisms.

This research offers valuable evidence contributing to the growing concern over microplastic pollution in freshwater ecosystems. These findings underscore the urgent need for enhanced waste management strategies and the enforcement of more stringent regulatory measures to effectively curb microplastic pollution.

# Data Availability Statement:

The manuscript includes all datasets generated or analyzed during this research, ensuring comprehensive documentation and transparency in the study's findings.

# Ethical Approval:

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)

Authors 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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