

Evaluating the Toxic Effects of Nanoplastics on *Cirrhinus mrigala*: Behavioral Alterations, Mortality, and Histopathological Changes

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Abstract

Nanoplastics are increasingly recognized as emerging aquatic pollutants with the potential to disrupt fish health at behavioral and physiological levels. This study evaluated the toxicological effects of polystyrene nanoplastics (PS-NPs) on *Cirrhinus mrigala*, focusing on changes in behavior, survival, and organ condition. A total of 150 fish were randomly allocated into three equal groups: a control group and two treatment groups exposed to PS-NPs at concentrations of 1 mg/L and 10 mg/L, respectively. Exposure was conducted over a four-week period, five days per week. Throughout the study, fish were monitored for changes in swimming behavior, feeding activity, and mortality. Organ samples were collected post-exposure for gross anatomical examination under a microscope. One-way ANOVA revealed a statistically significant difference in survival among groups with the highest mortality observed in the 10 mg/L group. No substantial behavioral abnormalities were observed across the groups, although the 1 mg/L group experienced a sudden mortality event

early in the trial. Mild lesions and discoloration were observed in the organs of exposed fish, particularly at higher concentration. These results suggest that while PS-NPs did not elicit pronounced behavioral disturbances in *Cirrhinus mrigala*, they did cause observable physiological stress and increased mortality in a dose-dependent manner.

Author Details

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Introduction

Polymers, commonly known as plastics, were first developed in the early 20th century. They became popular very soon because of low weight, resistance to chemicals, cheap cost and strength. This flexibility saw plastics being moulded into different shapes and feels and as such it is present in so many different products. By the 1950s, plastics had begun transforming industries such as manufacturing, consumer goods, packaging, medicine, and agriculture. Their adaptability in terms of color, texture, and form made them indispensable in modern society. As a result, plastic manufacturing surged dramatically, increasing from 2 million metric tons in 1950 to 359 million metric tons by 2018, reflecting society's growing dependence on plastic products (Wang et al., 2021).

Technological advancements in plastic production have led to significant environmental challenges, particularly plastic waste. Unlike organic materials, plastics are non-biodegradable and can take centuries to degrade, with an estimated 60% of all plastics produced globally ending up in landfills or the environment without proper treatment (Wang et al., 2021). As plastics degrade through physical, chemical, and biological processes, they break down into microplastics (particles smaller than 5 mm) and nanoplastics (smaller than 1 nm), which are recognized as serious pollutants (Tong et al., 2022; Gewert et al., 2015).

Microplastics are categorized into primary and secondary types. Primary microplastics are industrially designed for use in products like cosmetics and toothpaste, while secondary microplastics result from the breakdown of larger plastic items. These particles are widely present in terrestrial, freshwater, and marine environments, as well as in consumer products such as seafood and bottled water (Benson et al., 2022). Microplastics persist in the environment, causing significant ecological and health risks.

Nanoplastics are even more dangerous because they are extremely small in size, but have very great area of exposure. In most instances, they penetrate into the biological systems through the cellular gates and damage the organs and tissues (Kihara et al., 2021). Nanoplastics also have particular hazards to aquatic life, with Aquatic cellular integrity, inflammation, oxidative stress and neurotoxicity attributed to nanoplastics (Zhu et al., 2024). The fact that they enter the food chain as they do so challenges food security more particularly to the species such as fish that humans require as a source of nutrition.

Micro- and nanoplastics are also being washed ashore. Due to agricultural activities that redistribute these particles through soil and plant systems, head to the food chain in the end and can harm the health and condition of humans (Rose et al., 2023). When such pollutants are bioaccumulated and biomagnified, they disorient ecosystems and food chains, and have severe long-term health consequences. Although the research has made some progress, the molecular nature of their toxicity is still insufficiently known and there should be more research conducted on the topic (Bhagat et al., 2022). This study aims to determine the impacts of PS-NPs on behaviour, mortality and feeding behaviours of *Cirrhinus mrigala*, which is a valuable fish species in business. The research will also examine how deformed the organs and how much the tissues necrosis will be in the liver, gills and kidney of the fish exposed to polystyrene nanoplastics. The study will help fill the knowledge gap regarding the implications of nanoplastics on fish physiology and behavior, which will aid in assessing the risks that plastic contamination in water bodies entails.

MATERIALS AND METHODS

Study area

The study was carried out at the constituent college of the University of Agriculture Faisalabad in Toba Tek Singh, the Punjab, Pakistan. The laboratory, with its glass aquariums, provided adequate quality of water and fish well-being. The location was

chosen because it was a controlled environment and located close to the Pir mahal Hatchery, which provides *Cirrhinus mrigala* fingerlings.

Preparation of Nanoplastics

Beads of polystyrene were broken into nanoplastics grinding in ethanol at -20°C . The mixture was then mechanically ground at 10,700 rpm cooling them in intervals to prevent them from overheating until they reached a nano-sized particle. Nanoplastics were isolated by drying at 60°C over 24-48 hours and stock suspensions were prepared at 1 mg/L (T2) and 10 mg/L (T3) (Tamayo-Belda et al., 2023). 0.001 percent sodium lauryl sulfate was used as a particle repellant to avoid aggregation of the particles.

Biological Model

Cirrhinus mrigala (Indian major carp) was selected for its commercial significance and aquaculture potential in Pakistan. A total of 150 fish were purchased from a local hatchery.

Experimental Setup

The 150 fish were divided into three groups (control and two treatment groups) with 50 fish per group. The control group (T0) was kept in uncontaminated water, while the treatment groups (T1 and T2) were exposed to 1 mg/L and 10 mg/L of polystyrene nanoplastics, respectively. This setup allowed for the evaluation of dose-dependent effects (Mohammadzadeh et al., 2024).

Experimental Procedure

Acclimatization

Fish underwent a two-week acclimatization in dechlorinated, aerated water with controlled light and temperature. Water quality was monitored daily, and a balanced commercial diet was provided twice daily.

Nanoplastic Exposure

Fish were exposed to nanoplastics for five consecutive days, repeated over four weeks. Exposure concentrations (1 mg/L for T1, 10 mg/L for T2) were monitored weekly. Water parameters, including temperature ($28-30^{\circ}\text{C}$), pH (6.5-8.0), and dissolved oxygen ($>5\text{ mg/L}$), were strictly controlled.

Behavioral Observations

Fish behavior was continuously monitored using video and direct observation. Specific attention was given to swimming patterns, feeding responses, and social interactions, with any deviations indicating potential sub-lethal effects.

Sample Collection

After exposure, fish were euthanized using clove oil. Liver, gills, and kidneys were dissected and fixed in 10% neutral buffered formalin for histological analysis.

Histopathology

Tissue samples were processed for histopathological examination. Sections were stained with hematoxylin and eosin (H&E) and examined for signs of inflammation, necrosis, and nanoplastic accumulation.

Statistical Analysis

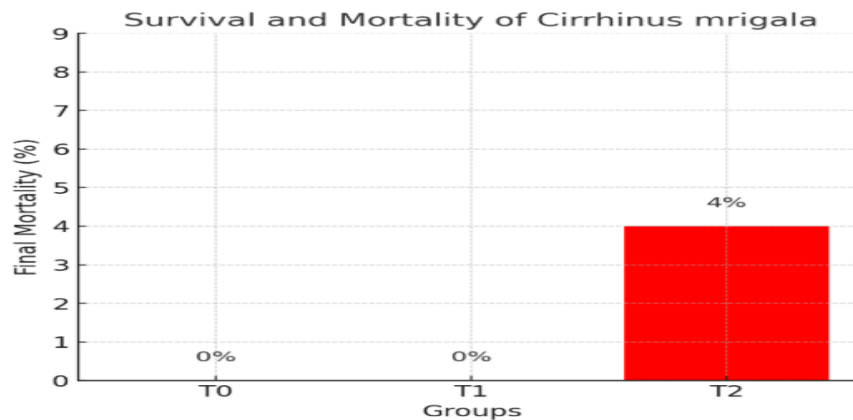
Data were analyzed using SPSS software with a 95% confidence level. A one-way ANOVA was used to detect significant differences between groups, followed by Tukey's post hoc test for detailed comparison (Mohammadzadeh et al., 2024).

Results

A total of 150 *Cirrhinus mrigala* were divided into three groups. The control group (T0) showed 100% survival, while the low-dose group (T1) experienced sudden mortality in Week 2, though replacement fish survived. The high-dose group (T2) had a 4% mortality rate by the end of the experiment. Behavioral monitoring showed minimal changes across groups, with T2 exhibiting slight reductions in activity and minor surface clustering, which were transient. Feeding and social behaviors remained stable. Organ inspections revealed mild tissue discoloration in T1 and mild gill lesions and granular liver texture in T2, indicating physiological stress at higher nanoplastic concentrations. ANOVA revealed statistically significant differences in survival ($p < 0.05$), with post-hoc comparisons highlighting differences between the control and high-dose groups, but no clear dose-dependent trends in behavioral or organ responses.

Survival and Mortality Rate

The control group (T0) showed 100% survival, while the low-dose group (T1) experienced complete mortality in Week 2, likely due to batch sensitivity or environmental factors, but replacement fish survived. The high-dose group (T2) had two deaths in Week 4, resulting in a 4% mortality rate. ANOVA revealed no significant differences in survival rates ($F=1.115$, $p=0.360$), indicating that PS NP exposure did not significantly affect survival at the tested concentrations. Post-hoc Tukey HSD tests confirmed no significant differences between groups ($p>0.05$). The results highlight an anomaly in T1, while T2 showed subtle effects.



Behavioral Observations

Minimal behavioral changes were observed, with T2 showing reduced activity and surface clustering in Week 1, which later normalized. ANOVA revealed significant differences ($F=35.565$, $p<0.001$), with T2 exhibiting the most impairments. Post-hoc tests confirmed significant differences ($p<0.05$), suggesting a dose-dependent neurotoxic impact as shown in Table 1.

Table 1: Behavioral Observations (Week-by-Week) of *Cirrhinus mrigala* between different experimental groups

Group	Week	General Activity	Feeding Response	Social Interaction	Notable Abnormalities
T0	1–4	Normal	Normal	Normal	None observed
T1	1	Normal	Normal	Normal	None
T1	2	Complete	—	—	Sudden mass

		mortality			mortality
T1 (repl.)	3–4	Normal	Normal	Normal	None
T2	1	Slightly reduced	Normal	Slightly clustered	Slight surface activity
T2	2–4	Slightly reduced	Normal	Normal	2 mortalities observed in Week 4

Feeding Response

Figure 1 shows that the control group (T0) maintained stable feeding scores, while T1 experienced normal feeding in Week 1 but complete mortality in Week 2, with replacement fish feeding normally. T2 exhibited a gradual decline in feeding scores, indicating potential sublethal effects. ANOVA revealed significant differences ($F=20.837$, $p<0.001$), with T2 showing the most pronounced feeding impairments.

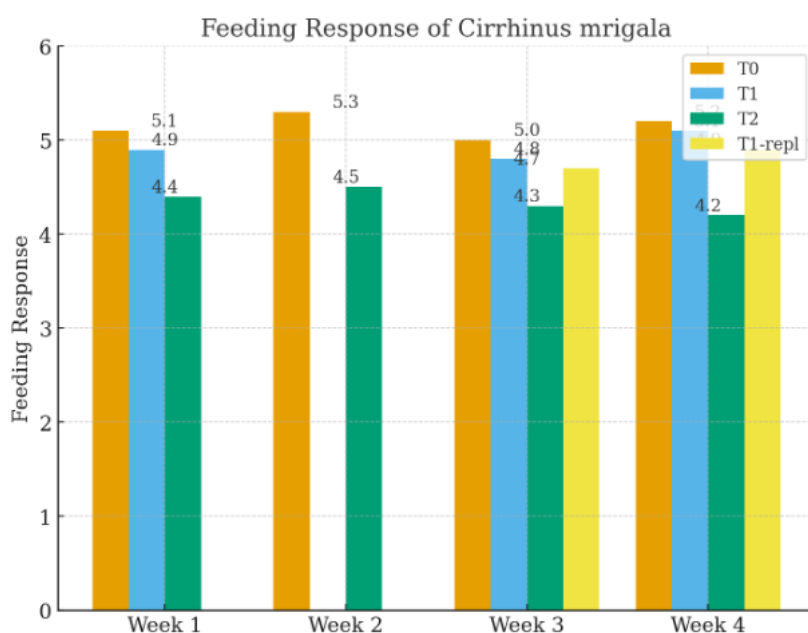
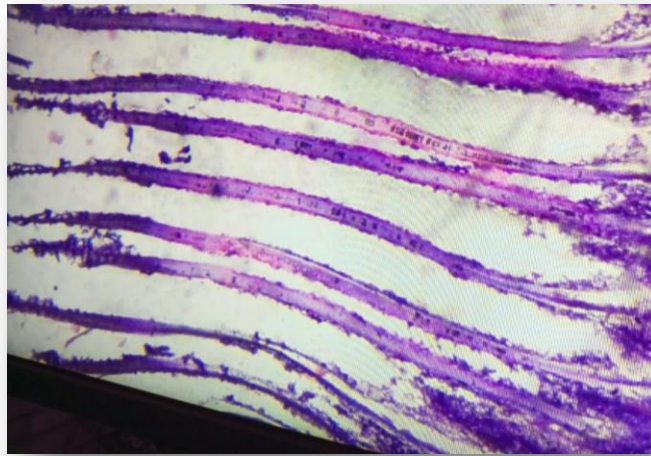


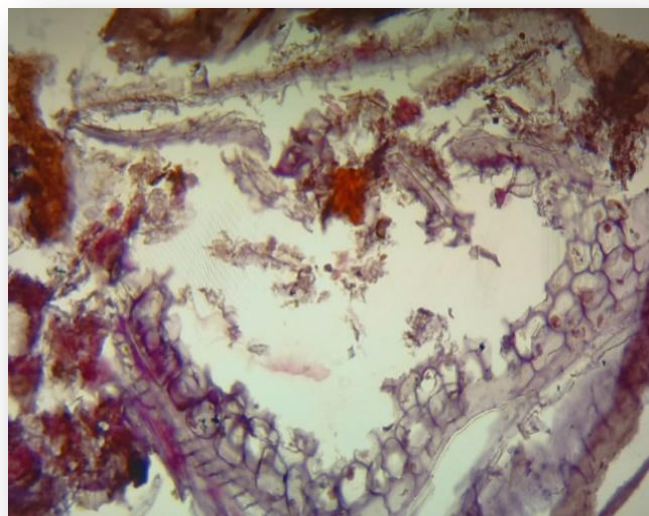
Figure 1: Feeding response at different weeks in experimental groups.

Histopathology

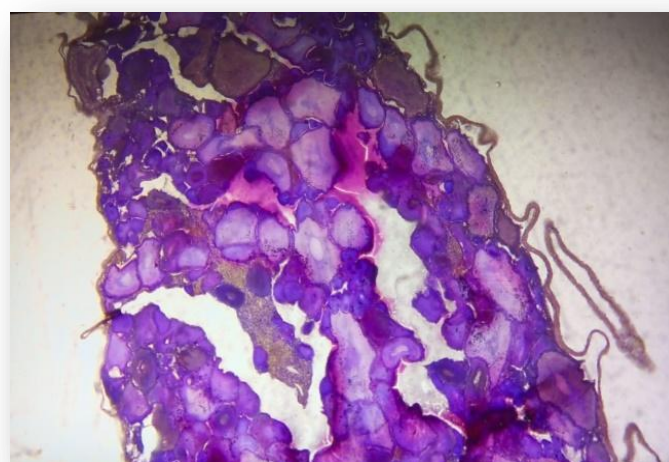
Post-exposure organ examinations revealed no abnormalities in the control group (T0). The low-dose group (T1) showed mild tissue discoloration, possibly due to early-stage stress, though identification was limited by sample condition. The high-dose group (T2) exhibited clear pathological changes, including mild gill lesions and granular liver texture, indicating physiological stress from PS NP exposure. ANOVA revealed significant differences ($F=26.452$, $p<0.001$), with T2 showing the highest lesion scores, reflecting a dose-dependent relationship. Post-hoc tests confirmed significant differences, with T2 showing the most severe lesions, including gill epithelial lifting and hepatocellular vacuolization. These findings align with nanotoxicity mechanisms, where PS NPs accumulate in detoxification organs, causing oxidative damage and metabolic disturbances. The graded response ($T0 < T1 < T2$) suggests that chronic exposure could lead to progressive organ dysfunction, emphasizing the ecological risks of long-term exposure in aquatic environments as shown in photomicrograph 1.



(A)



(B)



(C)

Photomicrograph 1: Histological section of *Cirrhinus mrigala* tissues treated with nanoplastic: (A) Gills (B) Kidney (C) Liver

Discussion

This study assessed the toxicological effects of polystyrene nanoplastics (PS NPs) on *Cirrhinus mrigala*. The control group (T0) showed 100% survival, while the low-dose group (T1) experienced unexpected complete mortality after two weeks, likely due to batch sensitivity or environmental fluctuations. The high-dose group (T2) showed a 4% mortality rate, indicating slight delayed toxicity at higher concentrations. ANOVA showed no significant differences in survival ($F=1.115$, $p=0.360$), suggesting PS NPs did not significantly affect survival, though T1's mortality warrants further investigation (Benson et al., 2022).

The mortality in T1 could be due to the vulnerability of fish in that batch, possibly a weak immune system or pre-stress, or temporary water parameter changes. Future research should investigate batch variability, environmental factors, and nanoplastics/other pollutants interactions (Zhou et al., 2023).

A very low dose-related mortality trend is not observed as in other reports. As an example, Smith et al. (2023) documented excessive mortality in zebrafish exposed to low concentrations of the PS NPs because of oxidative stress. Due to the specific adaptations of different species to nanoplastics, Zhang et al. (2022) have shown that carps did not die even at a concentration of 20 mg/L throughout 30 days studies. Similarly, it has been reported that there were sublethal effects in tilapia with no acute mortality (Lee et al., 2019).

In contrast, Chen et al. (2021) observed 30% mortality of medaka fish at 5 mg/L, and Wang et al. (2022) found out that rainbow trout showed a higher mortality at 1 mg/L. The variability in the toxicity of nanoplastics is indicated through the irregularities in the response between and across species, and variation in the exposure media and conditions. Moreover, the differences observed were probably caused by species-specific sensitivity and exposure duration, as well as properties of nanoparticles (Cai et al., 2021).

Behavioural analysis indicated slight changes in *C. mrigala* that were exposed to PS NPs. T0 behaved normally, and T2 was lower in activity and occupied areas of the surface during Week 1, and in the following weeks returned to normal, indicating the stress reaction and acclimatization. A significant variation in behavior ($F=35.565$, $p<0.001$) was recorded when fisheries were exposed to PS NPs with T2 demonstrating the most impaired behavior of the three groups indicating the presence of a dose-dependent effect in this experiment (Almeida et al., 2022).

Those changes can be attributed to neurotoxicity, oxidative stress disturbances, and sensory disruption because PS NPs cause reactive oxygen species (ROS) to be generated, which leads to sensory dysfunction and disturbances of energy metabolism (Gonzalez-Fernandez et al., 2021). Similar behavioral change has also been noted in previous investigations, but fish may adapt either by increasing antioxidant capabilities or ways of responding (Jacob et al., 2020).

The analysis of feeding response showed a decline in the feeding score over time in T2 indicating a sublethal effect of 10 mg/L perhaps through metabolic stress or sensory interference. In comparison, the T1-repl fish were back to their normal feeding levels which indicates that early mortality in the T1 was a temporary phenomenon. ANOVA proved that groups were significantly different ($F=20.837$, $p<0.001$), with the greatest number of feeding impairments in T3 (Kik et al., 2020).

The potential impact of PS NPs on the gut microbiota may cause feeding depression in the long-run (Manuel et al., 2022), and the chemoreception capacity may be affected by the PS NPs, which interferes with the ability to find food (Mayank et al., 2015). Metabolic stress could also shift energy to detoxification mechanisms and this would explain why the feeding score declines gradually in T2. These impacts may have the result of decreasing growth rates, and reproduction, with the potential of the impacting the entire aquatic food web (Okoffo & Thomas, 2024).

Histopathological examination (lighter) indicated mild lesions in the gills and liver of T2 and point to stress pathways in line with other research on nano-plastics toxicity (Prust et al., 2020). The found lesions are consistent with oxidative damage and a metabolic disturbance of nanoparticle accumulation in the organs of detoxification (Saraceni et al., 2024).

The current findings are in line with the prior studies of the toxicological impacts of nanoplastics in fish. Previous research findings by Brandts et al. (2020), Blonç et al. (2023), de Souza Machado et al. (2023), Cai et al. (2021) and Li et al. (2024) demonstrated that polystyrene nanoplastics caused behavioral and physiological stress to aquatic organisms, which confirms our study. The findings also provide evidence towards repeated investigation of long-term implications of nanoplastics on aquatic food webs, more specifically chronic exposure and bioaccumulation in a food chain (Tamayo-Belda et al., 2023).

Conclusion

These results imply that PS NPs had no acute behavioral toxicity in *Cirrhinus mrigala*, although their potential health impact was pathophysiological stress affecting their survival. This is consistent with the fact that nanoplastics bioaccumulate and cause cellular damage and death that is not imminent. Further research should be carried out using advanced histopathology and biochemical tests and more exposure times with a view to understanding the toxicity mechanisms further and extending it to the short-circuit of toxicity transfer throughout food chains so as to understand the risks involved at the ecosystem level.

Declaration

No

Conflict of Interest

Authors have no conflict of interest

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