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REVIEW

Danio rerio as a model animal for assessing microplastic toxicity

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Abstract
Microplastics are presently a major worldwide hazard, whether they come from massive plastic breakdown or directly from consumer and industrial items. By altering their physicochemical characteristics, weathering processes of plastics cause the fragmentation of polymers into microplastics. Due to delayed disposal of plastic waste, a lack of detecting equipment and specific removal techniques and a slow disposal rate, microplastics are prevalent in the environment. Microplastics are tiny enough to be easily absorbed. As a result, the presence of microplastics poses a hazard to both live species present in benthic zones and the water column. Microplastics therefore have non-lethal impacts on these creatures. In the present paper, various studies have been reviewed to find the effects of microplastics on *Danio rerio* (Zebrafish). Various microplastics caused epithelial damage, lipid accumulation in the liver, metabolic changes, adverse effects on gonads, obstructed fin regeneration and contraction parameters in the heart tissue of *Danio rerio*. Exposure to polystyrene decreased the bioavailability and bioaccumulation of F-53B which significantly reduced the body weight of *Danio rerio* larvae and resulted in oxidative stress and inflammation. Other sublethal effects included significant decrease in swimming distance as well as speed. Acetylcholinesterase activity of *Danio rerio* was remarkably inhibited by the microplastic exposure which further induced the immune responses. These findings emphasise the health hazards associated with microplastic pollution in aquatic ecosystems. Further studies are needed to assess the health impacts associated with microplastic exposure and their possible remedies.

Keywords: Microplastics; Toxicity; Pollution; *Danio rerio*; Hazard; Zebrafish

Introduction
Due to the mechanical and photochemical processes that lead to the production of microplastics, plastics that enter the coastal environment may continue to exist for millions of years after they are broken down (Singh, 2022). Plastics may contain polyurethane, polyethylene terephthalate (PET), polyester and polypropylene (Drobne et al., 2022). Face masks used during COVID times are another threat to the environment. The face masks left over normally contain a lot of polypropylene which generates tremendous toxicity in ocean beds and great lakes, when left as such. Various forms of microplastics were present in the inner frontal, middle filtering, and outer layers of masks. In a study, fibres were collected from the inner and outer layer but uneven fragments were seen in the middle layer. It was clear that the material was damaged during cryo-milling since the form of the collected microplastics was different from the original fibrous structure of the whole medical mask layers. The inner layer contained chemicals related to antimicrobial function and flavouring. The other two layers were also found to be composed of antioxidants and their degradation products, plasticizers, cross-linking agents, antistatic agents, lubricants and non-ionic surfactants. Discharge of microplastics from 18 brands of the medical mask into the water was observed (Chen et al., 2021). Degradation processes are the result of UV rays, temperature changes, increasing humidity, biodegradation, physical abrasion, and chemical oxidation. Weathering influences the physicochemical properties of plastics finally leading to their fragmentation into microplastics. The degradation of plastics is extremely dependent on the polymer type and any chemical additives. Polypropylene is vulnerable to photodegradation, heat and atmospheric oxygen. UV weathering of the masks has been found to affect the release of microplastics. They discovered that a single, unopened disposable mask might release about 483,888 plastic particles if it starts to degrade, while a damaged mask could release 1,566,560 plastic particles. More work should be done to examine the polypropylene microplastics from surgical masks' environmental dangers and how to combat this new source of environmental challenge (Kokalj et al., 2022).

It has also been shown that microplastics are present in the tissues of many wild fish species. Organisms continuously ingest microplastic from the environment directly or by trophic transfer due to widespread microplastic pollution (Chen et al., 2021). This ingestion causes severe effects on the health of organisms including reduced growth, decreased survival rate, intestinal damage, changed protein profile, blocked digestive tract, limited food intake and decreased reproduction (Cunningham et al., 2022). Toxicological and environmental research on microplastic has mainly focused on thermoplastics such as polyethylene and polystyrene. High density polyethylene and polystyrene can also cause changes in the expression of genes of the immune system of *Danio rerio* (Zebrafish). These plastics were found to increase the number of neutrophils in gills and intestinal epithelium of *Danio rerio*. Findings from transcriptome and histological studies suggest that immune response and the integrity of the mucosal epithelium might harm the *Danio rerio*'s defences against microbes and change how the energy reserves are used (Limonta et al., 2019). Over the past 15 years, extensive research efforts have been made to account for the global occurrence, distribution and potential environmental dangers of microplastics and their associated chemicals. Research on the potential unfavourable effects of microplastics on organisms has been very intense over the past decade which has confirmed physiological concern in organisms disclosed to microplastics and potential links to altered ecosystem functions (Kokalj et al., 2022).

Table 1. Studies revealing toxic effects of microplastics

Sr. No.	Author	Year	Animal model	Inference
1.	Limonta et al.	2021	*Danio rerio*	Uptake of MPs caused epithelial damage and neurotoxicity.
2.	Lu et al.	2016	*Danio rerio*	PS-MPs disturbed the lipid and energy metabolism.
3.	Limonta et al.	2019	*Danio rerio*	MPs indicates variations in the expression of immune system genes.
4.	Gu et al.	2020	*Danio rerio*	MPs exposure obstructed fin regeneration.
5.	Dimitriadi et al.	2021	*Danio rerio*	PS-MPs reduced heart functions.
6.	Qiang et al.	2021	*Danio rerio*	MPs caused adverse effects on reproductive organs.
7.	Qiao et al.	2019	*Danio rerio*	Three shapes (bead, fragment and fibre) of microplastics caused multiple toxic effects in the intestine of fish.
8.	Pie et al.	2022	*Danio rerio*	Remarkable changes in the richness and diversity of gut microbiota were observed after PS-MPs.
9.	Yu et al.	2022	*Danio rerio*	Waterborne MPs exposure directly effects the tissues while foodborne MPs exposure effects the swimming behaviour of fish.
10.	Yang et al.	2020	*Danio rerio*	Combined exposure of PS-MPs and F-53B increased oxidative stress and inflammatory response in *Danio rerio* larvae.
11.	Qiang et al.	2019	*Danio rerio*	MPs significantly decreases larval swimming competence.
12.	Chen et al.	2020	*Danio rerio*	On exposure to MPs, *Danio rerio* became hyperactive and stayed at manic and active states for much longer times.
13.	Limonta et al.	2021	*Danio rerio*	MPs exposure could induce the activation of immune response and xenobiotic metabolism.

Effects of microplastics on *Danio rerio*

Due to large production, improper use, and disposal of plastics, microplastics have become global environmental pollutants affecting both freshwater and marine ecosystems. Numerous studies have established the biological impacts of microplastic absorption in wild animals, including epithelium injury, inflammation, metabolic changes, and neurotoxicity (Limonta et al., 2021). Histopathological analysis showed that Polystyrene microplastics (PS-MPs) caused inflammation and lipid accumulation in fish liver. Superoxide dismutase and catalase activity were both considerably elevated following MP treatment with PS-MPs, demonstrating that oxidative stress was generated. In addition, metabolomic profiles in fish liver disturbed the lipid and energy metabolism. These findings reveal information on the harmful effects of MPs on *Danio rerio* (Lu et al., 2016). Twenty days were spent exposing adult *Danio rerio* to two

concentrations of microplastics composed of polystyrene and high-density polyethylene. According to transcriptomic findings, immune system genes are expressed differently while genes associated to epithelial integrity and lipid metabolism are downregulated. Mucosal epithelium integrity and immune response could potentially decrease the organism defence against micro-organisms. Histological analyses of fish proved that MPs might cause a potent inflammatory response in the target organs (Limonta et al., 2019).

Fin regeneration was severely hindered by microplastic exposure, both morphologically and functionally. Additionally, changes were made to the signalling networks that control the regeneration of fins, as well as to the signalling of reactive oxygen species and the immunological response, all of which are crucial for tissue repair and regeneration. Genes relevant to fin regeneration were transcriptionally altered in response to microplastic exposure, according to transcriptomic studies of the regenerating fin, and metabolic pathways were also heavily implicated (Gu et al., 2020). *Danio rerio* exposed to sublethal quantities of polystyrene microplastics were assessed for metabolic processes, oxidative stress levels, and heart tissue contraction characteristics. Particularly, exposure to PS-MPs resulted in a considerable decline in swimming ability and heart function, whereas the hearts of challenged species showed increased levels of metabolic adjustments and oxidative stress markers. Stress indices were more susceptible to DNA damage (Dimitriadi et al., 2021). Fish gonad exposure to microplastics can result in molecular reactions and histological changes, suggesting a possible negative influence on fish reproductive organs. At concentrations above 100 µg/L, significantly enhanced reactive oxygen species level was found in both male and female liver and gonads. At a dose of 1000 µg/L, male testes showed considerably higher levels of apoptosis, which enhanced the expression of p53-mediated apoptotic pathways. Histological changes, including a considerable reduction in testis basement membrane thickness, were also seen (Qiang et al., 2021).

In another study, *Danio rerio* were exposed to microplastics in three different forms (bead, fragment and fibre) that were organised roughly in one dimension. It was seen that microplastics aggregate and become hazardous in the stomach. Shape-dependent accumulation in the gut was observed with the order of fibres (8.0 µg/mg) > fragments (1.7 µg/mg) > beads (0.5 µg/mg). Microplastics damaged the fish mucosa, increased permeability, triggered inflammation, and upset the metabolism (Qiao et al.,2019). The toxicity of PS-MPs microplastics on gut microbiota, antioxidant activity and innate immune response in *Danio rerio* were observed in a study. After subjection to polystyrene plastic particles, the pathological and morphological differences of intestine and gills were detected, and the injury severity was found to be associated with the concentration and particle size of plastics. After exposing *Danio rerio* to polystyrene plastics, remarkable alterations in the richness and diversity of the gut microbiota were observed. Compared to the control bodies, the plastics-treated bodies had more pronounced oxidative stress. In addition, the mRNA expression level of most pro- and anti-inflammatory factors, including IL-8, NF-kb, and IL-10, elevated while the mRNA expression of TNF-α, a pro-inflammatory factor, declined (Pie et al., 2022). PS-MPs greatly enhanced the sorption of F–53B (6:2 chlorinated polyfluorinated ether sulphonate acid) which decreased the bioavailability and bioaccumulation of F–53B in Zebrafish larvae. Mixture of F–53B and PS-MPs notably reduced the body mass of *Danio rerio* larvae. Combined disclosure of PS-MPs and F–53B resulted in a noteworthy reduction in superoxide dismutase and lysozyme activity, demonstrating the occurrence of oxidative stress and inflammatory reaction in *Danio rerio* larvae (Yang et al., 2020). The aggregation of microplastics ensuing from foodborne exposure (0.01±0.01 µg/mg; 0.1±0.1 particles/mg) was remarkably lower than that through waterborne exposure (0.06±0.02 µg/mg; 0.8±0.3 particles/mg), indicating that intake of microplastics in their tissues occurs primarily through direct environmental uptake rather than food chain transfer. On the other hand, higher sublethal effects, including the significantly defective hyperactive swimming behaviour (107±5% induction; $p < 0.05$), were noticed in the foodborne group than waterborne group (Yu et al., 2022).

When *Danio rerio* embryos were subjected to microplastics starting from 4 h post fertilization then microplastics, it was observed that first attached to the embryo chorion, afterwards entered the stomach and intestinal tract of the larvae. In the free-swimming test, exposure to 1000 µg/L of microplastics resulted into a remarkable decrease in both swimming distance and speed of larvae under the dark condition by 3.2% and 3.5% respectively. Exposure to 100 and 1000 µg/L of microplastics reduced swimming distance by 4.6% and 2.6% and decreased active speed by 4.9% and 2.8% in the alternating light-to-dark photoperiod stimulation experiment, perhaps as a result of impaired dark avoidance in treated *Danio rerio* larvae. Exposure to microplastics might have led to the upregulation of genes linked to oxidative stress and inflammation at the molecular level. It proved that exposure to microplastics remarkably drops larval swimming competence, which may have eminent impacts on its population fitness in the aquatic environment and further development (Qiang et al., 2019). In another study, it was seen that aggregation of microplastics in gastrointestinal tract did not cause apparent damages to intestinal villi. However, the thickness of the muscularis layer in the foregut was lowered by 32% after 1 mg/L (1.45 × 104 particles/mL) microplastic exposure. The *Danio rerio*'s swimming distance increased to 1.3–2.4 times more than that of the control following the microplastic exposure and they remained in manic and active states for a significant amount of time (Chen at al., 2020). In another set of experiment, the biological effects were investigated through the expression levels of a set of selected genes in head kidney samples and two enzymatic biomarkers, acetylcholinesterase and lactate dehydrogenase, in head and body homogenates respectively. It was observed that the genes involved in xenobiotics catabolism processes (cyp2p8) and adaptive immunity were upregulated at the lowest microplastic concentration. Acetylcholinesterase activity was inhibited by the highest microplastics exposure. But there are no noteworthy effects on lactate dehydrogenase activity. The findings of this study are consistent with the theory that exposure to MPs might trigger the immune system's response and xenobiotic metabolism, suggesting that the cytochrome P450 enzyme cyp2p8 and acetylcholinesterase may be sensitive to MPs contamination (Limonta et al., 2021).

Conclusion

Microplastic contamination in marine habitats is currently a significant and widespread pollution issue. There aren't enough efficient therapy options. After going through the studies, it is clear that exposure of adult *Danio rerio* to microplastics can change the expression of genes associated to immunity and metabolic pathways in the liver, affect the swimming behaviour, tissue integrity of GIT and gills, where large no. of neutrophils were detected and have also an impact on D. *rerio* behaviour. Overall, this study reveals that aquatic creatures are prone to hazards when exposed to microplastics at various biological levels. Management of plastic waste, that is low cost, high quality and ecologically sustainable is necessary to combat the negative effects of microplastics.

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ZS conceived the concept. HG, SK and ZS wrote and approved the manuscript.

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