Original Article

Acute Toxic Effects of Polyurethane Microplastics on Adult Zebra fish (*Danio rerio*)

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Abstract

Aim: Microplastics (MPs) have become an emerging environmental contaminant and there are increasing concerns about potentially toxic effects on living organisms. This study is aimed to determine uptake, tissue accumulation, and toxic effects of polyurethane microplastics (PUR-MPs) on zebra fish. **Materials and Methods:** The zebra fish were exposed to different concentrations of PURs-MPs of different sizes for 10 days. Mortality and behavioral changes were monitored. Ingestion and tissue accumulation of PUR-MPs were studied by fluorescent tagging of PUR-MPs. Pathological damages of tissues were investigated with hematoxylin-eosin staining. **Results:** Microsize PUR-MPs can be ingested by zebra fish and fluorescent-tagged PUR-MPs were found in gills and gastrointestinal (GI) tract of fish after 10 days of exposure. A significant negative correlation was found between fluorescence intensity in fish tissues and size of PUR-MPs. Gills, GI tract, and liver were the most affected tissues by PUR-MP toxicity. GI damages included epithelial detachment, thinning of the bowel wall, congestive inflammation, epithelial damage, and lesions of villi in the gastric wall. Necrosis, adhesion, and partial fusion of secondary lamellae were the dominant pathological damages in the gills. Liver also was affected by cellular necrosis, infiltration, and lipid droplets. **Conclusion:** Exposure of zebra fish to PUR-MPs leads to ingestion of these particles by fish and significant increase in fish mortality and tissue damages. Particle size and MP concentration were the key determinant factors in PUR-MP toxicity. The results of the present study provide novel insights into environmental toxicity of PUR-MPs and toxic effect of PUR-MPs in aquatic organisms.

Keywords: Environmental toxicity, microplastics, pathological damages, polyurethane, zebra fish

Introduction

Microplastic (MP) pollution is an emerging concern that has received increasing attention during the last decade.[1] The common definition of MP is a water-insoluble, solid polymer particle 100 nm–5 mm in size.[2] MPs are classified as primary and secondary MPs. The primary MPs are intentionally manufactured for particular applications (for example in abrasive cosmetic products), whereas secondary MPs are formed by fragmentation and degradation of macroplastics by physical and chemical processes in the environment (such as sea waves action, ultraviolet [UV] degradation, biological degradation by microorganisms, and mechanical abrasion).[3] Due to the high persistence and durability of MPs, they can persist for centuries in the environment and as such, MP pollution has been highlighted as a global environmental contaminant with significant economic concern.[4]

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Today MP pollution is ubiquitous in almost all marine environments, accumulating on the surface of the rivers and oceans, throughout the water column, and found in benthic sediment.[5] There are strong evidences about the releasing of MPs into the environment at all steps of a plastic product life cycle (from production to waste management) with the potential for trophic transfer and human health exposure.[6] MPs could accumulate and transfer toxic agents (such as persistent organic pollutants, heavy metals, and pesticides) in the environment by the absorption of these chemicals

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on the surface of the plastic particles.[7] Higher affinity of these chemical contaminants for the plastic media leads to accumulation of them on the plastic particle up to one million times higher than water.[8]

Due to small size of MPs, ingestion of them has been reported in a long list of aquatic fauna such as cetaceans,[9,10] seabirds,[11] molluscs,[12] echinoderms,[13] zooplankton,[14] and corals.[15] Ingestion of these MPs by marine organisms has been reported to cause several toxic effects including physical injury,[16] reduction of feeding activity, genotoxicity, oxidative stress, and knock-on effects for growth and reproduction.[17,18]

Physical and chemical properties of MPs significantly affect their bioavailability and toxicity.[19] The toxic effects of MPs mainly depend on the type of polymer due to different properties of additive chemicals such as phthalates, heavy metals, and UV stabilizers.[20] Furthermore, chemicals used in the production process (such as solvents and surfactants) can contribute to toxic effects of MPs, but the toxic effects of different types of MPs remain mainly unknown.[21]

MPs consist of a large variety of polymer types, including polyethylene (PE), polypropylene (PP), polystyrene (PS), poly (vinyl chloride) (PVC), polyethylene terephthalate (PET), and polyurethanes (PURs).[22] PURs are a diverse family of synthetic polymers that are formed by the reaction between the OH (hydroxyl) groups of a polyol with the NCO (isocyanate functional group) groups of an isocyanate.[23] Nowadays, PURs are used as everyday life products, being one of the most important and popular classes of polymers. Furniture and interior design, construction, and home and consumer products are the main uses of PUR polymers. The worldwide consumption of PURs was estimated to be 60.5 billion USD in 2017 and its global usage is expected to expand from 13.65 million tons in 2010 to 17.95 million tons by 2016.[24]

Assessment of environmental and health hazards of plastic polymers based on the chemical composition of polymers has put the PURs in the first rank of hazardous polymers in the environment.[25] Toxic effects of other MPs on different living organisms have reported in several previous studies. Karami *et al.* evaluated the effects of PE MP exposure on zebra fish and reported no significant toxic effects.[26] In contrast, in a study by Lu *et al.,* significant toxic effects such as oxidative stress and inflammation have sine in zebra fish after exposure to PS MPs.[27] Lei *et al.* investigated the toxic effects of five common types of MPs and the results showed no or low acute lethality in zebra fish but caused intestinal damage including cracking of villi and splitting of enterocytes.[28]

zebra fish (*Danio rerio*) is a small tropical freshwater fish. The ease of care and transport, small size, year-round prolific breeding, and external development have made these fish a popular vertebrate model for toxicological studies.[29] Despite the severe environmental effects of the PUR-MPs, based on the best of our knowledge, there is no study in the literature evaluating toxic effects of these MPs on living organisms. In the present study, the acute toxic effects of polyurethane MPs (PUR-MPs) on adult zebra fish were investigated.

Materials and Methods

Preparation and characterization of PUR-microplastics

A block of rigid PUR foam was purchased from a local producer and ground into very small particles using mortar and mill and then sifted through different meshes (18, 48, and 150) sieves.[28] By this method, three classes of PUR-MPs were produced: 100 μm > particles (Class A-PUR-MPs), 100–300 μm particles (Class B-PUR-MPs), and 300–1000 μm particles (Class C-PUR-MPs). The size and morphology of gold-coated PUR-MPs was evaluated by scanning electron microscopy (SEM, Hitachi S-3400-II, USA). To prevent charging of PUR-MPs with the electron beam, samples were first subjected to gold coating (~5 nm) before being examined with SEM. Particle size was analyzed by using ImageJ software (NIH, Bethesda, Maryland, USA) (n = 100).[30]

Fish husbandry

Adult healthy zebra fish were purchased from a local ornamental fish dealer in Isfahan, Iran (298.2 ± 41.3 mg and 2.5 ± 0.5 cm in body length)[26] and acclimated in 10 L glass tanks for 2 weeks before the experiment. The fish were maintained at 23°C ± 1°C with a 12 h light/dark photoperiod. UV-sterilized dechlorinated tap water was used and the aquarium was aerated by an air pump. The pH of water, dissolved oxygen, and water hardness were controlled at 7.2 ± 0.4, 6.6 ± 0.5 mg/L, and 185 ± 10 mg/L, respectively.[28,31] The fish were fed twice a day with a commercial diet (Cargill, crude protein: 38%–40%).

Fish exposure to PUR-microplastics

For the experiments, zebra fish were randomly distributed into 1.5 L glass aquariums filled with 1 L water (6 fish per aquarium, 3 replicates for each exposure concentration, 5 exposure concentrations). Throughout the experiments, aquariums were gently aerated with a centralized pump using an air stone. The fish were exposed to different concentrations of PUs-MPs (0, 1, 10, 100, and 1000 μg/L) of different sizes for 10 days. During the experiments, the aquariums were continuously aerated to maintain the dispersion of the particles in water. Control groups were reared in water alone. New exposure solutions were prepared every day and the exposed fish were moved into them.[32]

Fluorescent tagging of PUs-microplastics with Nile Red

Previously prepared MPs were immersed in 70% ethanol for 24 h to remove possible contamination. The stock solution of Nile Red was prepared at 1 mg/mL in acetone and filtered using a 0.22 μm Polytetra-fluorethylene (PTFE) filter syringe. For the staining of MPs, 500 μL of Nile Red working solution (100 μg/ml) was added to 100 mg PUR-MPs in a clean glass screw-top vial and incubated for 24 h at room temperature. Then, the excess Nile Red was removed and sedimented MPs were washed several times with n-hexane and allowed to rest for 48 h covered with a watch glass under a fume cupboard until

all moisture evaporated. The quality of staining was examined by fluorescence microscope under green emission (Optika, IM-3FL4, Italy).[33,34]

Determination of accumulation kinetics by fluorescent spectroscopy

After fasting for 24 h, the acclimated fish were randomly selected and distributed into two 1.5 L glass aquariums filled with 1 L water (5 fish per aquarium). The treatment group was exposed to 100 mg/L fluorescent-tagged MPs in culture water and the control group was exposed to culture water only without MPs for 10 days. After the exposure, the fish were sacrificed and were transferred to agar-padded slides, immobilized with 100 mM sodium azide, and then sealed with coverslips for fluorescent spectroscopy observations.

Evaluation of acute toxic effects (fish mortality and histopathological analysis)

Acute toxicity of PUR-MPs in zebra fish was investigated using revised US-EPA methods for conducting 10 days water toxicity.[28] Fish mortality was monitored and recorded during the 10 days of exposure. Ten days' median lethal concentration (LC_{50}) of PUR-MPs was calculated with probit regressions with 95% confidence intervals using SPSS Statistics software 20.0 (IBM, Armonk, NY, USA) on either untransformed or log-transformed data.[35]

For histopathological analysis, dead fish were fixed in 10% formalin quickly, embedded in paraffin wax, sectioned at 5 μm thickness, and then stained with hematoxylin and eosin for microscopic observation. When necessary, additional serial sections were cut to reveal tissues of concern. The fish tissues were graded for inflammation and/or morphological changes by an expert pathologist. The grading system included four categories: 0 = normal morphology, 1 = mild inflammatory infiltrate, 2 = moderate inflammatory infiltrate, and 3 = marked inflammatory infiltrate.[36] Tissues examination and observation were done using a LEICA DM2500 bright field microscope.

Behavioral and morphological observation

Changes in zebra fish behavior and morphology were monitored during the PUR-MP experiments by two independent observers. Observation was conducted for 60 min every day by each observer and the changes were recorded. Behavioral changes including resting time, swim activity, erratic movements (sharp changes in direction), and vertical or sideways swimming were categorized.[37,38] Morphological changes (tails bent) also were monitored and recorded.

Data analysis

In this study, the results are shown as the means ± standard deviation. *T*-test or one-way analysis of variance was used to compare the means between the treatment groups and the control group. The level of significance was set at $P \leq 0.05$. The statistical software SPSS (version 20, IBM, USA) was used for the statistical analyses.

Results

PUR-microplastic characteristics

PUR-MP was a light yellow powder. SEM was used to investigate the size and surface morphology of PUR-MPs. The images [Figure 1] revealed various sizes, shapes, and surface roughness for scanned particles. All fragments displayed a rough surface with sharp edges. Average particle size of MPs in Class A was 86 μm, in Class B was 209 μm, and in Class C was 423 μm. Aggregation behavior was not observed between particles.

Acute toxic effects (fish mortality)

Acute exposure to PUR-MPs particles resulted in a time- and dose-dependent increase in fish mortality. Significantly higher mortality was observed in fish exposed to Class A and Class B-PUR-MPs compared to Class C-PUR-MPs. Exposure to 100 and 1000 μg/L Class A-PUR-MPs killed (100%) of fish after 6 and 10 days, respectively, while 88% of fish were alive after 10 days exposure to 1000 μg/L Class C-PUR-MPs [Table 1 and Figure 2]. Acute exposure to PUR-MPs resulted in a 10 days' LC_{50} of 16.59 μg/L and 100.2 μg/L for Class A and B particles, respectively.

Acute toxic effects (histopathological analysis)

Morphological changes in tissues of exposed fish compared to unexposed fish were observed and graded by an expert pathologist. The pathologist has reported normal overall body morphology in unexposed fish and surviving fish which exposed to Class C-PUR-MPs. However, the fish that had died during the exposure time showed swollen abdomens. Mark histopathological alterations were observed in surviving and dead fish which exposed to Class B-PUR-MPs and Class A-PUR-MPs in comparison with the control group. Gills, gastrointestinal (GI) tract, and liver were the most affected organs and significant pathological damages were observed in the tissues of these organs. Staining of the gut tissues demonstrated significant alterations of the intestinal mucosa including increases in the volume of mucus and epithelial detachment. Thinning of the bowel wall, congestive inflammation, epithelial damage, and lesions of villi in the gastric wall were observed [Figure 3].

About 98% and 66% of observed sections from zebra fish exposed to Class A-PUR-MPs and Class B-PUR-MPs, respectively, presented significant intestinal damages indicating the role of particle size on PUR-MP toxicity. Regression analysis showed a significant positive correlation between pathological damages and PUR-MP concentration ($P < 0.05$) [Table 2].

Significant damages also were observed in the gill epithelium of zebra fish exposed to PUR-MPs including necrosis, adhesion, and partial fusion of secondary lamellae and mucous hypersecretion [Figure 4]. The liver of fish is also affected obviously by PUR-MP toxicity. Cellular necrosis, infiltration, and lipid droplets were observed in hepatocytes in exposed fish, indicating that PUR-MP toxicity caused inflammation and lipid peroxidation in fish liver [Figure 5]. Similar to intestinal damages, gills and liver alterations are dose and size dependent.

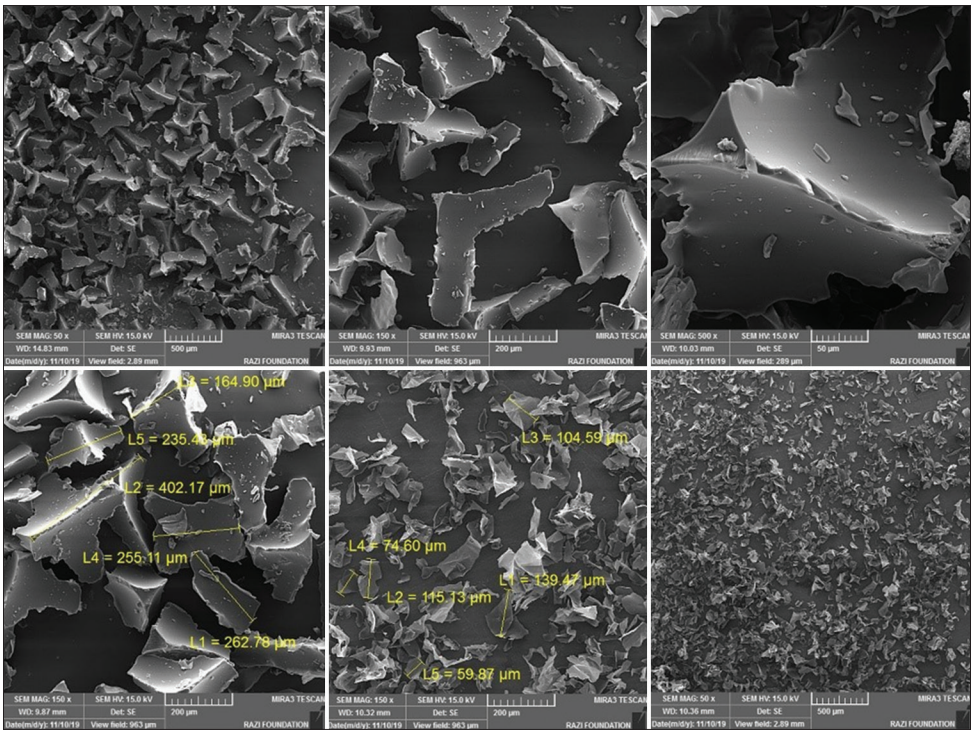

Figure 1: Scanning electron microscope images of investigated PUR-microplastics

Table 1: Fish mortality during the 10 days of exposure to various concentrations of polyurethane microplastics (cumulative results from the three independent experiments)

PUR-MPs concentration (µg/L)	Days of exposure											Class of MPs
	1	2	3	4	5	6	7	8	9	10	Sum	
0	0	0	0	0	0	0	1	0	0	0	1	A
1	0	0	0	0	0	0	1	0	1	2	2	
10	0	0	0	0	0	0	0	3	3	3	9	
100	0	0	0	0	0	3	2	4	4	5	18	
1000	0	0	2	5	5	6	-	-	-	-	18	
0	0	0	0	0	0	1	0	0	0	0	1	B
1	0	0	0	0	0	0	0	1	0	0	1	
10	0	0	0	0	0	0	0	0	1	1	2	
100	0	0	0	0	0	0	1	1	3	2	7	
1000	0	0	0	0	0	1	2	2	3	4	13	
0	0	0	0	0	0	1	0	0	0	0	1	C
1	0	0	0	0	0	0	1	0	0	0	1	
10	0	0	0	0	1	0	0	0	1	0	2	
100	0	0	0	0	0	0	0	0	1	1	2	
1000	0	0	0	0	0	0	0	0	1	1	2	

PUR-MPs: Polyurethane-Microplastics

Almost all gills and liver tissues sections of zebra fish exposed to 1000 µg/L Class A-PUR-MPs presented Grade 3 and 4 pathological damages and severity of injuries reduced by reduction of MP concentration or increase of MP size.

Fluorescent tagging and determination of accumulation kinetics by fluorescent spectroscopy

Quality of fluorescent tagging of PUR-MPs was examined under green fluorescence on black PC filter paper. PUR-MPs were effectively stained and identified under the given staining condition [Figure 6].

MP distribution in fish body tissues was studied by assaying fluorescently tagged PUR-MPs. After 10 days of exposure, PUR-MPs particles were clearly visible in the gills and digestive system of fish which confirm the swallowing of MPs by fish [Figure 7]. Among three classes of PUR-MPs, Class A particles showed the strongest fluorescence intensity in the

Table 2: Percentage of observed fish sections with guts pathological damages

	PUR-MPs concentration (μg/L)														
	0			1			10			100			1000		
	PUR-MPs class			PUR-MPs class			PUR-MPs class			PUR-MPs class			PUR-MPs class		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
No damage	100	100	100	91	100	100	79	96	100	46	79	94	8	24	76
Grade1	0	0	0	9	0	0	11	4	0	31	9	6	41	46	20
Grade2	0	0	0	0	0	0	9	0	0	13	8	0	24	14	4
Grade3	0	0	0	0	0	0	1	0	0	6	4	0	16	10	0
Grade4	0	0	0	0	0	0	0	0	0	4	0	0	11	6	0

PUR-MPs: Polyurethane-microplastics

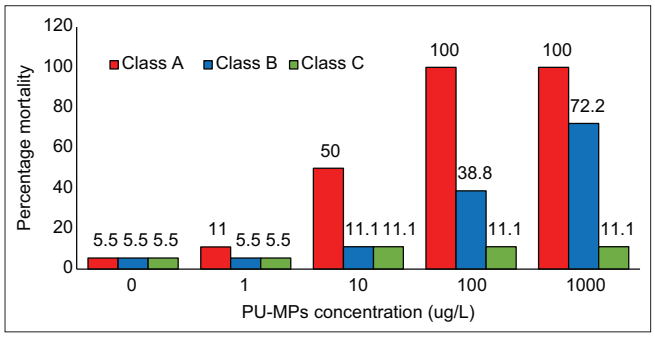

Figure 2: Fish percentage mortality (mean ± standard deviation) after 10 days of acute exposure to different concentrations of PUR-microplastics

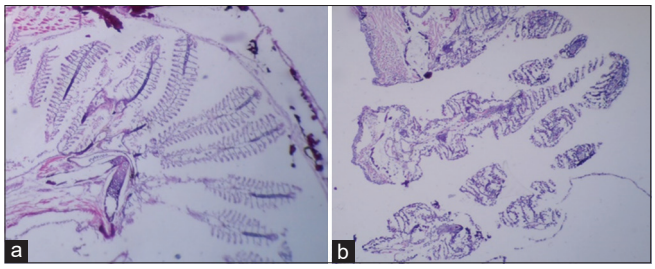

Figure 4: Alterations observed in the gills of zebra fish exposed to 1000 μg/L Class A-PUR-microplastics for 10 days. (a) Control, unexposed fish. (b) Gill of an exposed fish

gills and intestine of fish, while other classes of PUR-MPs showed relatively weak fluorescence intensity, which indicated the effect of MPs size on accumulation in the fish tissues. PUR-MPs were not observed in fish liver and kidney. In contrast, no green fluorescent objects were seen in the fish tissues from the unexposed fish.

Behavioral and morphological observation and analysis

PUR-MPs exposure significantly affected behavioral and morphological characteristics of zebra fish. Significant alterations were observed in behaviors and morphology of fish exposed to Class A polyurethane MPs [Figure 8], but these alterations were not significant for Class B and C-PUR-MPs. Due to the significant mortality of fish exposed to PUR-MPs concentrations above 10 μg/L, only the results of behavioral and morphological alterations in fish exposed to 10 μg/L of PUR-MPs are reported in Figure 8. The changes began around

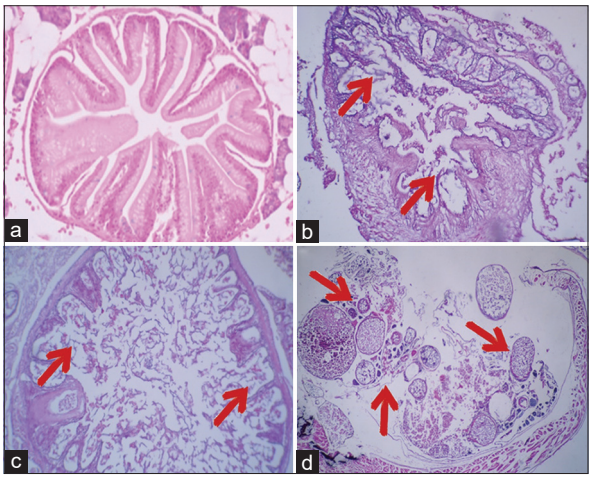

Figure 3: Intensive pathological damages in the guts of zebra fish due to exposure to 1000 μg/L Class A-PUR-microplastics for 10 days. (a) Normal pathology of zebra fish gut (unexposed). (b) Epithelial detachment. (c) Thinning of the bowel wall, congestive inflammation, (d) epithelial damage, and lesions of villi in the gastric wall

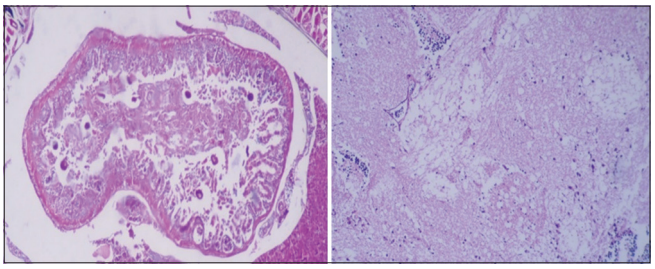

Figure 5: Cellular necrosis, infiltration and lipid droplets in hepatocytes of zebra fish after exposure to 1000 μg/L Class A-PUR-microplastics for 10 days

days 3–4 of exposure and included abnormal swimming behavior, gradual increase in resting time and erratic movements, and decrease in swim activity and vertical swimming. Tails bent was the dominant morphological change in exposed fish and increase significantly by dose and time of exposure.

Discussion

This study deals with acute toxic effects of polyurethane MPs on marine organisms and used zebra fish as a common model for evaluating aquatic toxicity of polyurethane MPs. The effects

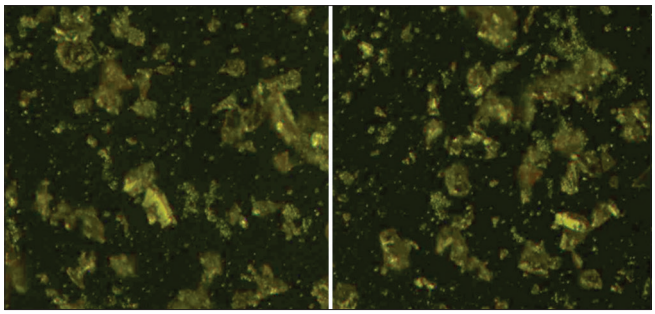

Figure 6: Stained PUR-MPs with fluorescent dye (Nile red) under a fluorescent microscope with green emission

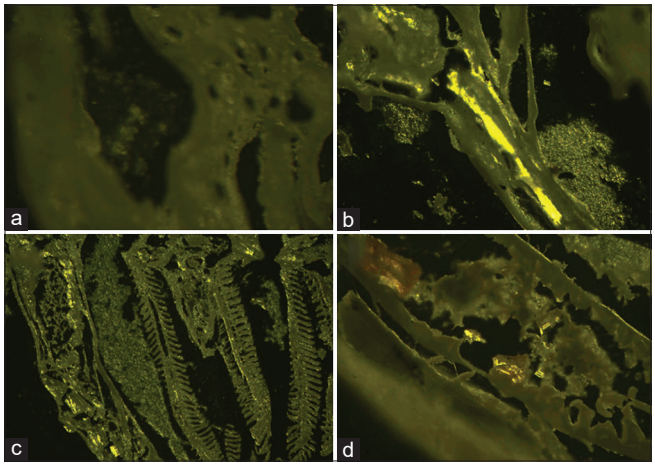

Figure 7: Photographs of fish gastrointestinal tissues under green fluorescence after 10 d of the exposure to to 100 mg/L fluorescent-tagged microplastics. Bright fluorescent plastic particles are cleary visible in photographs b-d (gills and guts tissues of exposed fish) but not in photographs a (control, unexposed fish)

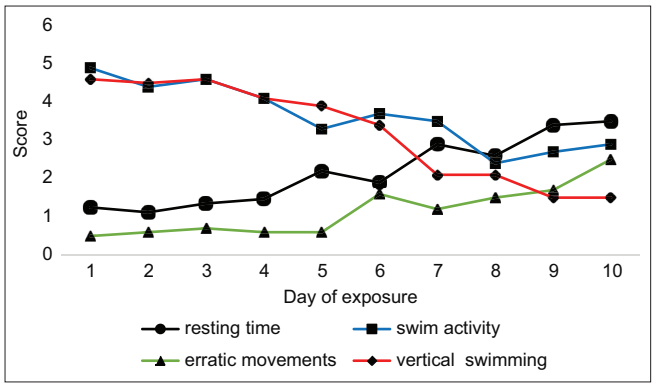

Figure 8: Behavioral alterations in fish exposed to 10 µg/L of PUR-microplastics for 10 days

of size and concentration of PUR-MPs on toxicity and also the patterns of toxic effects were studied.

To our knowledge, there is no study in the literature investigating the toxic effects of PUR-MPs *in vitro* or *in vivo* and this is the first study in this field (despite the widespread use of polyurethane foams and their significant release to the environment).

The results of this paper clearly showed that zebra fish readily ingest PUR-MPs. Florescent-labeled PUR-MPs were found inside the gut of the fish (even the relatively large particles; around 500 µm in length). Ingestion of other types of MPs such as PS, PE, PP, PVC, and polyamides by different species of fish was reported in previous studies,[27,28,32] and ingestion of PUR particles by zebra fish confirmed in this study. These evidences indicate that fish ingest PUR-MPs. Fish have a sensitive gustatory system and can segregate food from inedible items efficiently upon oral uptake. However, despite such a developed sense of taste, MPs are ingested significantly by almost all fish species.[39] It is not clear what mechanisms cause to fish could not distinguish inedible plastics from food particles. It is suggested that co-occurrence of MPs and food in the oral cavity of the fish may affect the gustatory system of fish and decreases the detectability of inedible items, and allows the MPs to be swallowed accidentally.

Fluorescence intensity in tissues of fish exposed to Class A-PUR-MPs was significantly stronger than those exposed to Class C polyurethane MPs elucidated that size is the key determining factor in MP ingestion and toxicity. It seems that larger plastic particles have lower deleterious effects on living organisms, but unfortunately plastics are persistent for hundreds of years in the environment and larger plastic debris is degraded into smaller and smaller pieces by different mechanisms.

In the present study, acute toxic effects and lethality of PUR-MPs were studied. The LC_{50} of Class A and Class B polyurethane MP was determined 16.59 and 100.2 µg/L, respectively. Exposure to 1000 and 100 µg/L Class A-PUR-MPs killed all fish after 6 and 10 days, but 89% of fish were alive after 10 days of exposure to 1 µg/L PUR-MPs, indicating that toxicity was occurred in a dose- and size-dependent pattern.

Based on the best of our knowledge and deep reviewing of the literature, there is no study that determined the levels of PUR-MPs in river and seawater and only in some limited studies, the presence of PUR particles in environmental samples is mentioned.[40] But obviously, the levels of PUR-MPs in real samples are significantly lower than reported LC_{50}s. Environmental monitoring and further studies on sublethal toxic effects of PUR-MPs on marine organisms are necessary.

GI tract is the most important fish tissue which could be affected by the MPs directly. It has been reported that ingestion of MPs has interfered with the normal functioning of the digestive systems of fish.[41] According the results of previous studies, MPs exposure could cause functional and histopathological alterations in fish GI tract.[32] A study by Lei *et al.* had shown that different types of MPs (such as PVC, PET, PS and PP) accumulate in zebra fish intestine and exerts functional damage via reduction in calcium levels, inflammation, and induction of oxidative stress.[28]

Lei *et al*. and Qiao *et al*. found that 21 days' exposure to MPs induced significant intestinal damage including cracking of

villi and splitting of enterocytes in zebra fish,[1] and the results of our study showed PUR-MPs similar to other types of MP could induce epithelial detachment, thinning of the bowel wall, congestive inflammation, epithelial damage, and lesions of villi in the gastric wall in the gastric system of fish in a time- and dose-dependent manner. The size of plastic particles also played an important role.

Lu *et al.* have reported hepatotoxic effects of MPs (PS MPs) in zebra fish for the first time including early inflammatory responses such as necrosis, vacuolation, and infiltration.[27] Similar effects in zebra fish have not yet been reported for other types of MPs. Hepatotoxic effects of PUR-MPs were investigated and confirmed in the present study for the first time. Similar to polystyrene MPs, PUR-MPs also induced pathological and inflammatory damages in zebra fish liver (cellular necrosis, infiltration, and lipid droplets). In the study by Lu *et al*., no conspicuous differences of pathological damages were reported in hepatic tissues of fish treated with the different sizes of MPs, but on contrary, our observations indicated that these pathological alterations were significantly associated with PUR-MPs size and concentration. In the 91% of liver tissues sections from fish exposed to 1000 μg/L Class A-PUR-MPs, different grades of pathological damages were observed, while only in 8% of sections from fish exposed to 10 μg/L Class A-PUR-MPs, low-grade pathological damages were observed.

MPs caused structural damage to gills of zebra fish. These damages depended on the size and concentration of MPs. PUR-MPs were detected in the gills of fish and caused the breakage of gill filaments, likely due to direct contact. Similar findings have also been reported by Erkmen *et al*.[43] and Jabeen *et al*.[41] Direct contact of MPs to gill tissues of fish induces hyperemia, epithelial lifting, edema, telangiectasia, epithelial hyperplasia, and fusion of secondary lamellae. Chemical composition of MPs plays a significant role in toxicity due to direct contact and chemical reaction between the chemicals in the surface of particles and fish tissues. The particles with sharp edges could induce physical damages to the gill tissues.[42]

Zebra fish is a suitable model for the study of behaviors changes due to chemical toxicity.[44] In the present study, the severity of abnormal behavior and the percentage of fish with abnormal behavioral changes increased with the concentration of PUR-MPs. Behavioral changes (abnormal swimming behavior, gradual increase in resting time and erratic movements, and decrease in swim activity and vertical swimming) in zebra fish due to PE MP exposure and toxicity have been reported previously[37] and confirmed for PUR-MP by our results.

Conclusion

In this paper, we present for the first time the toxic effects of polyurethane MPs on adult zebra fish. The results showed that zebra fish ingests PUR-MPs. Relatively large particles, (about 500 μm), were found in the gut of zebra fish. Exposure to the PUR-MPs results in increased mortality of fish and the size and concentration were the key determinant factors in MPs toxicity. PUR-MPs caused intestinal damage including epithelial detachment, thinning of the bowel wall, and lesions of villi, as well as hepatic and gills damages and behavioral alterations. The results of the present study provide novel insights into the environmental toxicity of PUR-MPs in aquatic organisms.

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Conflicts of interest

There are no conflicts of interest.

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