Subacute toxic effects of polyvinyl chloride microplastics (PVC-MPs) in juvenile common carp, *Cyprinus carpio* (Pisces: Cyprinidae)

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ABSTRACT

Pollution of the aquatic environment by microplastics (MPs) is one of the most serious environmental issues worldwide and has raised many concerns about their availability and hazards for aquatic biota. In addition, fish is an important source of protein for humans, hence, the accumulation, and toxic effects of the MPs in fish deserve special attention. This study aims to examine the ingestion, tissue accumulation and toxic effects of polyvinyl chloride microplastics (PVC-MPs) in juvenile common carp. Fluorescent-tagged PVC-MPs were found in various tissues of fish only after 4-5 days of exposure. The size of MPs showed a significant role in acute toxicity and mortality due to PVC-MPs. 100% mortality were observed after 7 and 10 days exposures to 1000 and 100 μg L^{-1} class A-PVC-MPs (100>μm) respectively, while 1000 μg L^{-1} of class A-PVC-MPs (300-1000 μm) killed only 16.6% of fish after ten days. Different grades of tissue damage were found in the gills, gut, and liver of fish in proportion to size, time, and concentration of PVC-MPs. Epithelial detachment, thinning of the bowel wall, and lesions of villi in the gastric wall were the dominant damages in the gastrointestinal tract. Gills also were affected in the form of necrosis, adhesion, and partial fusion of secondary lamellae. Hepatic damages (cellular necrosis and infiltration) were found only due to exposure of fish to class A-PVC-MPs. Altogether, these findings suggest that common carp intake significant levels of environmental microplastics (intentionally or accidentally), which seriously affect fish health and raise significant concerns about marine ecosystem health and seafood safety due to microplastic pollution.

Keywords: Polyvinyl chloride (PVC), Microplastics, Environmental toxicity, Common carp, Pathological damages.
Article type: Research Article.

INTRODUCTION

A dramatic increase in the world's plastics production has received increasing concerns about its environmental toxicity over the past decades. In 2017, plastic production reached up to 370 million tons (Ji *et al.* 2021). Nowadays, plastics are involved in almost all aspects of modern life, including agriculture, clothing, construction, furniture, packaging, and so on. This growth in plastic production and application has led to a considerable increase in the release of plastic litters into the environment (Barnes *et al.* 2009). Continuous fragmentation and degradation of macroplastics in the environment by physical or chemical processes (such as sea waves action, sunlight degradation, biological degradation by microorganisms, and mechanical abrasion) lead to the production of secondary microplastics (MPs; < 5 mm in diameter; Zeng 2018). Primary microplastics are intentionally manufactured for particular applications (such as the plastic microbeads in cosmetic and personal care products) (Cole *et al.* 2015).

MPs are highly persistent in the environment and ubiquitously distribute all the environmental medias, particularly aquatic ecosystems, making them available to all aquatic organisms such as fish. MPs also could accumulate and transfer toxic agents (such as persistent organic pollutants, heavy metals, and pesticides) in the environment by the absorption of these chemicals on the surface of the plastic particles (Hüffer & Hofmann, 2016). The affinity of chemical pollutants for plastic particle surfaces leads to their accumulation on the plastic particle up to one million times higher than in water (Hirai *et al.,* 2011). Due to MPs small size and resemblance to natural food items, fish readily ingest them in incidental or intentional ways (Jabeen *et al.* 2018). Furthermore, since fish is an important protein source for human beings, the presence and ecotoxicological impacts of MPs in fishes may have consequences on aquatic food safety (Barboza *et al.* 2018). Common carp, *Cyprinus carpio* L. 1758 is a freshwater fish species, belonging to the Cyprinidae family, which is considered the largest freshwater fish family. It is the third most-widely cultivated and commercially-important freshwater fish species in the world. In some European countries, over 80% of total fish production comes from common carp (Rahman 2015), since it exhibits a better capacity for resistance to pollutants than other laboratory fish, such as zebrafish. Because of the dominance of this species in the natural environment, omnivorous, and bottom-feeding characteristics, carp is considered a good model for evaluating the ecotoxic effects of pollutants (Lee *et al.* 2012). Pollution of aquatic ecosystems and living environments of *C. carpio* (water and sediments) by the different types of MPs has been reported in several previous studies (Mataji *et al.* 2020; Mehdinia *et al.* 2020; Manbohi *et al.* 2021; Rasta *et al.* 2020, 2021a). As a consequence, significant levels of MPs have been found in the tissues of fish. Rasta *et al.* reported significant levels of MPs in the gastrointestinal (GI) tract of exposed fish to the MPs (Rasta *et al.* 2021b). Nematollahi *et al.* (2021) determined MP fibers in the gut of common carp from the southern coast of the Caspian Sea, reporting a significant relationship between the carp health status and MP frequency. MP physical and chemical properties significantly affect their bioavailability and toxicity (Gatidou *et al.* 2019). The toxic effects of MPs mainly depend on the type of polymer due to the different properties of additive chemicals such as phthalates, heavy metals, and UV-stabilizers (Rochman 2015). Also, chemicals used in the production process (such as solvents and surfactants) can contribute to the toxic effects of MPs, but the effects of different types remain mainly unknown (Hamlin *et al.,* 2015). MPs consist of a large variety of polymer types, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and Polyurethanes (Pus; Kramm *et al.* 2018). Polyvinyl chloride (PVC) was one of the first plastics discovered and is produced by the polymerization of vinyl chloride monomers. It is one of the most extensively-used plastics and is the world's third most-widely produced synthetic plastic polymer, after polyethylene and polypropylene. PVC resins are used widely in building materials, construction, and home furnishings, making PVC resins nearly ubiquitous in our society (Fralish & Downs 2019). Global production of PVC reaches 37 million tons year^{-1} (Lithner *et al.* 2011). PVC has been reported to cause chronic bronchitis, birth defects, genetic changes, cancer, skin diseases, deafness, vision failure, ulcers, liver dysfunction, and indigestion in humans. Vinyl chloride monomer (VCM) is now classified as an International Agency for Research on Cancer (IARC) Group 1 carcinogen (Alabi *et al.* 2019). Assessment of environmental and health hazards of plastic polymers based on the polymers chemical composition has placed the PVC on the top rank of hazardous polymers in the environment (Lithner *et al.* 2011). The toxic effects of other MPs on different living organisms have been reported in several previous studies. Karami *et al.* (2017) evaluated the effects of polyethylene MPs exposure on zebrafish, reporting no significant toxic effects. In contrast, in a study by Lu *et al.* (2016), significant toxic effects such as oxidative stress and inflammation have been observed in zebrafish after exposure to polystyrene MPs. Lei *et al.* (2018) investigated the toxic effects of five common types of MPs, reporting no or low acute lethality in zebrafish, however, displaying intestinal damage, including cracking of the villi and splitting of enterocytes. Based on the best of our knowledge, there are no studies in the literature evaluating the toxic effects of PVC-MPs on common carp or other fish with nutritional value for humans. In the present study, we investigated the PVC microplastics in juvenile common carp as a model for evaluating environmental toxicity of PVC-MPs.

MATERIALS AND METHODS
Preparation and characterization of PVC-MPs
A piece of rigid PVC pipe was provided by a local producer and ground into very small particles using a mortar and mill and then sifted through different mesh (18, 48, and 150) sieves (Lei *et al.* 2018). By this method, three classes of PVC-MPs were produced: 100>µm particles (Class A-PVC-MPs), 100-300 µm particles (Class B- PVC -MPs), and 300-1000 µm particles (Class C-PVC-MPs). The size and morphology of gold-coated PVC-MPs were

evaluated by scanning electron microscopy (SEM, Hitachi S-3400-II, USA). To prevent charging PVC-MPs with the electron beam, samples were first subjected to gold coating ($\sim$ 5 nm) before being examined with SEM. Then, particle size was analyzed using ImageJ software (NIH, USA; n = 100; Hwang *et al.* 2019).

Fish husbandry
Juvenile healthy common carps were purchased from a local ornamental fish dealer in Tehran, Iran, and acclimated in 10 L glass tanks for two weeks before the experiment. The fish were maintained at 23 ± 1°C with a 12-h light/dark photoperiod. Ultraviolet-sterilized dechlorinated tap water was used, and an air pump aerated the aquarium. The pH of water, dissolved oxygen (DO), and water hardness were maintained at 7.2 ± 0.4, 6.6 ± 0.5 mg L^{-1}, and 185 ± 10 mg L^{-1}, respectively (Chen *et al.* 2017; Lei *et al.* 2018). The fish were fed twice a day with a commercial diet (Cargill, crude protein: 38-40%).

Fish exposure to PVC-MPs
Common carps were randomly distributed into 1.5-L glass aquariums filled with 1 L water (3 aquariums for each replicate per treatment, 6 fish per aquarium). Throughout the experiment, aquariums were gently aerated with a centralized pump using an air stone. The fish were exposed to different concentrations of PVC-MPs (0, 1, 10, 100, and 1000 µg L^{-1}; Ding *et al.* 2018) by different sizes for ten days. During the experiment, 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 replaced (Jin *et al.* 2018).

Fluorescent tagging of PVC-MPs 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^{-1} in acetone and filtered using a 0.22 µm PTFE filter syringe. In order to stain MPs, 500 µL Nile Red working solution (100 µg mL^{-1}) was added to 100 mg PVC-MPs in a clean-glass screw-top vial and incubated for 24 h at room temperature. Thereafter, the excess Nile Red was removed, and MPs sediments were washed several times by n-hexane, allowing to rest for 48 h covered with a watch glass under a fume cupboard until all moisture evaporated. Quality of staining was examined using fluorescence microscope under green emission (Optika, IM-3FL4, Italy; Maes *et al.* 2017, Shim *et al.* 2016).

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^{-1} fluorescent-tagged MPs in culture media, while the control group was exposed to culture media only without MPs for 10 days. After the exposure, the fish were sacrificed and the samples were transferred to agar-padded slides, immobilized by 100 mM sodium azide, and sealed with coverslips for the fluorescent spectroscopy observations.

Evaluation of subacute toxic effects (fish mortality and Histopathological analysis)
Subacute toxicity of PVC-MPs in common carp was investigated using revised US-EPA methods for conducting 10 days of water toxicity (Au *et al.* 2015). Fish mortality was monitored and recorded during the 10 days of exposure. 10-day median lethal concentration (LC$_{50}$) of PVC-MPs were calculated with probit regressions with 95% confidence intervals using SPSS Statistics software (version 20, IBM, USA) on either untransformed or log-transformed data (Kefford *et al.* 2019). For histopathological analysis, dead fish were fixed in 10% formalin quickly, embedded in paraffin wax, sectioned at 5 µm thickness, and stained with hematoxylin and eosin for microscopic observation. When necessary, additional serial sections were cut to reveal tissues of concern. The fish tissues were evaluated and graded for pathological damages by an expert pathologist. The grading system included four categories: 1 = Normal morphology, 2 = Mild pathological damages, 3 = Moderate pathological damages and 4 = Marked pathological damages. Tissue examination and observation were performed using a LEICA DM2500 bright field microscope.

Data analysis
In this study, the results are shown as the mean ± standard deviation (Mean ± SD). T-test or one-way analysis of variance (ANOVA) was used to compare the means between the treatment and control groups. The level of significance was set at p ≤ 0.05. The statistical software SPSS (version 20, IBM, USA) was used for the statistical analyses.

RESULTS AND DISCUSSION

PVC-MPs characteristics

PVC-MPs were a gray powder. Scanning electron microscopy (SEM) was used to investigate the size and surface morphology of PVC-MPs. The images (Fig. 1) revealed various sizes, shapes, and surface roughness for scanned particles. All fibers displayed a relatively smooth surface without sharp edges. The average particle size of MPs in classes A, B, and C were respectively 72, 186, and 402 μm. Aggregation behavior was not observed between particles.
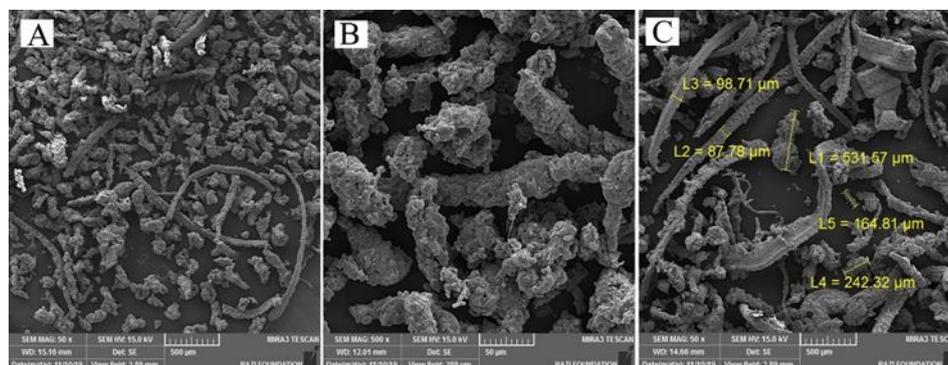

Fig. 1. Scanning electron microscope (SEM) images of investigated PVC-MPs, A: ×50 magnification. B: ×500 magnification, C: sizes determined by SEM.

Subacute toxicity (fish mortality)

Subacute exposure to PVC-MPs particles resulted in a time and dose-dependent increase in fish mortality. Mortality among fish exposed to different classes of PVC-MPs was significantly different ($p < 0.05$). Lethality of class A-PVC-MPs was significantly higher than those of class B and C, while lethality of class B-PVC-MPs was significantly higher than class C ($p < 0.05$). Exposure to 100 and 1000 μg L^{-1} class A-PVC-MPs killed 100% of fish after 10 and 7 days, respectively, while 88.9% of fish survived after 10 days of exposure to 1 μg L^{-1} class A-PVC-MPs. Only 72% of fish died after 10 days exposure to 1000 μg L^{-1} class B-PVC-MPs. The mortality of fish exposed to 1000 μg L^{-1} class C-PVC-MPs was 16.6% (not significantly different from the control group, $p > 0.05$) (Table 1 and Fig. 2). Subacute exposure to PVC-MPs resulted in a 10-day LC$_{50}$ of 18.7 μg L^{-1} and 98.6 μg L^{-1} for the class A and B particles, respectively.

Table 1. Fish mortality during the 10 days of exposure to various concentrations of PVC-MPs (cumulative results from the three independent experiments).

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

Subacute toxic effects (histopathological analysis)

Morphological changes in tissues of exposed- compared to -unexposed fish were observed and graded, reporting normal overall body morphology in unexposed fish and surviving fish exposed to Class C-PVC-MPs. However, the fish died during the exposure time, showed swollen abdomens. Marked histopathological alterations were

observed in surviving and dead fish exposed to Class A-PVC-MPs and Class B-PVC-MPs compared to the control group. Gills, gastrointestinal tract, and liver were the most affected organs with the significant pathological damages. Staining the gut tissues demonstrated significant alterations of the intestinal mucosa, including increases in the volume of mucus and epithelial detachment. Thinning of the intestinal wall, congestive inflammation, epithelial damage, and lesions of villi in the wall were observed (Fig. 3). 98% and 67% of observed sections from common carps exposed to 1000 µg L^{-1} class A-PVC-MPs and Class B-PVC-MPs respectively, presented grade 4 intestinal damages indicating the role of particle size on PVC-MPs toxicity. These pathological damages also are in proportion to PVC-MPs concentration ($p<0.05$). Full results of pathological observation from gastrointestinal damages are presented in Table 2.
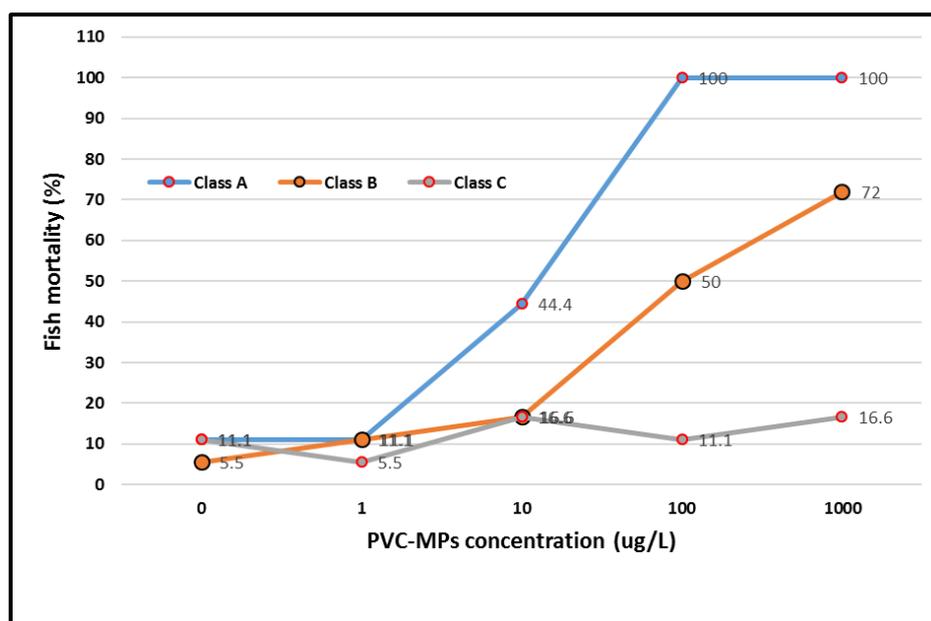
Fig. 2. Fish mortality (%) resulted from the PVC-MPs concentration after 10 days of acute exposure

Significant damages also were observed in the gill epithelium of common carps exposed to PVC-MPs, including necrosis, adhesion, and partial fusion of secondary lamellae and mucous hypersecretion (Fig. 4). The liver of fish was also affected obviously by PVC-MPs toxicity. Cellular necrosis was observed in hepatocytes in exposed fish, indicating that PVC-MPs toxicity caused inflammation and lipid peroxidation in the fish liver (Fig. 5). In the 95% of liver tissue sections from fish exposed to 1000 µg L^{-1} class A-PVC-MPs, different grades of pathological damages were observed, while we found low-grade pathological damages, only in 3% of the sections from fish exposed to 1000 µg L^{-1} class C-PVC-MPs. Similar to intestinal damages, gills and liver alterations were dose and size-dependent. Almost all gills and liver tissue sections of common carps exposed to 1000 µg L^{-1} Class A-PVC-MPs presented grade 3 and 4 pathological damages and severity of injuries decreased by reducing MPs concentration or increasing MPs size.

Table 2. Grading pathological damages in gastrointestinal tract of common carp due to 10 days exposure to different concentrations and classes of PVC MPs (% of observed tissue sections).

MPs Concentration	1 µg L^{-1}				10 µg L^{-1}				100 µg L^{-1}				1000 µg L^{-1}			
	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4
Class A	8	67	13	12	0	15	39	46	0	2	21	77	0	0	2	**98**
Class B	50	44	5	1	45	21	22	12	0	24	22	54	0	0	33	**67**
Class C	74	26	0	0	75	23	2	0	13	64	21	2	0	12	65	**23**

Note: G1; Grade 1= Normal morphology, G2; Grade 2 = Mild pathological damages, G3; Grade 3=Moderate pathological damages and G4; Grade 4 = Marked pathological damages.

Fluorescent tagging and determination of accumulation kinetics by fluorescent spectroscopy
The quality of fluorescent tagging of PVC-MPs was examined under green fluorescence on black PC filter paper. PVC-MPs were effectively stained and identified under the given staining condition (Fig. 6).

Microplastic distribution in fish body tissues was studied by assaying fluorescently-tagged PVC-MPs. After ten days of exposure, PVC-MP particles were clearly visible in the gills and digestive system of fish which confirm the swallowing of MPs by fish (Fig. 7). Among three classes of PVC-MPs, class A particles showed the strongest fluorescence intensity in the gills and intestine of fish, while other PVC-MP classes showed relatively weak fluorescence intensity, indicating the effect of MPs size on the accumulation in the fish tissues. PVC-MPs were not observed in fish liver, and kidney.
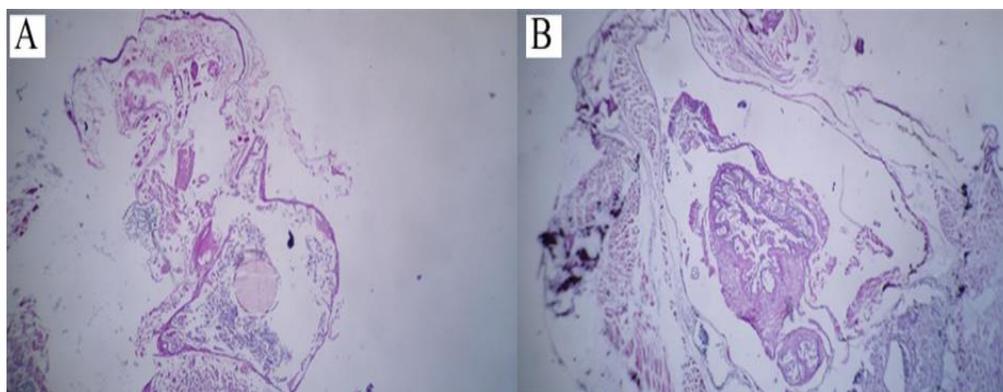
Fig. 3. A: Intensive pathological damages in the guts of common carp due to exposure to 1000 μg L^{-1} Class A-PVC-MPs for 10 days; B: Epithelial detachment, thinning of the intestinal wall, congestive inflammation, epithelial damage and lesions of villi in intestinal wall. (Original magnification: × 100).
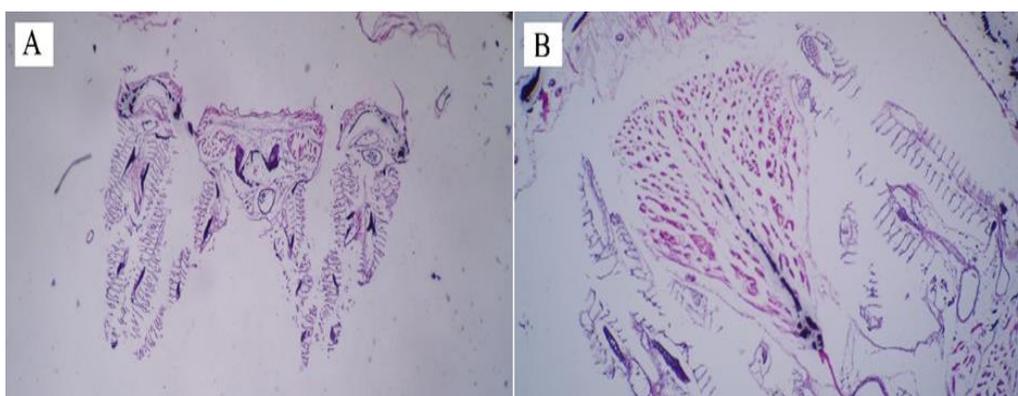
Fig. 4. Alterations observed in the gills of common carp exposed to A: 100 μg/L Class A-PVC-MPs for 10 days, and B: 1000 μg L^{-1} Class A-PVC-MPs for 10 days. Necrosis, adhesion, and partial fusion of secondary lamellae and mucous hypersecretion. (Original magnification: × 100).
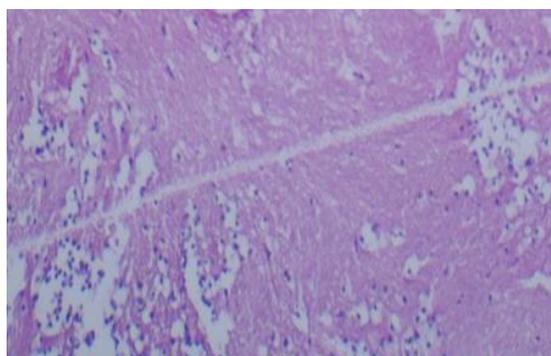

Fig. 5. Cellular necrosis in hepatocytes of common carp after exposure to 1000 μg L^{-1} Class A-PVC-MPs for 10 days. (Original magnification: × 200).

Behavioral observation and analysis

The behavioral characteristics of fish were significantly affected by PVC-MPs. Significant alterations were observed in the behaviors of fish exposed to class A PVC-MPs. However, these alterations were not significant for class B and C-PVC-MPs. After four days of exposure, abnormal swimming behavior, gradual increase in resting time and erratic movements, and decreased swim activity and vertical swimming were observed.
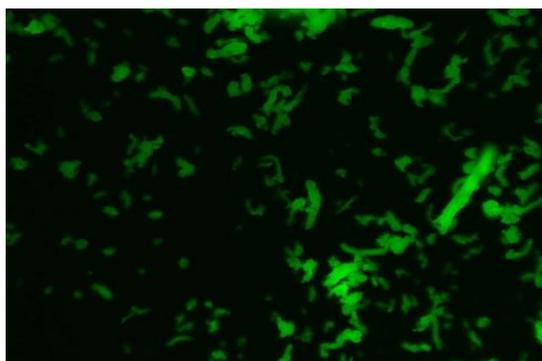
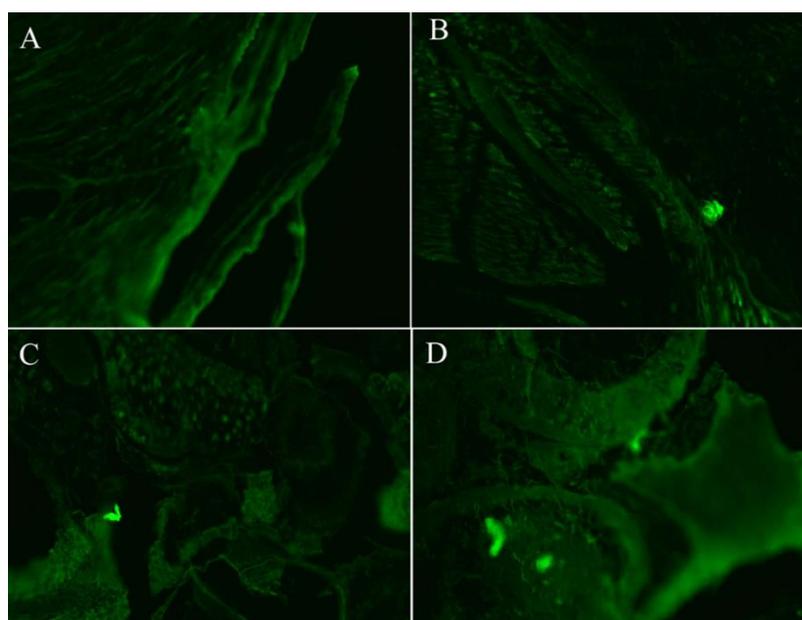**Fig. 6.** Stained PVC-MPs with florescent dye (Nile Red) under florescent microscope with green emission.

Fig. 7. Photographs of fish GI tissues under green fluorescence after 10 d of the exposure to 100 mg L^{-1} fluorescent-tagged MPs. Bright fluorescent plastic particles are clearly visible in photographs B, C and D (gills and guts tissues of exposed fish) but not in photograph A (control, unexposed fish).

DISCUSSION

This study deals with the subacute toxic effects of PVC-MPs using common carp as a suitable model for evaluating the aquatic toxicity of MPs. In this study, the effects of dose and size of PVC-MPs as well as the patterns of pathological damages were assessed. Reviewing the literature showed that there is not any study investigating the toxic effects of PVC-MPs in common carp, and this is the first report in this field. Contrary to zebrafish and other laboratory animal models, common carp is a fish with nutritional value for humans. Although weekly consumption of fish is recommended, contaminants such as MPs in seafood have raised many concerns regarding the benefits of fish consumption. Observation of the florescent-labeled PVC-MPs in the different tissues of common carp indicated that this fish readily ingests PVC-MPs. Ingestion of other types of MPs such as polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polyamides (PA) by different species of fish were reported in previous studies (Lu *et al.* 2016; Jin *et al.* 2018; Lei *et al.* 2018). Although the fish have a sensitive gustatory system and could distinguish food particles from inedible items efficiently (Houlihan *et al.* 2008), however, our findings in accordance with the results of several recent studies (Wang *et al.*, 2020) indicate that fish ingest MPs regardless of their types and composition. The mechanisms causing fish not to separate inedible plastics from food items, is not fully understood. However, we suggest that the co-occurrence of MPs and food in the fish oral cavity may affect the gustatory system of fish, decreasing the detectability of inedible items, hence allowing MPs to be swallowed accidentally. In the fluorescent microscopic observations, the fluorescence intensity in the fish tissues exposed to Class A and B-PVC-MPs was significantly higher than those exposed to class C, indicating the key role of particle size consuming by fish. Although the larger MPs have less

chance to be swallowed, unfortunately, plastics are persistent for hundreds of years in the environment, and larger plastic debris is degraded into smaller and smaller pieces by different chemical and physical mechanisms. In the acute lethality tests, the LC_{50} of classes A and B were 18.7 and 98.6 µg L^{-1}, respectively. No acute lethality was observed in fish exposed to class C. The average particle size of classes A and B were 72 and 186 µm, respectively. About two times reduction in particle size increased lethality about five times, indicating that the particle size is the determinant factor in MPs toxicity. Exposure to 1000 and 100 µg L^{-1} class A-PVC-MPs killed 100% of fish after 7 and 10 days. However, 88.9% of fish were alive after ten days of exposure to 1 µg L^{-1} class A indicating that toxicity occurred in a dose and time-dependent pattern. In a relative study (Ding *et al.* 2018), red tilapias were exposed to polystyrene-MPs for 14 days and time-dependent increase in MPs concentration was observed in fish tissues following the order gut > gills > liver ≈ brain. In the present study, pathological damages in common carp tissues were occurred according to the same pattern. The gastrointestinal tract suffered the most pathological damages. Common carp ingested a significant amount of PVC-MPs, and the GI tract was in direct contact with MPs. Intake of MPs interfere with the normal functioning of the fish digestive system (Jabeen *et al.* 2018), including functional and histopathological alterations in its GI tract (Jin *et al.* 2018). Fish Intestinal damages, including cracking the villi and splitting enterocytes, were reported due to MPs exposure (Qiao *et al.* 2019). A study by Lei *et al.* (2018) showed that MPs (PVC, PET, PS, and PP) could accumulate in the zebrafish intestine, inducing functional damage via induction of inflammation and oxidative stress. The results of our study showed that PVC-MPs in fish, similar to other types of MPs could induce epithelial detachment, thinning of intestinal wall, congestive inflammation, epithelial damage, and lesions of intestinal villi in a time and dose-dependent manner. Lu *et al.* (2016) reported hepatic damages in fish exposed to MPs (polystyrene MPs) for the first time, including early inflammatory responses such as necrosis, vacuolation and infiltration. The hepatotoxic effects of PVC-MPs were investigated and verified in the present study for the first time. Marked pathological damages (including cellular necrosis) were observed in the liver of common carps exposed to class A-PVC-MPs, while these damages were significant for classes B and C. Those with sizes above 100 µm could not be absorbed from the GI tract and enter bloodstream. Consequently, internal tissues of fish such as the liver may not be affected by PVC-MPs with sizes above 100 µm. Lu *et al.* (2016) working on zebrafish, reported that after seven days of exposure to polystyrene MPs, those with 5 µm in diameter accumulated in fish gills, liver, and gut, while those with 20 µm in diameter accumulated only in fish gills and gut. In the present study, class A-PVC-MPs included different sizes of MP particles (from 2 to 104 µm). Further studies are necessary to determine the exact sizes of the particles which can be adsorbed from the fish GI tract. MPs caused structural damage to the gills of common carp, depending on their size and concentration. PVC-MPs were detected in fish gills, leading to the breakage of gill filaments, likely due to direct contact. Similar findings have also been reported by Erkmen *et al.* (2017) and Jabeen *et al.* (2018). The present study showed that the severity of abnormal behavior and the percentage of fish with abnormal behavioral changes increased by PVC-MP concentrations. Behavioral changes (abnormal swimming behavior, gradual increase in resting time and erratic movements, decreased swim activity; and vertical swimming) in zebrafish due to polyethylene microplastic exposure and toxicity have been reported previously (Mak *et al.* 2019) similar to our results.

CONCLUSION

In this study, we present the toxic effects of PVC-MPs on common carp for the first time. We showed that common carp ingests PVC-MPs, and the relatively large PVC particles were found in the fish gut. Exposure to the PVC-MPs resulted in upraised mortality of fish. Size and concentration were the key determinant factors in MPs toxicity. PVC-MPs caused intestinal damage, including epithelial detachment, thinning of the intestinal wall, villi lesions, hepatic and gills damages, and behavioral alterations. The results of the present study provide novel insights into the environmental toxicity of PVC-MPs in aquatic organisms.

AKNOWLEGMENT

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