The Toxicity of Polyethylene Microplastic Exposure and Its Concurrent Effect With *Aeromonas Hydrophila* Infection To Zebrafish

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Abstract

Microplastics that are widely distributed in the environment has raised great concerns due to their potential negative effects to humans. Zebrafish was used as the model organism in this study to assess the toxicity of microplastic exposure. The adult zebrafish were exposed to the PE microplastics in smooth clustered sphere shapes with diameters of 75-100 μm for 35 days, and the survival rate of the zebrafish were not significantly affected, whereas the growth rate was. Further analyses on the oxidative stress related enzyme activities showed that the production of GSH, GSH-PX, and GST in the intestine were stimulated when exposing to the microplastics of lower concentrations (0.1 and 1 mg/L), while the production of SOD, CAT, GSH, GSH-PX were suppressed when exposing to those of 10 mg/L. The activities of enzymes in the muscle were much less affected. The intestinal injury and changes of colony structure in the intestine were observed by exposure to the tested concentrations of microplastics. By exposure to the microplasctics for 35 days, a further concurrent exposure to microplasctics and *Aeromonas hydrophila* did not exacerbate the mortality of zebrafish due to bacterial infection; on the contrary, the mortality was reduced. This study confirmed the intestinal enzyme activity changes of zebrafish, but showed no sign of inducing higher mortality or exacerbating bacterial infection by chronic exposure to the microplastics.

Introduction

Plastics are widely adapted in all aspects of life and production, such as packaging, textile, building and construction, and so on. Due to the indiscriminate disposal, the waste plastics are increasingly entering the environment. Plastic particles < 5 mm in size are usually defined as microplastics (Thompson et al. 2004). Plastics that are directly manufactured to be of a microscopic size are defined as primary microplastics, which always can be used in personal cleaners and cosmetics. Some microplastics derived from the breakdown of large plastic debris via exposure to environment, known as secondary microplastics (Cole et al. 2011, Sun et al. 2019). Due to the small particle size, low density, and strong durability, microplastics have been widely distributed around the world (Le et al. 2018).

Microplastics were firstly detected in seas and oceans, and have been well studied in the marine environment (Zhu et al. 2018, Courtene-Jones et al. 2017, Kazmiruk et al. 2018). Recently, microplastics have also been reported being present in freshwater bodies such as lakes, rivers, and treated drinking water. Microplastic pollution in the freshwater environment is as serious as in the ocean, and is mainly concentrated in lakes. Compared with microplastics in the marine environment, microplastics observed in freshwater are smaller, with the majority < 0.5 mm (Yan et al. 2019, Main et al. 2015, Baldwin et al. 2016), compared to the particle size 0.3-5 mm of those in the marine environment. In addition, microplastics have been found not only in aqueous environment, but also in terrestrial systems (Wang et al. 2020), and even in the atmosphere (Dris et al. 2016). More seriously, microplastics are continuously spreading. In addition to the water and soil environments of plains and coastal areas with intense human activities, microplastics have also been detected in places such as inaccessible plateaus, deep seas, and even poles

(Jiang et al. 2019, Van Cauwenberghe et al. 2013). Therefore, the negative effects of microplastics to animals and humans have raised great concerns.

Microplastics can be ingested easily by marine birds, mammals, fish, and reptiles owing to their small size, thus resulting in damage on the organisms due to the size, sharp ends and toxic additives of microplastics (Ding et al. 2020). Mao et al. (2018) confirmed that the growth rate of *Chlorella pyrenoidosa* was reduced under the exposure of polyethylene (PE) microplastics, and observed unclear pyrenoid, distorted thylakoids and damaged cell membranes, which might be attributed to the physical damage and oxidative stress. Von Moos et al. (2012) exposed blue mussels to PE microplastics in size of 0-80 μm, and showed the presence of PE particles on gills and inside the digestive system.

Over the recent years, zebrafish has been used as the model organism to study the toxicity of microplastics. Besides their small size, short life cycle, and inexpensive maintenance, the availability of transgenic lines, high-throughput sequencing and genetic similarities to humans made it being more advantageous to other rodent models (Bhagat et al. 2020). By using adult zebrafish as the model organism, studies have proved that microplastics of various sizes mostly affected the gut rather than other tissues. Although microplastics of polyamide (PA), PE, polypropylene (PP), and polyvinyl chloride (PVC) at ~70 μm showed low lethality on zebrafish, they did cause intestinal damage (Lei et al. 2018). By exposure to polystyrene (PS) particles from 100 nm to 200 μm at the concentration of 500 μg/L, Gu et al. (2020) observed dysfunction of intestinal immune cells of zebrafish. PE is the polymer produced with the largest quantities worldwide, and is the most widely detected microplastic pollution in the ocean and freshwater (Rezania et al., 2018). Limonta et al. (2019) also found that by exposing zebrafish to a mix of PE and PS microplastics with the medium size of ~50 μm for 20 days led to alteration in the intestinal mucosa, and the expression of genes related to immunity and metabolic pathways in the liver. Upon the observed effects, the authors pointed that a concurrent exposure of microplastics and pathogen may lead to changes of innate immune mechanisms. However, few studies have conducted to verify this hypothesis.

The objectives of this study were to investigate: (1) the effects of PE microplastics on the survival and growth of zebrafish, (2) changes of oxidative stress related enzyme activities in zebrafish by exposure to PE microplastics (3) the effects of PE microplastic exposure on intestinal injury and intestinal flora of zebrafish, and (4) the concurrent effect of PE microplastic exposure on the mortality of zebrafish infected with *Aeromonas hydrophila.*

Materials And Methods

Microplastics

Pristine PE microplastic particles (75-100 μm) were purchased from Zhangmutouhua plastics and rubber company, China. The microplastic particles were used after screening. The microplastic particles were identified by Fourier transform infrared spectroscopy (FTIR, 8700, SBE - 16, Thermofisher, USA) and

Raman spectroscopy (Bruker, Senterra II, Germay). The morphology of the microplastics were detected by the field emission scanning electron microscope (SEM) (Tescan Vega II, Czech Republic). The supplier claimed that no plasticizers have been added during the production of the microplastics.

Zebrafish

Zebrafish (3-4 months old) were purchased from China Zebrafish Resource Center. Before the exposure experiments, the zebrafish were domesticated in the laboratory for 2 weeks, and exposed to the normal circadian cycle. The water for culturing the zebrafish were retained from tap water aerated for 2-3 days to remove residual chlorine before use. Zebrafish were cultured in the water at 24 ± 1°C, pH 7.0 - 8.0, and aerated with dissolved oxygen at 6.24 ± 0.74 mg/L.

Aeromonas hydrophila

The standard strain of *Aeromonas hydrophila* used in this study was purchased from China General Microbiological Culture Collection Center (CGMCC No.: 1.2017). The colonies with neat edges were light yellow. The surface was smooth, and the middle was convex and opaque. Samples were stored at -80°C and cultured under 30°C at aerobic environment before use.

Exposure of zebrafish to microplastics

There were four groups in the exposure experiment of microplastics, including the control group and three experimental groups with different concentrations of PE microplastics (0.1 mg/L, 1 mg/L, and 10 mg/L, respectively). In each group, there were three parallel fish tanks, with 30 zebrafish in each tank. The zebrafish were cultured by exposure to the microplastics for 35 days. The survival rate and body weight of zebrafish in each experimental group were recorded on the 35th day. The control group represented the zebrafish without exposing to microplastics.

Concurrent bacterial infection of Aeromonas hydrophila and microplastic exposure

The spiking dose of *Aeromonas hydrophila* for infection of zebrafish was determined according to the growth curve of *Aeromonas hydrophila* and the corresponding curve of half lethal dose (LD_{50}) and the concentration *Aeromonas hydrophila*. After a 35-day exposure to the microplastics, zebrafish were cultured in the aerated water with the microplastic exposure at the LD_{50} of *Aeromonas hydrophila* for another 7 days. The mortality and the characteristics of dead zebrafish in each group were recorded.

Determination of oxidative stress related enzyme activities

The oxidative stress related enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-PX), and glutathione s-transferase (GST) were analyzed with the commercial kits (Nanjing Jiancheng Bioengineering Institute Ltd., China).

Determination of intestinal flora

The intestinal flora was determined by 16S rRNA amplification and sequencing. The zebrafish were immersed in 0.2% Tricaine solution and allowed to stand for 20-30 min to ensure that the fish had died. After that, the fish were taken out and dissected, the intestinal tract was completely stripped, and then the intestinal contents were extruded with pointed forceps, stored in a cryopreservation tube at -80°C. DNA was extracted from the samples by using the FAST DNA SPIN kit (MP bio, Irvine, CA, USA). Primers of bacteria 16S rRNA V3-V4 regions in samples were selected for amplification. PCR product purification used Qiagen gel recovery kit (USA), and library quantification used NanoDrop and Qubit, respectively. Appropriate library was added according to the data volume of 60,000 reads per sample, and HiSeq2500/MiSeq was selected for PE250 sequencing. Quality testing was performed by FASTQC which was based on the sequencing data. GreenGenes, a database, was used as a reference to group the sequences into multiple OTU (Operation Taxonomic Unit) with 97% sequence similarity by QIIME (V1.9.1). Species taxonomy of the OTU was conducted according to the existing reference taxonomy in the Ribosomal Database Project (RDP). The sequence abundance of each sample was calculated to build up Genus's sequence abundance matrix.

Histopathological analysis

The pathological examination of zebrafish intestines was carried out by histological paraffin section, and the intestinal injury was observed by slide scanning software k-viewer. The specific procedures included sampling and fixation, dehydration and transparency, wax immersion and embedding, sectioning and pasting. Briefly, the intestines were fixed with 4% paraformaldehyde and embedded in paraffin. Histological sections of 4-6-μm thickness were cut and stained with hematoxylin & eosin (H&E) and finally observed under microscopy (Li et al. 2016).

Statistical analysis

All data of the experiments were analyzed by SPSS and Origin software. The differences between the control group and the experimental groups were analyzed by single factor analysis of ANOVA, and the independent sample t-test was followed.

Results And Discussion

Characteristics of microplastics

The chemical composition of the microplastic particles were identified by FTIR spectroscopy and Raman spectroscopy, and the results were shown in Fig. 1 and Fig. S1 (Supplementary information), respectively. By comparing with the standard spectrum and observing the typical absorption peaks, the chemical composition was confirmed to be PE. No absorption peaks of ester were detected, which confirmed that no ester plasticizers were added during manufacturing of the microplastics. The shapes and sizes detected by SEM are shown in Fig. 2. The microplastics particles were irregular clustered spheres, with smooth surfaces.

Effects of microplastics on the growth and survival of zebrafish

After a 35-day exposure to different concentrations of the microplastics, only several dead zebrafish were found. The survival rate and weight growth rate of zebrafish are shown in Fig. 3. There is no significant difference in the survival rate of zebrafish ($P > 0.05$). Comparing the experimental results with that of the control, the weight growth rate of zebrafish was decreased in all the microplastic-exposed groups.

The acute toxicity of mortality of zebrafish by exposure to the microplastics were not observed in this study. It has been reported that microplastics at environmental concentrations are difficult to cause the lethal effects of zebrafish (Lei et al., 2018), and the lethal effects of microplastics are closely related to the factors such as their shape, particle size, concentration, and experiment duration (Gray and Weinstein 2017). Interestingly, fish could spontaneously cough up microplastics (Li et al. 2021), and the rejective behavior of fish greatly reduced their mortality.

Although the lethal effect of microplastics on zebrafish was not observed in this study, the weight growth rate of zebrafish decreased after exposure to the microplastics. At the microplastic concentration of 0.1 $mg{\cdot}L^{-1}$, the growth rate of body weight was the lowest, followed by the concentration of 10 $mg{\cdot}L^{-1}$, which showed no significant difference compared with that at the concentration of 1 $mg{\cdot}L^{-1}$ (Fig. 3). The weight growth loss of zebrafish seems to be independent of microplastic concentration in this study. Au et al. (2015) also observed a non-dose-dependent effect on the growth of *Hyalella azteca* after a 42-day exposure to PE microplastic beads. However, as growth rate was affected by many factors, further analyses were carried out to explore the effects of the microplastic exposure on zebrafish.

Effects of microplastics on oxidative stress related enzyme activities in zebrafish

Organisms produce certain intermediate products and free radicals in the process of oxidation, such as hydrogen peroxide and other reactive oxygen species (ROS). Excessive ROS leads to oxidation damage to the body of organisms. Cells mainly rely on their own enzymatic antioxidant defense system to prevent oxidative stress. The changes of activities of SOD, CAT, GSH, GSH-PX, and GST in the intestinal and muscle tissues of zebrafish by exposure to the microplastics are shown in Fig. 4.

As shown in Fig. 4A, exposure to the microplastics at 10 mg/L strongly reduced SOD activity in the intestine over the exposure period, whereas exposure to microplastics at 0.1 and 1 mg/L did not greatly affect SOD concentration in the intestine of zebrafish. Similar trend was also observed with the activity of CAT in the intestine of zebrafish (Fig. 4B). SOD is a cellular antioxidant enzyme that can catalyze the dismutation of superoxide into molecular oxygen and hydrogen peroxide, consequently protecting organisms from over-production of ROS induced by xenobiotics (Piddington et al. 2001). The main function of catalase (CAT) is to catalyze the decomposition of H_2O_2 and O_2, so that H_2O_2 can not react with O_2 to produce harmful to hydroxyl radicals. The results indicated that exposure to lower concentration (0.1 and 1 mg/L) of the microplastics did not affect much on the concentrations of SOD and CAT in the intestine of the zebrafish, however, a much higher dose (10 mg/L) exposure may exceed the oxidative stress abilities and lead to the malfunction of SOD and CAT production.

The concentrations of both GSH and GSH-PX in the intestine increased by exposure to lower concentration (0.1 and 1 mg/L) of the microplastics, while strongly reduced by exposure to higher concentration (10 mg/L) of the microplastics (Fig. 4C and D). GSH is not only a low molecular scavenger, but also a substrate for the synthesis of GST and GSH-PX, which can eliminate the effect of ROS (Massarsky et al. 2017). In this study, exposure to the microplastics at lower concentrations caused slight oxidative damage to the zebrafish, stimulating organisms to produce oxidative stress response, and leading to the activation of the zebrafish's own antioxidant defense system thus increasing GSH and GSH-PX concentrations. While as the concentration of the microplastics became much higher, the ROS produced in zebrafish exceeded its antioxidant defense ability, the production of GSH and GSH-PX in the intestine of zebrafish. The activity of GST almost followed the same trend as that of GSH and GSH-PX, which showed a slight elevation in the intestine after exposure to the microplastics at lower concentrations (0.1 and 1 mg/L), and a reduction when the concentration of microplastics became higher (10 mg/L) (Fig. 4E).

Compared with the enzyme activities in the intestine, their functions in the muscle were much less affected by exposure to the microplastics (Fig. 4), indicating that microplastics may not cause severe oxidative damage to the muscle tissue of zebrafish. As the size of microplastics applied in this study was comparatively large (75 - 100 μm), microplastics were not able to enter the muscle tissue of the zebrafish through blood circulation. Chen et al. (2020) found that microplastics with a much smaller size might cause alternation of muscle tissues of zebrafish, through affecting energy supply and motor-related pathways of muscle tissues.

Effects of microplastics on the intestinal injury of zebrafish

In the intestinal section of the control group (Fig. 5), the intestinal structure was clear, the intestinal inner wall was complete, and the folds and the number of mucus cavities were normal. After exposure to different concentrations of the microplastics for 35 days, the intestines of zebrafish showed different degrees of damage. With the increase of microplastic concentration, the number of mucus cavities in the intestine greatly increased. When the microplastic concentration was 10 mg/L, a large number of mucus cavities were observed. The microplastics also damaged the cell structure of zebrafish intestinal epithelial cells. When the microplastics concentration was 1 m/L, it was observed that the folds of the intestinal walls and some epithelial cells of zebrafish were damaged. The results showed that the effect of the microplastics on the intestine of zebrafish was dependent on the their concentrations. At a lower concentration (0.1 mg/L), the effect of microplastics on zebrafish was difficult to be observed and characterized by pathological sections. Qiao et al. (2019a) confirmed that microplastics exposure could cause vacuolization, cilia defects, and mast cells in the gut. Jin et al. (2018) also proved that microplastic particles can damage the intestines of zebrafish.

Effects of microplastics on intestinal flora of zebrafish

In order to observe the effect of different concentrations of microplastics on the intestinal flora of zebrafish, the composition of intestinal flora of zebrafish was detected by 16s rRNA amplification and

sequencing. The microbial communities in the intestine of both the control and microplastic exposure groups were dominated by *Proteobacteria* at the phylum level (Fig. 6). By exposure to 0.1 mg/L microplastics, the proportion of *Firmicutes* and *Actinobacteria* increased. By exposure to 10 mg/L microplastics, the proportion of *Verrucomicrobia* and *Bacteroidetes* increased. However, by exposure to 1 mg/L microplastics, the proportion of *Proteobacteria* was strongly increased, and the richness of intestinal microbial community decreased.

Intestinal tract is an important digestive place for aquatic animals. Relevant studies have shown that environmental factors will affect the diversity of microorganisms in intestinal tract and even change the composition of microbiota, but its transformation mode varies with different species (Raymann et al. 2017). Jin et al. (2018) has found that by exposure to PS microplastic beads for 14 days, the abundance of *Bacteroides* and *Proteobacteria* decreased and *Firmicutes* increased; whereas Qiao et al. (2019b) indicated that the relative abundance of *Proteobacteria* was significantly elevated by exposure to microplastic beads, fibre, and fragments. These studies demonstrated that microplastics may affect the composition of colonies in the intestinal tract of aquatic organisms and change the structure of intestinal colonies. On the other hand, however, the inconsistency of variation of intestinal flora may be related to inflammation in individual bodies of zebrafish (Jin et al. 2017). In this study, the increase of mucus cavity and oxidative stress reaction were observed in the intestine of zebrafish exposed to microplastic environment, which changed the environment in the intestine, resulting in the change of flora diversity and the change of colony structure in the intestine of zebrafish.

Effect of microplastics on zebrafish infected with Aeromonas hydrophila

Normal and healthy zebrafish were soaked and cultured with different concentrations of *Aeromonas hydrophila*, and the half lethal dose (LD_{50}) of *Aeromonas hydrophila* to infect zebrafish was determined to be 6.049×10^{6} CFU/mL. The growth curve of *Aeromonas hydrophila* (Fig. S2) and the method for determination of LD_{50} are detailed in the Supplementary information. Zebrafish that exposed to different concentrations of the microplastics for 35 days were concurrently exposed to *Aeromonas hydrophila* at LD_{50} in aerated water, and was continuously observed for 7 days. The results on the mortality of zebrafish infected with *Aeromonas hydrophila* are shown in Table 1. Compared with the control group, the mortality of zebrafish exposed to the microplastics decreased, and the lethal rate was the lowest by exposure to PE microplastics at the concentration of 1 mg/L, which could be resulted from the colony structure of this group (Fig. 6).

Table 1
Lethal rates of zebrafish infected by *Aeromonas hydrophila* upon concurrent exposure to the microplastics

Concentration of PE microplastics	Items of zebrafish	Dead items	Lethal rates	Average lethal rate
0 mg/L	23	7	30.43%	52.41%
	23	10	43.48%	
	24	20	83.33%	
0.1 mg/L	22	8	36.36%	40.10%
	24	19	79.17%	
	21	1	4.76%	
1 mg/L	23	0	0	4.55%
	23	0	0	
	22	3	13.64%	
10 mg/L	23	0	0	21.74%
	23	1	4.35%	
	23	14	60.87%	

One of the reasons of the reduction of lethal rates and the variation of among the parallels in each group could be due to absorption of *Aeromonas hydrophila* to the microplastics in the fish tanks. Due to their high specific surface area and hydrophobicity, microplastics have strong absorption to many pollutants, such as heavy metals and organics (Qiao et al. 2019c). In addition, they may also absorb microorganisms and serve as carriers of pathogenic bacteria. It has been proved that microplastics could serve as a vector of *Aeromonas hydrophila* in marine environment (Virsek et al. 2017). Brandts et al. (2020) reported that PS nanoplastic exposure for 24 h did not affect the infection of zebrafish larvae by *Aeromonas hydrophila*. Sendra et al. (2020) found that the effect of infection of *Mytilus galloprovincialis* hemocytes with *Vibrio splendidus* exposed previously to nanoplastics for 3 h had effects in gene expression, while exposure to the combination of microplastics and *Vibrio splendidus* had no effect on the mortality of zebrafish. The authors also indicated that a chronic exposure to microplastics may be of greater importance. In this study, after exposure to the microplastics for 35 days, a subsequently concurrent exposure to the microplastics and *Aeromonas hydrophila* by the zebrafish did not show any exacerbating on the mortality of zebrafish, compared with those infected by *Aeromonas hydrophila* alone.

Conclusion

In this study, the effects of exposure to the microplastics on zebrafish were investigated. By exposure to clustered smoothly sphere PE particles (75-100 μm) for 35 days, no acute toxicity of mortality on the zebrafish was observed, while the growth rates were negatively affected. The oxidative stress related enzyme activities in the intestine of the zebrafish were influenced. The production of GSH, GSH-PX, and GST were stimulated when exposing to microplastics of lower concentrations (0.1 and 1 mg/L), while the production of SOD, CAT, GSH, GSH-PX were suppressed while exposing to those of a higher concentration of 10 mg/L. However, the activities of the enzymes in the muscles of the zebrafish were much less impacted. In addition, the intestinal injury was observed in the zebrafish by exposure to microplastics, especially at higher concentrations of 1 and 10 mg/L. The intestinal microbial flora changed, but the trend was hard to define due to the individual differences of zebrafish. It was observed that exposure to microplastics reduced the mortality of the zebrafish by *Aeromonas hydrophila* infection, however, adsorption of *Aeromonas hydrophila* to the microplastics might contribute to the reduction. There was at least no sign of exacerbating effect of concurrent exposure to microplastics to bacterial infection.

Declarations

Author contribution ND designed the study and wrote the manuscript; LJ conducted the experiments and wrote part of the manuscript; XW conducted the experiments and analyses; CW provided guidance on the analyses of the toxicity of zebrafish and designed the bacterial infection experiments; YG conducted the analyses of enzyme activities; JZ did fish breeding and husbandry; YS provided guidance of this study; YZ and QY provided advice on the design of the research; and HL provided assistance on oxidative stress related enzyme activity analyses. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

1. Au SY, Bruce TF, Bridges WC, Klaine SJ (2015) Responses of Hyalella azteca to acute and chronic microplastic exposures. Environ Toxicol Chem 34:2564–2572
2. Baldwin AK, Corsi SR, Mason SA (2016) Plastic debris in 29 great lakes tributaries: relations to watershed attributes and hydrology. Environ Sci Technol 50:10377–10385

3. Bhagat J, Zang L, Nishimura N, Shimada Y (2020) Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. Sci Total Environ 728:138707
4. Brandts I, Garcia-Ordonez M, Tort L, Teles M, Roher N (2020) Polystyrene nanoplastics accumulate in ZFL cell lysosomes and in zebrafish larvae after acute exposure, inducing a synergistic immune response in vitro without affecting larval survival in vivo. Environ Sci-Nano 7:2410
5. Chen Q, Lackmann C, Wang W, Seiler T-B, Hollert H, Shi H (2020) Microplastics Lead to Hyperactive Swimming Behaviour in Adult Zebrafish. Aquat Toxicol 224:105521
6. Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: A review. Mar Pollut Bull 62:2588–2597
7. Courtene-Jones W, Quinn B, Gary SF, Mogg AOM, Narayanaswamy BE (2017) Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environ Pollut 231:271–280
8. Ding N, An D, Yin XF, Sun YX (2020) Detection and evaluation of microbeads and other microplastics in wastewater treatment plant samples. Environ Sci Pollut Res 27:15878–15887
9. Dris R, Gasperi J, Saad M, Mirande C, Tassin B (2016) Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar Pollut Bull 104:290–293
10. Gray AD, Weinstein JE (2017) Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio). Environ Toxicol Chem 36:3074–3080
11. Gu W, Liu S, Chen L, Liu Y, Gu C, Ren H-q, Wu B (2020) Single-Cell RNA Sequencing Reveals Size-Dependent Effects of Polystyrene Microplastics on Immune and Secretory Cell Populations from Zebrafish Intestines. Environ Sci Technol 54:3417–3427
12. Jiang CB, Yin LS, Li ZW, Wen XF, Luo X, Hu SP, Yang HY, Long YN, Deng B, Huang LZ, Liu YZ (2019) Microplastic pollution in the rivers of the Tibet Plateau. Environ Pollut 249:91–98
13. Jin YX, Wu SS, Zeng ZY, Fu ZW (2017) Effects of environmental pollutants on gut microbiota. Environ Pollut 222:1–9
14. Jin YX, Xia JZ, Pan ZH, Yang JJ, Wang WC, Fu ZW (2018) Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environ Pollut 235:322–329
15. Kazmiruk TN, Kazmiruk VD, Bendell LI (2018) Abundance and distribution of microplastics within surface sediments of a key shellfish growing region of Canada. PLoS One 13:e0196005
16. Le LL, Liu MT, Song Y, Lu SB, Hu JN, Cao CJ, Xie B, Shi HH, He DF (2018) Polystyrene (nano)microplastics cause size-dependent neurotoxicity, oxidative damage and other adverse effects in Caenorhabditis elegans. Environ Sci-Nano 5:2009–2020
17. Lei L, Wu S, Lu S, Liu M, Song Y, Fu Z, Shi H, Raley-Susman K, He D (2018) Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*.. Sci Total Environ 619-620:1–8
18. Li B, Liang W, Liu Q-X, Fu S, Ma C, Chen Q, Su L, Craig NJ, Shi H (2021) Fish Ingest Microplastics Unintentionally. Environ Sci Technol 55:10471–10479

19. Li Q, Wang PP, Chen L, Gao HW, Wu LL (2016) Acute toxicity and histopathological effects of naproxen in zebrafish (Danio rerio) early life stages. Environ Sci Pollut Res 23:18832–18841
20. Limonta G, Mancia A, Benkhalqui A, Bertolucci C, Abelli L, Fossi M, Panti C (2019) Microplastics induce transcriptional changes, immune response and behavioral alterations in adult zebrafish. Sci Rep 9:15775
21. Mani T, Hauk A, Walter U, Burkhardt-Holm P (2015) Microplastics profile along the Rhine River. Sci Rep 5:17988
22. Mao YF, Ai HN, Chen Y, Zhang ZY, Zeng P, Kang L, Li W, Gu WK, He Q, Li H (2018) Phytoplankton response to polystyrene microplastics: Perspective from an entire growth period. Chemosphere 208:59–68
23. Massarsky A, Kozal JS, Di Giulio RT (2017) Glutathione and zebrafish: Old assays to address a current issue. Chemosphere 168:707–715
24. Piddington DL, Fang FC, Laessig T, Cooper AM, Orme IM, Buchmeier NA (2001) Cu, Zn superoxide dismutase of Mycobacterium tuberculosis contributes to survival in activated macrophages that are generating an oxidative burst. Infect Immun 69:4980–4987
25. Qiao RX, Deng YF, Zhang SH, Wolosker MB, Zhu QD, Ren HQ, Zhang Y (2019a) Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. Chemosphere 236:124334
26. Qiao RX, Lu K, Deng YF, Ren HQ, Zhang Y (2019b) Combined effects of polystyrene microplastics and natural organic matter on the accumulation and toxicity of copper in zebrafish. Sci Total Environ 682:128–137
27. Qiao RX, Sheng C, Lu YF, Zhang Y, Ren HQ, Lemos B (2019c) Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. Sci Total Environ 662:246–253
28. Raymann K, Shaffer Z, Moran NA (2017) Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. PLoS Biol 15(3):e2001861
29. Rezania S, Park J, Din MM, Taib SM, Talaiekhozani A, Yadav KK, Kamyab H (2018) Microplastics pollution in diferent aquatic environments and biota: A review of recent studies. Mar Pollut Bull 133:191–208
30. Sendra M, Saco A, Yeste MP, Romero A, Novoa B, Figueras A (2020) Nanoplastics: From tissue accumulation to cell translocation into *Mytilus galloprovincialis* hemocytes. resilience of immune cells exposed to nanoplastics and nanoplastics plus *Vibrio splendidus* combination. J Hazard Mater 388:121788
31. Sun J, Dai XH, Wang QL, van Loosdrecht MCM, Ni BJ (2019) Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Res 152:21–37
32. Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, McGonigle D, Russell AE (2004) Lost at sea: Where is all the plastic? Science 304:838

33. Van Cauwenberghe L, Vanreusel A, Mees J, Janssen CR (2013) Microplastic pollution in deep-sea sediments. Environ Pollut 182:495–499
34. Virsek MK, Lovsin MN, Koren S, Krzan A, Peterlin M (2017) Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida. Mar Pollut Bull 125:301–309
35. Von Moos N, Burkhardt-Holm P, Kohler A (2012) Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis L. after an experimental exposure. Environ Sci Technol 46:11327–11335
36. Wang WF, Ge J, Yu XY, Li H (2020) Environmental fate and impacts of microplastics in soil ecosystems: Progress and perspective. Sci Total Environ 708:10
37. Yan M, Nie H, Xu K, He Y, Hu Y, Huang Y, Wang J (2019) Microplastic abundance, distribution and composition in the Pearl River along Guangzhou city and Pearl River estuary, China. Chemosphere 217:879–886
38. Zhu L, Bai H, Chen B, Sun X, Qu K, Xia B (2018) Microplastic pollution in North Yellow Sea, China: Observations on occurrence, distribution and identification. Sci Total Environ 636:20–29

Figures

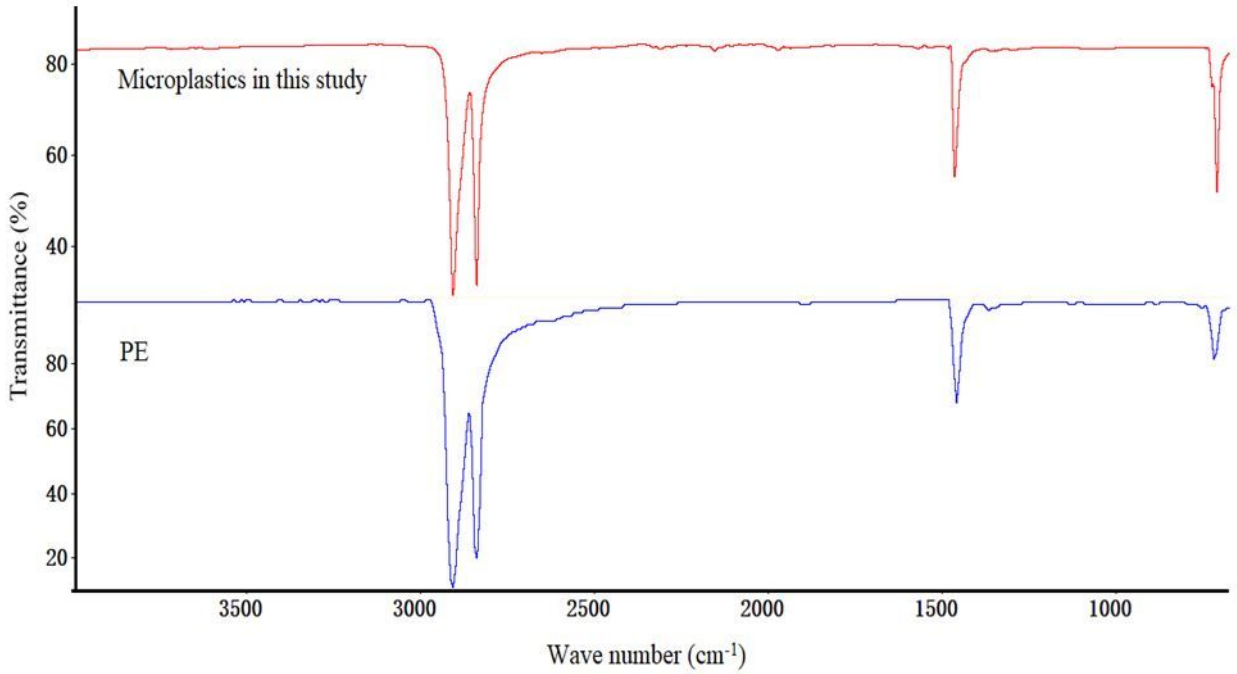

Figure 1

FTIR spectrum of the microplastics

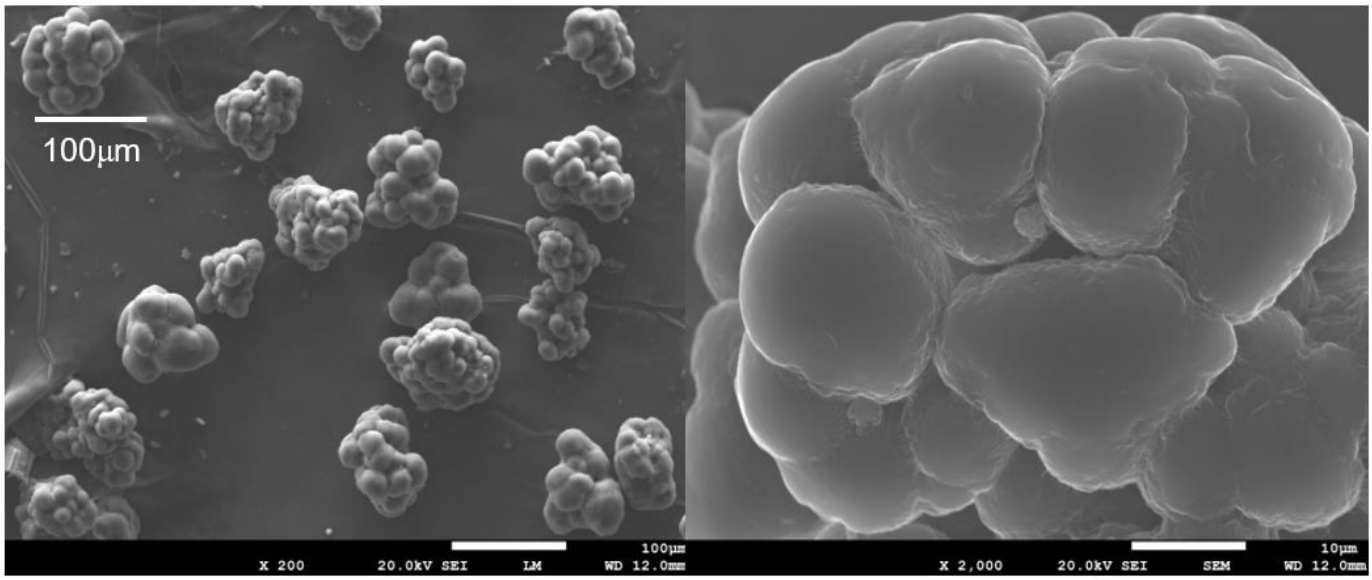

Figure 2

SEM images of the microplastics

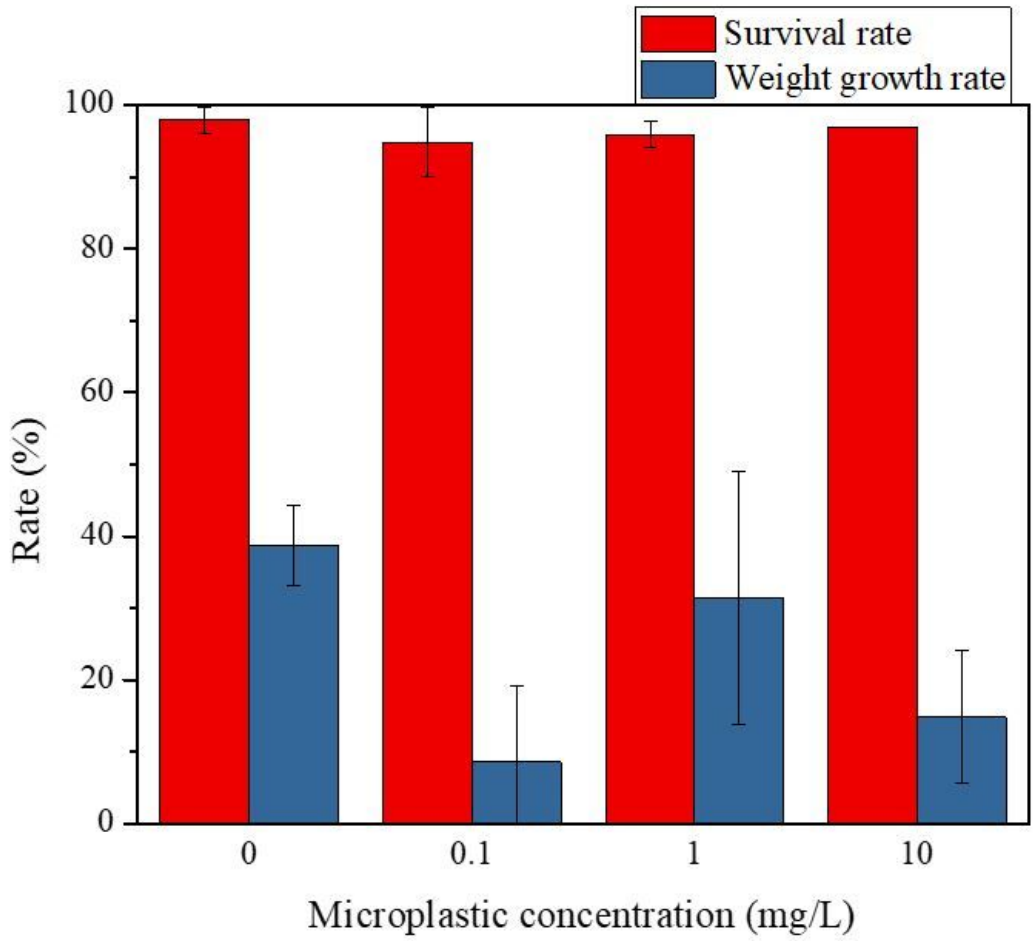

Figure 3

The survival rate and weight growth rate of zebrafish exposed to microplastics

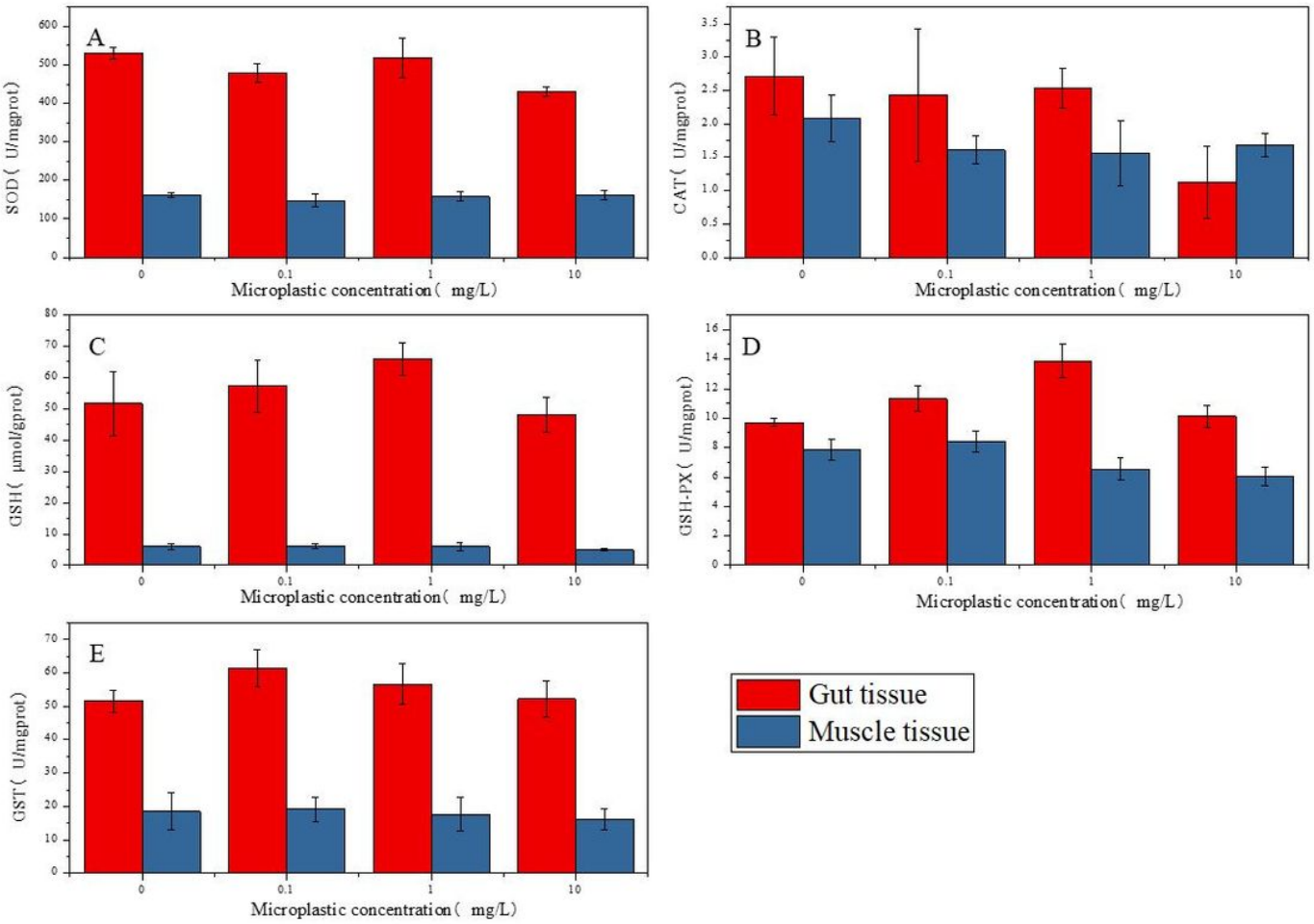

Figure 4

Changes in the concentration of (A) SOD, (B) CAT, (C) GSH, (D) GSH-PX, and (E) GST in the intestine and muscle of fish by exposure to the microplastics for 35 days. Error bars indicate ± SD.

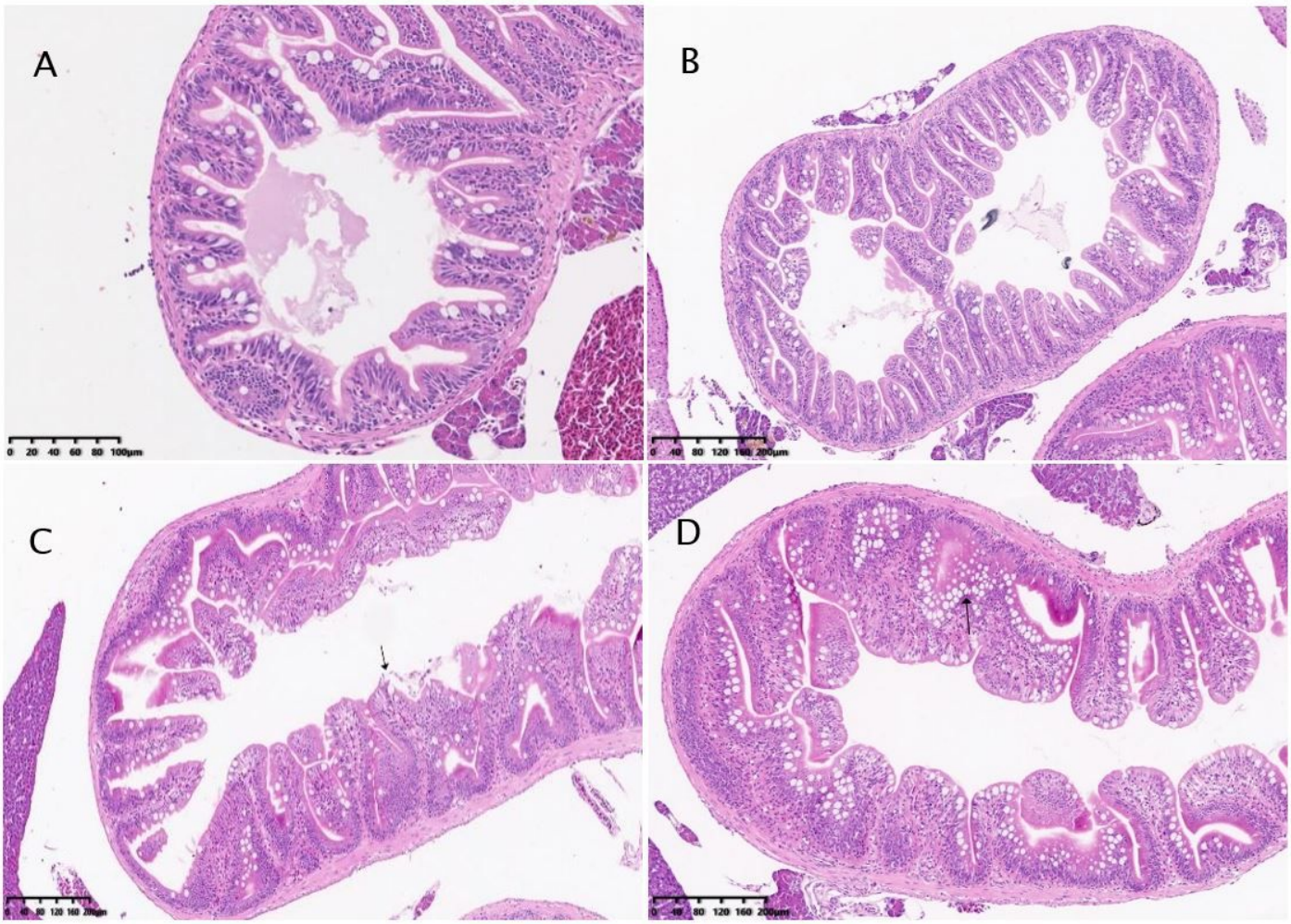

Figure 5

Histological effects of different concentrations (A: 0 mg/L, B: 0.1 mg/L, C: 1 mg/L, and D: 10 mg/L) of the microplastics on the intestines of the zebrafish. The arrows indicate intestinal epithelial cell damage and mucus cavity increase.

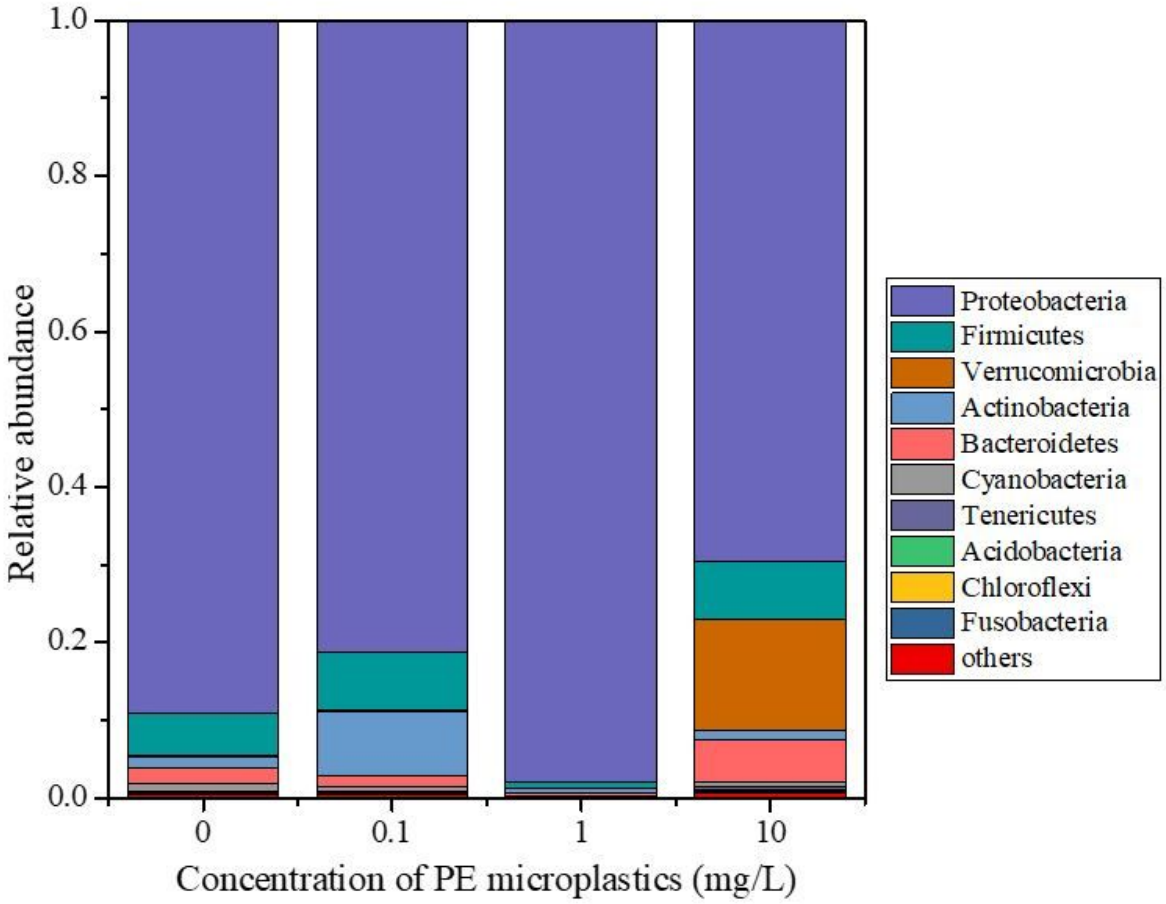

Figure 6

Effects of microplastics on the intestinal flora of zebrafish at phylum level

Supplementary Files

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- SupplementaryInformation.docx