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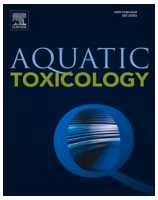

Multi-biomarkers hazard assessment of microplastics with different polymers by acute embryo test and chronic larvae test with zebrafish (*Danio rerio*)

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ABSTRACT

Microplastics as emerging contaminants show various composition features in the environment. However, influence of polymer types on the toxicity of microplastics is still unclear, thus affecting evaluation of their toxicity and ecological risks. In this work, toxic effects of microplastics (fragment, 52–74 μm) with different polymer types including polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP) and polystyrene (PS) to zebrafish (*Danio rerio*) were studied using acute embryo test and chronic larvae test. Silicon dioxide (SiO_2) was used as a control representing natural particles. Results showed microplastics with different polymers had no influence on embryonic development at environmental relevant concentration (10^2 particles/L), but could lead to accelerated heartbeat rate and increased embryonic death when exposed to SiO_2, PE and PS at higher concentrations (10^4 and 10^6 particles/L). Chronic exposure for zebrafish larvae indicated different polymers of microplastics did not affect zebrafish larvae' feeding and growth, nor induce oxidative stress. But larvae' locomotion level and AChE (acetylcholinesterase) activities could be inhibited by SiO_2 and microplastics at 10^4 particles/L. Our study demonstrated negligible toxicity of microplastics at environmental relevant concentration, while different polymers of microplastics have similar toxic effects as SiO_2 at high concentrations. We suggest that microplastic particles may have the same biological toxicity as natural particles.

1. Introduction

Plastic products bring great convenience to people but inappropriate disposal of plastic waste has caused serious pollution problems worldwide. Plastics break down in the environment as a result of weathering and generate large amount of microplastics (<5 mm) (Alimi et al., 2022). Plastic products are made from different polymers and have different morphological characteristics, which result in the diversity and complexity of environmental microplastics (Hahladakis et al., 2018). Microplastic samples with different shapes, such as films, fragments, spheres and fibers, are often observed in the environment (Cai et al., 2018; Rodrigues et al., 2019). Microplastics commonly detected are composed of polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), or polyamide (PA), which are widely used in the production of common plastic products (Moore et al., 2018; Petersen et al., 2021). Analysis of microplastics in environmental samples is complex and time consuming, there is still no widely accepted standard methods and the identification of small microplastics remains challenging (Rocha-Santos et al., 2015; Li et al., 2020).

The effects of microplastics on aquatic organisms have received great concern. Unlike traditional chemical pollutants, microplastics are particles and their toxicity could be affected by their morphology and components (Arias-Andres et al., 2019; Huang et al., 2021). It is now generally accepted that the toxicity of microplastics is size-dependent, and nano-plastics can migrate and accumulate within the circulatory system and thus cause toxic effects, while large microplastics are more likely to reside on body surface, gills and digestive tract of organisms, which could induce mechanical damage (Leung et al., 2018; Mao et al., 2018; Zhao et al., 2021). Among all shapes, fibrous microplastics are

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more likely to retain in the intestinal tract of organisms compared with fragments and spheres (Jabeen et al., 2018; Xiong et al., 2019). In addition, microplastics with an irregular shape may cause more serious impacts on organisms than spherical ones (Frydkjær et al., 2017; Choi et al., 2018).

It remains unclear whether the toxic effect of microplastics is related to their polymer types. Most of studies used PS microplastics for toxicological experiments, and only few works studied the toxicity of microplastics with other polymer types. PS was regarded as the most hazardous microplastic types compared to PET and PE due to the induced genotoxicity in larvae fish of sea trout *Salmo trutta* (Jakubowska et al., 2020). Plastic chemicals or additives extracted from PVC can increase microplastic toxicity, while effects of polyurethane and polylactic acid microplastics were induced merely by particle properties (Zimmermann et al., 2020). Variations on calcium levels and expression of *gst-4* gene in nematodes (*C. elegans*) were dependent on particle size rather than chemical composition of microplastics (Lei et al., 2018). Since microplastics in the environment exist as a mixture with different polymers, it is important to explore the influence of polymer types on microplastic toxicity for a more accurate risk assessment.

Most types of plastics are considered as chemically inert. Therefore, we hypothesized that toxicity of microplastics is independent on polymer types. To test the hypothesis, we used both acute embryo test and chronic larvae test to study the toxicity effects of microplastics on zebrafish (*Danio rerio*) at environmental relevant levels and hypothetical high levels. Multiple biomarkers including embryonic development, growth and feeding, energy reserve, locomotion levels, and oxidative stress were selected as toxicity endpoints to assess their sensitivity to microplastic exposure. Inorganic particles of silicon dioxide (SiO_2) were used as a control to compare the difference of toxicity effects between microplastics and natural particles.

2. Material and methods

2.1. Particles and zebrafish

Four types of widely used polymers, including PE, PET, PP and PS were selected for the experiment. They are predominant polymer types of microplastics which are frequently detected in the environment. Pure microplastics (no chemical additives added) and SiO_2 powder were purchased from Huachuangsuhua vendor (Dongguan, China). The size distributions of particles were determined using a laser particle size analyzer (S3500, Microtrac Inc, USA). Particle size ranged from 52 to 74 μm which had the highest proportion varying from 15.45% to 23.98% (Figure S1). Morphology of microplastics were examined using scanning electron microscope (JSM-IT 300HR, JEOL, Japan). PE microplastics were near-spherical while other microplastics had an irregular shape (Figure S2). Contact angles of microplastics were analyzed using OCA Contact Angle Tester (Dataphysics, Germany), for characterizing the hydrophobicity of microplastics (Table S1). Polymer types of microplastics were confirmed using Fourier Transform Infrared (FTIR, Thermo Fisher, German). Considering environmental microplastic concentrations from survey studies, and exposure concentrations from ecotoxicity studies, particle concentrations were set as 10^2 particles/L, 10^3 particles/L, 10^4 particles/L and 10^6 particles/L for embryo exposure, and 10^2 particles/L, 10^4 particles/L for larvae exposure (Cunningham and Sigwart, 2019). Actual exposure concentrations were transformed as mass concentrations by the formula $m = \rho v$ (for the specific conversion process, see the supplementary material, Table S2). Wild-type zebrafish embryos and larvae were obtained from Wanwuyuan laboratory equipment business department (Wuhan, China). All studies were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institute for Food and Drug Control of China.

2.2. Zebrafish embryo acute toxicity test

Exposure solutions were prepared by adding different microplastics (PP, PE, PET, PS) or SiO_2 particles into aerated tap water separately (Santos et al., 2022). Then Triton X-100 was added (0.001% v/v) to improve dispersion, and methylene blue dye was added (0.1%) to prevent mold. Aerated tap water with Triton X-100 and methylene blue dye were set as blank control group (CK). Four replicates ($n = 4$) were performed for each group. Exposure experiments were performed when embryos were 4 h post fertilization (hpf). After microscopic examination, 30 viable embryos were put into one well of the 6-well plate containing 15 mL of exposure solution or control solution. Embryos were incubated at 28 °C in a water bath with 14 h light: 10 h dark. Exposure solutions were renewed daily. Exposure experiments lasted for 5 d and ended at 144 hpf.

Dead embryos and hatched larvae were recorded daily. One larva was picked up randomly from one well (four mixed samples for each group, $n = 4$) for recording the development toxicity including heart rate (96 hpf), blood flow activity (96 hpf), deformity rate (120 hpf) and total body length (120 hpf) using an optical microscope (SZ61, OLYMUS, Japan), and then analyzed using Danio scope software (Noldus, Wageningen, Netherlands).

Larva locomotion levels was measured after 5 d-exposure (144 hpf), using the DanioVision observation chamber (Noldus., Wageningen, The Netherlands) with a temperature control unit to maintain the water temperature at 28 °C. Eight live zebrafish larvae were selected from each group (two from each well, $n = 8$) and transferred to one well of a clean 6-well plate. Their locomotor activity was recorded for 10 min at 25 frames/s via a high-speed infrared camera after acclimatization for 10 min. The EthoVision XT 5 software (Noldus, Wageningen, The Netherlands) was used to acquire behavior parameters including the total movement distance and average speed.

2.3. Zebrafish larvae chronic toxicity test

A hundred zebrafish larvae which were 10 d post fertilization (dpf) were kept in a tank (21 cm × 13 cm × 13 cm) filled with 1 L aerated tap water for each treatment. Microplastics or SiO_2 particles were added into the tank and stirred gently using a glass rod. Aerated tap water without particles were set as blank control group, and three replicates were set for each group ($n = 3$). Larvae were fed with the same amount of live brine shrimp (*Artemia sp.*) twice a day. Feces and dead fish were discarded, and exposure solutions were renewed daily. Chronic larvae exposure experiments lasted for 28 days.

At the end of experiments, five larvae were picked up randomly from each treatment ($n = 15$), and anesthetized with 0.2 g/L tricaine (MS-222, Zhongshan Mingshun Biotechnology Co., LTD, China) for few seconds. Body weight and total length of larva were recorded. In order to analyze peristaltic capacity of the intestine, intestine parts of larvae were filmed for 1 min under a microscope, and then counted the peristalsis numbers. These larvae were then put back in 100 mL beakers for 24 h to quantify food intake. After gut emptying, a slightly excessive amount of brine shrimp (2 mL, 4 g/L) were added to each beaker. Leftover brine shrimps were collected with a rubber head straw after feeding for 10 min, and then counted.

Three larvae were collected randomly from each repetition group for testing larvae' locomotion ($n = 9$). The larvae were placed in a glass petri dish with a diameter of 9 cm. The locomotion levels of larvae were video tracked for 10 min after adaptation for 10 min in the DanioVision observation chamber. Larvae' total movement distance and average speed were analyzed by EthoVision XT 5 software.

An appropriate number (8–10 pairs) of larvae were randomly picked up and homogenized from each treatment for physiological tests ($n = 3$). Physiological parameters including total protein (TP, Coomassie brilliant blue method), glucose (GLU, glucose oxidase method), total cholesterol (T-CHO, GPO-PAP method), pyruvate, acetylcholinesterase

(AChE, Colorimetric method), superoxide dismutase (SOD, Hydroxylamine method), and malondialdehyde (MDA, TBA method) were measured using Nanjing Jiancheng Biological Kits (Biological Kit codes: A111-1, A045-2, A154-1-1, A081-1-1, A024-1-1, A001-1-2, A003-1-2) (Nanjing Jiancheng Bioengineering Institute, Nanjing) referring to the instructions.

2.4. Data analysis

Statistical analyses and plots were performed using R Studio (ver.4.1.0). The Shapiro-Wilk and Levene's Test were used to examine the normality and homogeneity of the data. One-way analysis of variance (ANOVA) was used to examine the difference at a significance level of $p<0.05$ and followed by the Tukey's Honestly Significant Difference (Tukey's HSD) post-hoc test. The integrated biomarker response index version 2 (IBRv2) was calculated referring to the method of Sanchez et al. (2013). It was based on the integration of multiple biological responses from the individual to the physiological level, and to assess the sensitivity of zebrafish to different microplastics and SiO_2 particles under different exposure concentrations.

3. Results and discussion

3.1. Embryonic developments

Microscopic images showed particles attached on the embryo chorion and aggregated into large granules with the increase of particle concentrations (Fig. 1). The embryo chorion was more densely covered by SiO_2 particles followed by PS and PET microplastics, and relatively less PE and PP microplastics were attached. The number of particles attached on embryo chorion depended on the concentration of the particles and their hydrophobicity and density. Both PE and PP microplastics are low-density and more hydrophobic (Table S1, S2), and could be more difficult to attach on the embryo chorion.

Figure 2 showed the sublethal and lethal effects of different polymers of microplastics on zebrafish embryo development at different exposure concentrations. For the heartbeat rate, blood flow activity, malformation rate and body length of zebrafish larvae (Fig. 2A, B, C, D), results showed negligible differences of sublethal effect of different microplastics and SiO_2 compared with CK group, except that the total length

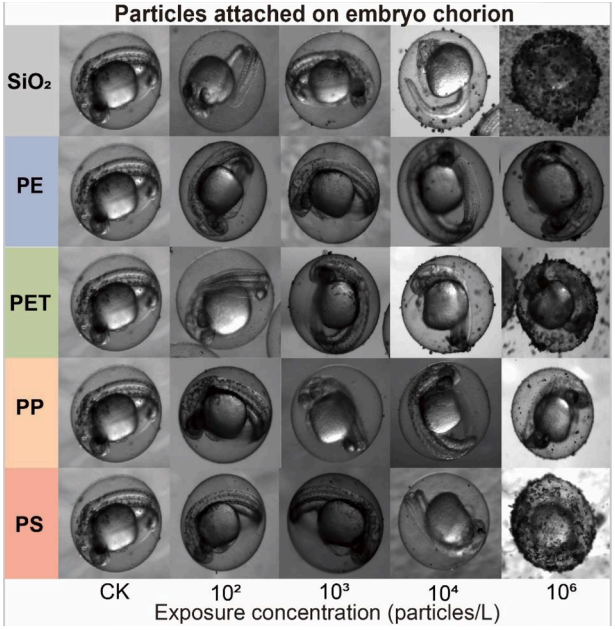

Fig. 1. Particles adsorbed on embryonic chorion.

in SiO_2 group reduced at 10^3 and 10^4 particles/L ($p<0.05$) (Fig. 2D). Larvae' heartbeat rate accelerated significantly for SiO_2 and PS exposure at 10^6 particles/L compared with those at 10^2 and 10^3 particles/L ($p<0.05$) (Fig. 2A).

Only PE and PS group showed remarkable lethal toxicity at high concentrations (Fig. 2E and F). About 20% embryonic mortality was observed in CK group after 24 -h exposure (Fig. 2E). At these concentrations of 10^2, 10^4 and 10^6 particles/L, PE group had higher embryonic mortality that CK group ($p<0.05$). The embryonic mortality of PS group was significantly higher than CK at 10^6 particles/L concentration ($p<0.05$). PE group had a higher embryonic mortality at 10^4 particles/L than 10^2 and 10^3 particles/L ($p<0.05$), and PS also had a higher embryonic mortality at 10^6 particles/L than lower concentrations ($p<0.05$). In different groups, zebrafish embryos hatched into larvae gradually from 48 hpf to 120 hpf (Figure S3). No incubation delay or advance was observed during exposure. Larvae' hatching rate at 120 hpf was 77.88 ±4.72% in CK group (Fig. 2F). PE group significantly inhibited embryo incubation at 10^4 and 10^6 particles/L compared with CK group ($p<0.05$). Both PE and PS group had lower hatching rate at 10^4 and 10^6 particles/L concentration than those at 10^2 and 10^3 particles/L ($p<0.05$).

According to the development toxicity of different polymers of microplastics on zebrafish embryos, it was found that different polymer types of microplastics had no influence on embryonic development at environment relevant concentration (10^2 particles/L). Effects such as accelerated heartbeat rate, increased embryonic death, and reduced hatching rate were induced by SiO_2, PE and PS exposure at higher concentrations (10^4 and 10^6 particles/L). Previous studies reported that pristine PA and PE particles did not affect zebrafish larvae' heartbeat and total length, while PET particles and PS sphere changed the velocities of blood flow and heartbeat rate (Malafaia et al., 2020; Zhang et al., 2020; Cheng et al., 2021). The mortality of zebrafish embryos increased with the increase of microplastic concentrations (Zhang et al., 2020), while microplastics with different polymers did not affect the mortality and hatching rates of sea trout *Salmo trutta* embryos (Jakubowska et al., 2020). As these particles used in studies were larger than the pore of chorionic channel (0.5–0.7 μm in diameter) (Cheng et al., 2021), particle coverage may be responsible for these effects. Few particles attached on embryo chorions at 10^2 particles/L, while at 10^4 and 10^6 particles/L, large amounts of SiO_2 and PS particles coverage may act as a physical barrier to oxygen transport, and in turn alter the microenvironment inside the embryo, causing changes in heart rate and blood flow and even leading to embryo death (Zou et al., 2020; Duan et al., 2020; Cheng et al., 2021). As for PE used in our study, it was more hydrophobic and low-density, and mainly floated on the water surface and formed a dense membrane, which may obstruct oxygen transport and lead to embryo death.

3.2. Feeding, energy reserve and growth for zebrafish larvae

Zebrafish larvae start to eat food from 120 hpf, and are vulnerable to external environmental stresses at this period. After 28 d exposure, food intakes of larvae fish were about 93.43±21.89 to 110.21±14.82 numbers of live brine shrimp within 10 min (Fig. 3A). There was no significant difference between treatment groups and CK group ($p>0.05$), and also no difference between different treatment groups ($p>0.05$) (Fig. 3A). The peristaltic frequencies of larvae intestine ranged from 10.11±1.07 to 12.33±1.15 times per minute (Fig. 2B). No significant difference was observed between different groups ($p>0.05$).

Results suggested that during prolonged exposure, SiO_2 particles and different polymers of microplastics did not affect zebrafish larvae' feeding ability and intestinal peristalsis at both environmental concentration and hypothetic high concentrations. This was contrary to the currently accepted views that the presence of microplastics could affect the feeding efficiency of organisms (de Sá et al., 2015; Carrasco et al., 2019). Previous studies held that exposure to high concentrations of

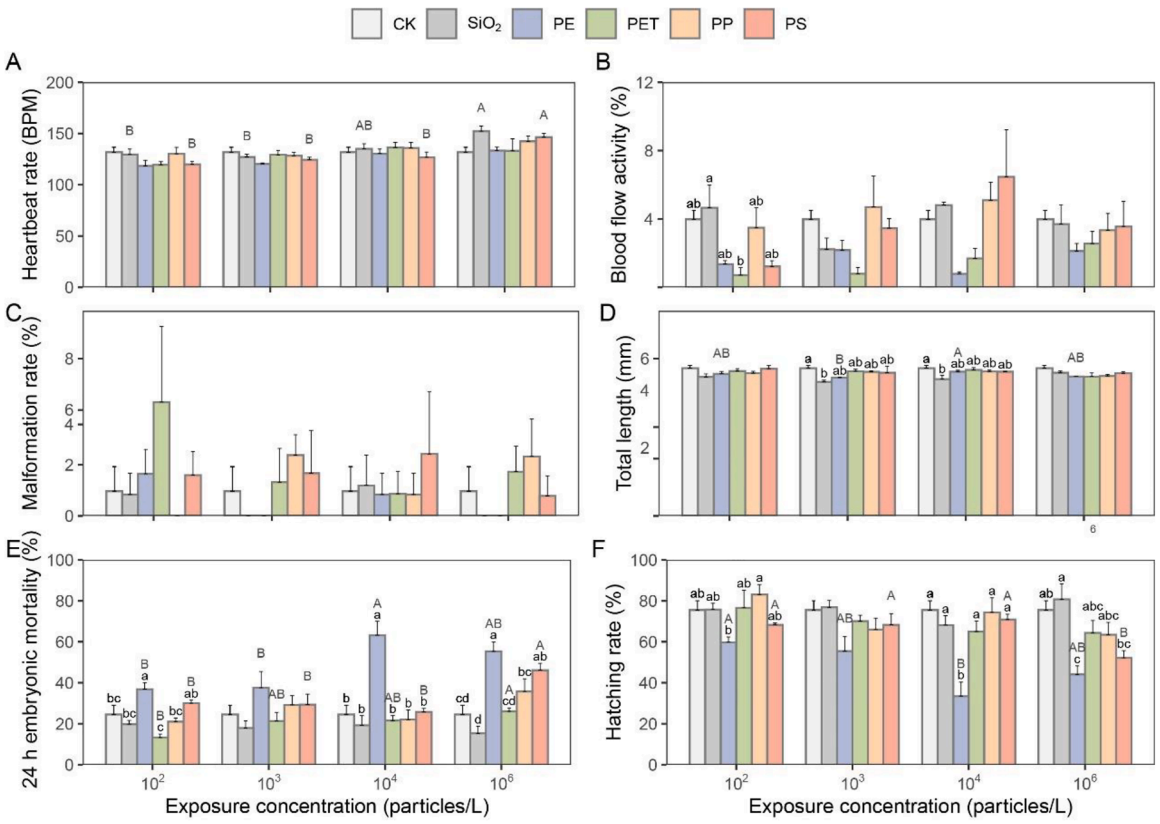

Fig. 2. Development toxicity of embryos under different exposure conditions: (A) Heartbeat rate; (B) Blood flow activity; (C) Malformation rate; (D) Total length; (E) Embryonic mortality after 24 h exposure; (F) Hatching rate at 120 hpf (Error bars stand for standard error. Lowercase letters indicated a significant difference for the different groups and capital letters indicated a significant difference for particles of different concentrations).

microplastic particles or a mixture of food and microplastics could make more passive ingestion of microplastics by organisms and thus reduce predation efficiency (de Sá et al., 2015; Carrasco et al., 2019). As reported by Rist et al. (2017), microalgae ingestion rate of *Daphnia magna* significantly decreased by 21% after exposure to 0.1 μm PS for 24 h. In our experiment, food intake tests was performed without microplastics or SiO_2 supply after finishing exposure, in order to avoid the effects of space occupation of microplastics in the intestine and visually reflect the effect of microplastic ingestion on the feeding ability of fish. According to our results, zebrafish showed the same scavenging ability for SiO_2 fragments and microplastic fragments with different polymer types. All tested particles were excreted rapidly along with feces. Therefore, microplastics and SiO_2 may not be able to affect the feeding capacity and intestinal motility of zebrafish with limited retention time in intestines.

For the energy reserve, larvae fish in different treatment groups had similar contents of TP, T-CHO, GLU and pyruvic acid as CK group at 10^2 particles/L ($p>0.05$) (Fig. 3C, D, E, F). At 10^4 particles/L, larvae fish had a higher content of TP in PP group than in CK ($p<0.05$), but had no difference with other treatment groups ($p>0.05$) (Fig. 3C). T-CHO content was higher in PS group than that in CK group and SiO_2, PE, PET groups ($p<0.05$), but was the same as in PP group ($p>0.05$) (Fig. 3D). In addition, TP and T-CHO in other treatment groups were not significantly different from that in CK group ($p>0.05$). GLU and pyruvic acid of larvae fish in different treatment groups were consistent with CK group ($p>0.05$) (Fig. 3E and F). Survival rates of larvae were over 90% in all groups after exposure 28 days, and showed no significant difference (between different groups ($p>0.05$) (Fig. 3G). Zebrafish larvae's total length and body weight also showed no significant difference in different treatment groups from that in CK group (Fig. 3H and I) ($p>0.05$).

Results suggested that different polymers of microplastics had negligible effect on energy reserve and growth of larvae fish, and were consistent with the intake and peristaltic frequency data as described above. This may be related to the rapid excretion of microplastics in the digestive system of zebrafish. Previous studies reported that the gross energy, crude protein and crude lipid of *S. schlegelii* dramatically decreased after 14-day exposure to PS microplastics (Yin et al., 2019). However, studies also found impacts of feeding PE and PS microplastics were limited for suspension-feeding like urchin larvae, and detritivorous invertebrates like amphipods (Kaposi et al., 2014; Blarer et al., 2016). Differences in the accumulation and excretion characteristics of microplastics in different organism species may be reasons for the different effects on feeding and energy reserves of organisms. For a better understanding the exposure risk of microplastics of different polymer types, the characteristics of accumulation and excretion kinetic processes of microplastics in different organisms need to be further studied. Our results suggested that polymer types of microplastics were not related to their effects on fish feeding and growth.

3.3. Locomotion and neurotoxicity

Behavior changes have proven to be more sensitive and act as important endpoints for toxicological study (Little and Finger, 1990). Zebrafish larvae at 120 hpf have finished the complete differentiation of pigment cells of eyes, and they can swim to hunt for food and escape from predators using eyes and lateral line (Westerfield, 2000). After exposure 5 days, microplastics of different polymer types did not affect the behavioral development of zebrafish embryos at environmental relevant concentration (10^2 particles/L) ($p>0.05$), but PET inhibited locomotion activity at 10^3 particles/L and PP stimulated locomotion activity at 10^6 particles/L compared with CK group ($p<0.05$) (Fig. 4A, B).

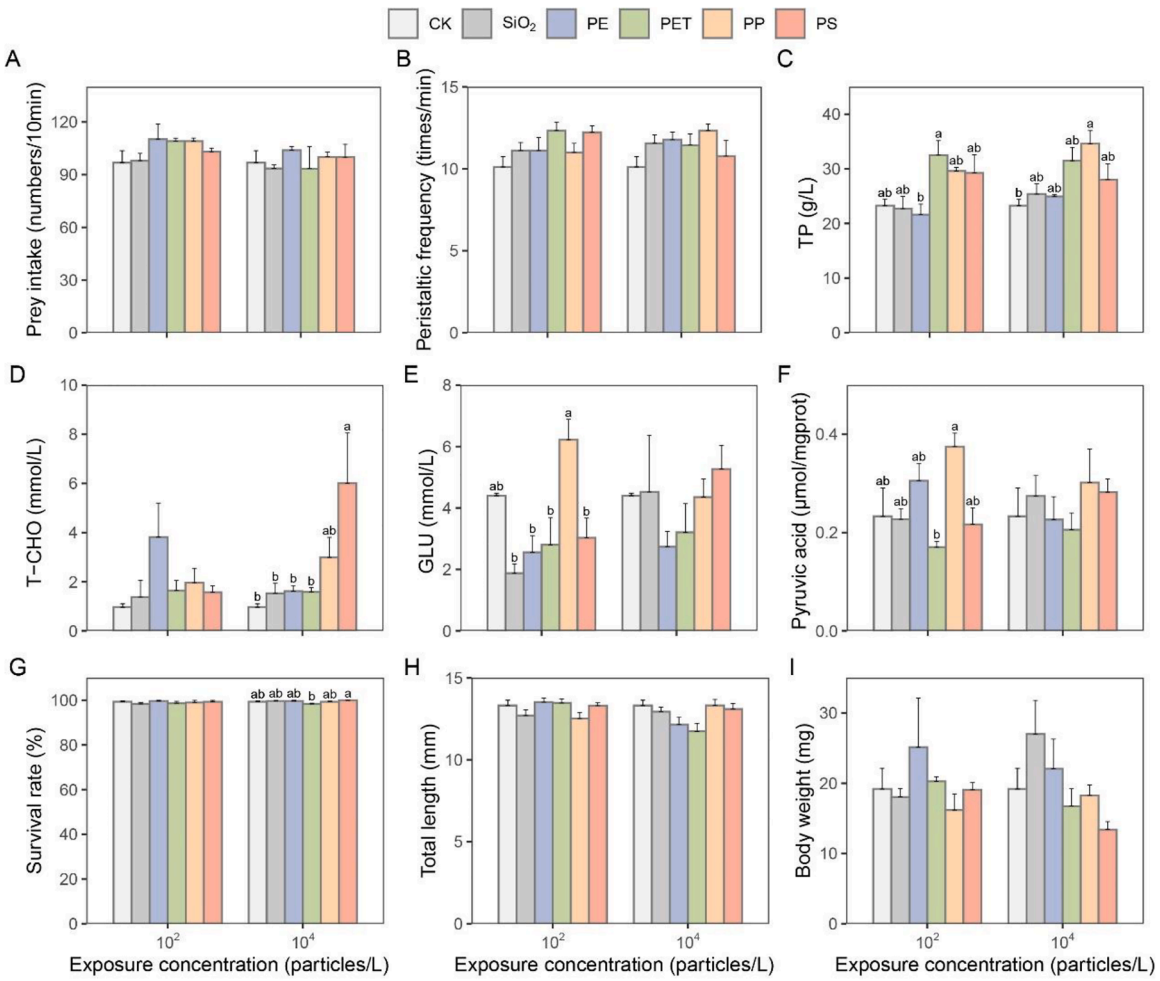

Fig. 3. Feeding and energy reserve of larvae after 28-d chronic exposure: (A) prey intake; (B) intestinal peristaltic frequency; (C) total protein; (D) total cholesterol; (E) glucose; (F) pyruvic acid. Survive and growth of larvae: (G) survival rate; (H) total length; (I) body weight (Error bars stand for standard error. Lowercase letters indicated a significant difference for the different groups and capital letters indicated a significant difference for particles of different concentrations).

After 28-day chronic exposure for zebrafish larvae, microplastics of different polymer types and SiO_2 led to the changes of locomotion activity at both environmental relevant concentration and high concentration, but only caused neurotoxicity at high concentration. Among treatment groups, PE, PET and PP inhibited larvae' locomotion levels at 10^2 particles/L, while PS stimulated larvae' locomotion ($p<0.05$) (Fig. 4C, D). At 10^4 particles/L, larvae' locomotion was inhibited by SiO_2 and different microplastics ($p<0.05$) (Fig. 4C, D). Among these treatments, SiO_2 group had similar larvae' locomotion levels as PS group ($p>0.05$), but differed from other treatment groups ($p<0.05$). PE group was the same as PET group in locomotion levels ($p>0.05$), but was different from other treatment groups ($p<0.05$). PP group had higher locomotion levels compared with other treatment groups ($p<0.05$). AChE activities of larvae were only inhibited at 10^4 particles/L ($p<0.05$), but there was no significant difference among different treatment groups ($p>0.05$) (Fig. 4E).

Overall, these results indicated that the effect of microplastics on the behavior zebrafish was negligible under acute exposure, but different polymers of microplastics were able to cause behavioral changes and neurotoxicity of zebrafish larvae under chronic exposure. The locomotor activity of aquatic organisms after exposure to microplastics could be either directly or indirectly influenced by several physiological changes like energy reserve reduction, metabolism disorder, gut microbiota dysbiosis, inflammation and neurotoxic response (Sun et al., 2021). According to our study, chronic exposure to different polymers of microplastics did not influence the feeding, energy reserve and growth of larvae. Neurotoxicity may account for behavioral changes of zebrafish larvae. AChE can degrade acetylcholine and terminate the excitatory effect of the neurotransmitter on the postsynaptic membrane. When AChE activity is inhibited, it can lead to neural hyperexcitability thus causing behavioral abnormalities. In this work, different polymers of microplastics showed no effect differences on neurotoxicity in zebrafish larvae after chronic exposure. But different polymers of microplastics and SiO_2 treatment groups led to variances in behavior changes, which further confirmed the sensitivity of behavioral indicators in toxicological studies.

3.4. Oxidative stress

SOD activities and MDA contents were not significantly different between treatment groups and the CK group ($p>0.05$), and no significant difference was observed between the different treatment groups ($p>0.05$) (Fig. 5A, B). Since we only tested once at the end of exposure, changes in the antioxidant capacity of fish during the intermediate process of exposure were unclear. Larvae may have adapted to the exposure during the experiment.

Many studies have found microplastic exposure could induce oxidative stress on organisms. It was reported that chronic exposure to 0.5 μm PS particles led to increased SOD and glutathione in the liver of the Chinese mitten crab *Eriocheir sinensis* at low concentrations (40 and 400 μg/L), while induced a decrease in high concentrations (4×10^3 and 4×10^4 μg/L) (Yu et al., 2018). Lei et al. (2018) reported that ingestion

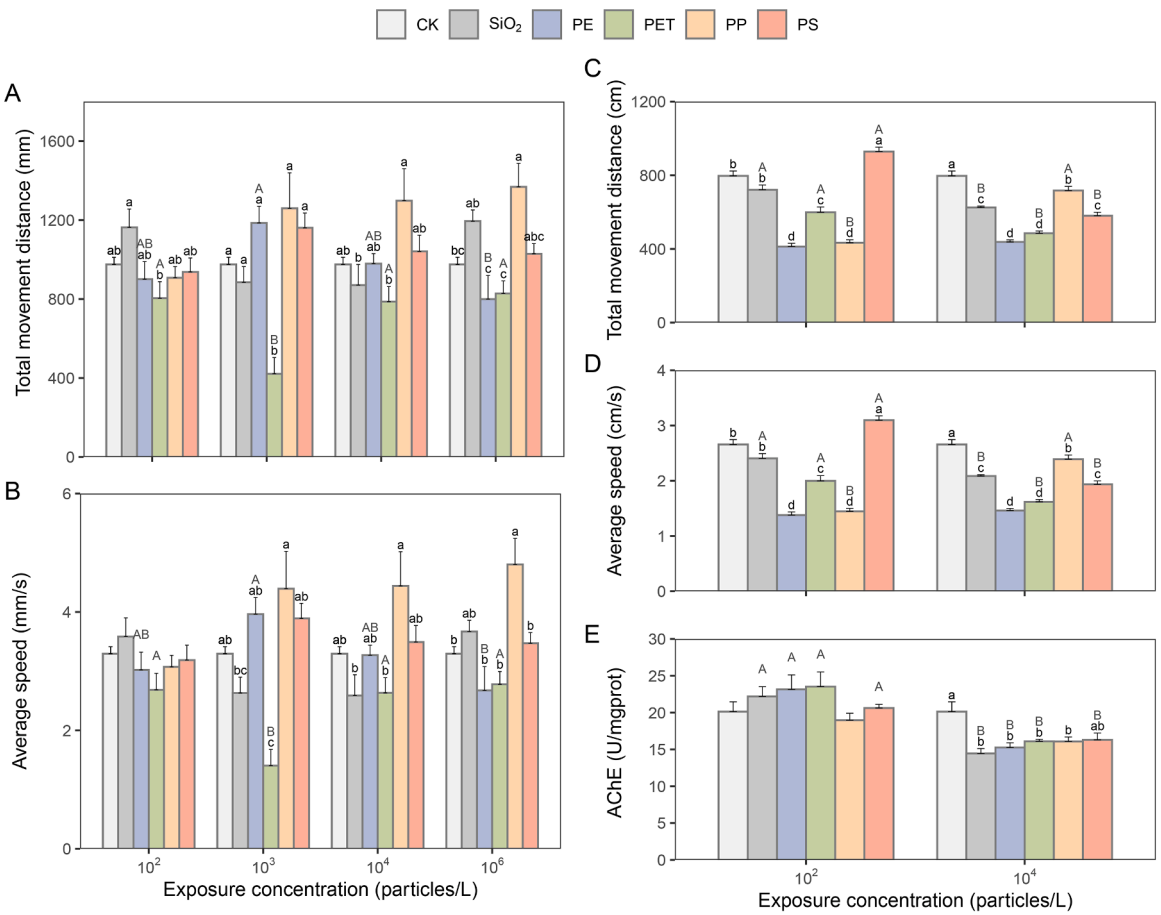

Fig. 4. Larvae' locomotion levels after 5-day acute exposure for zebrafish embryos (A: total movement distance; B: average speed). Larvae' locomotion levels and neurotoxicity after 28-day chronic exposure for zebrafish larvae (C: total movement distance; D: average speed); E: AChE in brain). (Error bars meant standard error. Lowercase letters indicated a significant difference for the different groups and capital letters indicated a significant difference for particles of different concentrations).

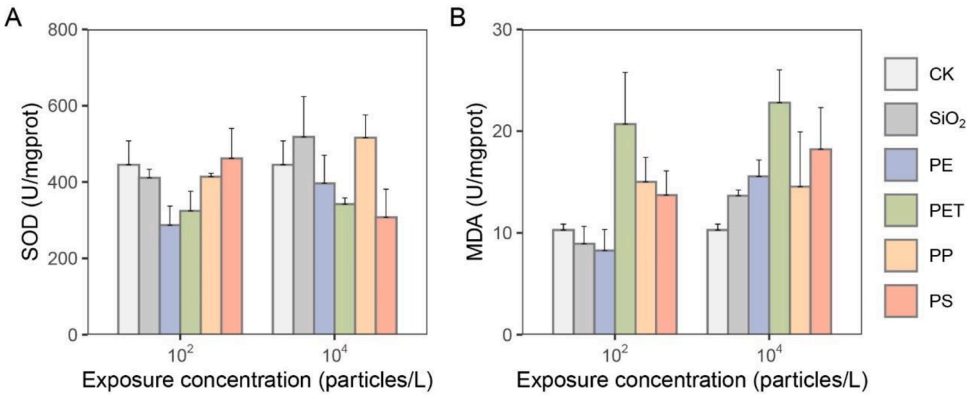

Fig. 5. SOD activities (A) and MDA contents (B) of zebrafish larvae after 28-day chronic exposure (Error bars stand for standard error).

of microplastics by nematode *Caenorhabditis elegans* induced the increase of *gst* expression, but the toxicity of microplastics was closely dependent on their size rather than their polymer types. Oxidation stress caused by different polymers of microplastics were not comparable among different studies due to different experimental conditions used (Proki et al., 2019).

3.5. Low toxic effect caused by different microplastics and SiO_2

For both embryonic acute exposure and larvae chronic exposure, we could find that different treatment groups had similar and low levels of IBRv2 values at the same exposure concentration (Figure S4 A, F). Radar plots also showed little difference in the reference deviations of the same bioindicator for different particle exposure groups (Figure S4 B, C, D, E, G, H). This may be related to these less impacts of particles on zebrafish embryos and larvae at individual and physiological levels.

4. Conclusion

Microplastics of different polymer types that are frequently detected

in the environment showed negligible toxicity on zebrafish at environmental relevant concentration, and they showed similar toxic effects as SiO_2 particles at high concentrations. Our results suggested that microplastics might have similar biological effects and toxicological mechanisms as natural particles, and the influence of microplastic polymer types could be ignored for toxicity assessment of microplastics at environmental relevant concentration. We argue that microplastics from environmental samples could be surveyed without identifying the polymer types, which could greatly reduce the workload and cost of microplastic analysis.

CRediT authorship contribution statement

Yuling Chen: Writing – original draft, Writing – review & editing, Investigation, Methodology, Visualization. **Ming Duan:** Investigation, Project administration, Methodology. **Xiangrong Xu:** Writing – review & editing. **Chenxi Wu:** Writing – review & editing, Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2023.106595.

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