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Original Article

Initial Assessment the Effects of Polyethylene Microplastics on the Growth of Zebrafish Embryos *Danio rerio*

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Abstract: The aim of this study to evaluate the effect of polyethylene (PE) microplastics to mortality rates and morphological changes of zebrafish (*Danio rerio*) embryos by determining the mortality ratio of embryos and changes in embryo morphological structure after 24, 48, 72 and 96 h of exposure. Zebrafish were obtained from the Faculty of Biology - Hanoi University of Science. 24-hour-old fish embryos were exposed to PE microplastic concentrations of 5; 10; 20; 50 and 100 µg/mL. The results showed that when exposed to microplastics at concentrations of 10 µg/mL or higher, only about 5-10% of mortality embryos and the ratio did not increase with increasing concentration. These embryos died in the first 24 h and the number of dead embryos did not increase throughout the experiment. A similar trend was also observed when evaluating the ratio of morphologically abnormal embryos, embryos with abnormal pericardial edema were observed throughout the experiment at the highest concentration of 100 µg/mL. The results showed the potential risk in the using plastic products for the ecological environment.

Keywords: effect, zebrafish (*Danio rerio)*, mortality ratio, microplastic, exposure.

1. Introduction

Environmental pollution not only causes unusual weather status, deteriorating the living environmental quality but also causes negative consequences for human health and the ecosystem. To limit the impacts of climate change, it is an urgent requirement to develop

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sustainable sectors. In addition, the excessive use of plastic and plastic products in production and daily life has caused significant impacts on ecosystems both on terrestrial and aquatic ecosystems. Among the pollutants, plastics and plastic products have become more popular in estuarine areas through daily life and discharge from industrial zones or clusters and other activities in agriculture discharges waste into rivers and then into the sea [1]. These pollutants over time break down into smaller particles called microplastics that can pose a threat to ecosystems and the growth of organisms. Microplastics are very small pieces less than 5 mm in size and have the potential to pollute the environment [2]. After more than 100 years of appearing, plastic waste is becoming a major hazard, with serious consequences affecting the environment, ecosystem and, above all, human health [3].

Fish is one of the foods with special economic importance and is quite sensitive to a variety of pollutants released into the ecosystem. The concentration of pollutants in the fish body depends on care habits, digestive ability, food intake, physicochemical properties of water, metabolic rate of the animal and toxicity of the pollutants [4, 5]. In water bodies, fish often die due to water pollution including pesticides, heavy metals, waste, toxins... contained in the water [6]. These are dangerous to embryos and immature fish and can significantly reduce the number of fish populations, even causing extinction of all fish in polluted environments. Zebrafish (*Danio rerio*) originates from the genus Brachydanio, which is closely related to the genus Devario [7]. In the wild, zebrafish live in moderately flowing, clear, shallow waters with a lot of vegetation [8]. Zebrafish are important vertebrate model organisms, widely used in scientific research such as in drug development, especially preclinical development [9]. In order to have management policy for the estuarine environment in general and enhance the quality of seafood in particular, it is necessary to conduct studies about toxicity of some toxins in the aquatic ecological environment. This research data will contribute to protecting the environment and estuary resources, guiding the sustainable development strategy of aquaculture area planning. Therefore, this study was conducted to evaluate the effects of microplastics PE on the growth of zebrafish embryos *Danio rerio*.

2. Methodology

2.1. Preparation of Zebrafish Embryos (Danio rerio)

AB adult zebrafish from 18 - 24 months old were raised in plastic tanks with a capacity of 3 liters of water and a density of 10-15 individuals per tank at the Department of Biology, Hanoi University of Science. The culture conditions: pH 7-7.4; The temperature 26-28 °C; Lighting conditions were light/dark: 14/10 h. Adult fish have fed twice a day at 10am and 5pm with TetraMin® Tropical Flakes food.

To increase egg production, female fish were often fed a small amount of boiled egg yolk about 2-3 days before pairing. The parent fish chosen for breeding must be healthy fish, female fish over 6 months old and male fish over 9 months old. Fish were paired according to the sex ratio of 1 female: 2 males. The fertilized embryos were collected and washed, then placed in a petri dish containing solutions according to the designed experiment.

2.2. Preparation of PE

Microplastic particles PE have size of 10 - 27 μm was bought from Cospheris LLC (USA) with characteristic: Clear Polyethylene Microspheres 0.96 g/cc 10-45 μm.

2.3. Setup Experiments

The experimental procedure of this study was built according to the steps in the OECD No.236 (Organization for Economic Cooperation and Development) 2013 protocol [10] and the research published by Freire et al., (2023) [11] for testing chemical toxicity on fish *Danio rerio* as follows: Healthy fish embryos were selected and translated them to the experimental wells SPL 6-well plates (Korea) containing PE microplastic particles at concentration: 5; 10; 20; 50, 100 µg/mL and the control sample (absent PE microplastic). Each experiment was repeated three times, each well had 20 organisms/concentration. The toxicity of PE microplastic particles was calculated by survival/mortality ratio after 24, 48, 72 and 96 h.

The effect of PE microplastic on fish embryos (*Danio rerio*) was based on 3 criteria: the lethal embryo ratio, the hatching embryos ratio and the malformation embryos ratio. The malformation structure of fish embryos was observed directly under an Olympus electron microscope at 40x magnification.

2.4. Statistics

All experiments were repeated three times. The collected data was processed by using GraphPad Prism 6 software.

3. Result and Discussion

3.1. Effect of PE Microplastics on the Growth and Development of Fish Embryos

The results of toxicity experiments on zebrafish embryos were evaluated according to three criteria: the lethal embryo ratio, the hatching embryos ratio and the malformation embryos ratio. In general, the low concentrations of PE microplastics did not have a significant effect on the lethal embryo ratio and the malformation embryos. At the same time, PE microplastic particles have little impact (only about 5-20% of embryos lethal) on the hatchability of zebrafish embryos after from 24 to 96 h (Figure 1, 2 and 3).

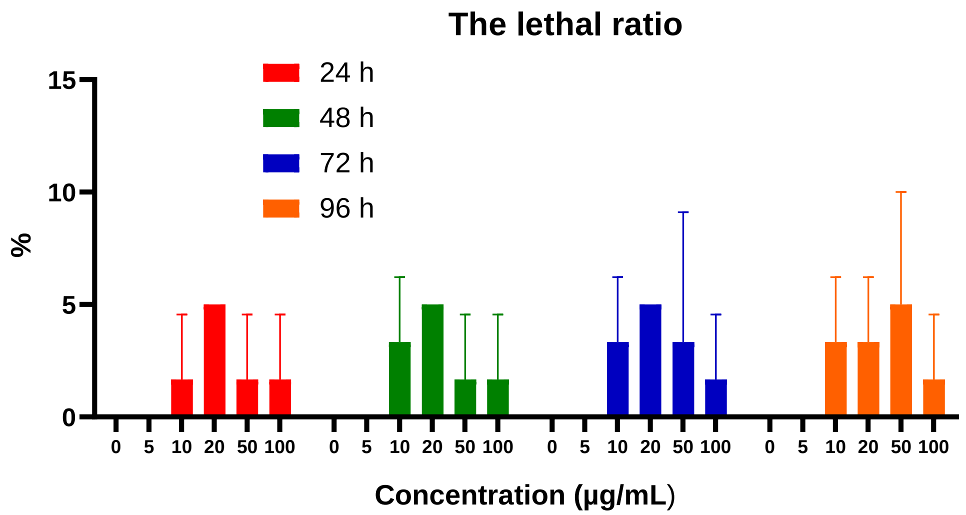

Figure 1. The variation of the lethal rates of zebrafish embryos (*Danio rerio*) after 24, 48, 72 and 96 h exposure to 0; 5; 10; 20; 50 and 100 µg/mL PE microplastic particles; values are reported as the mean ± standard deviation (SD) of three replicates (n=3).

When exposed to microplastics at a concentration of 10 µg/mL or more, only about 5 - 20% of embryos lethal when compared with the control sample (0 µg/mL and the survival rate of 100%) and the rate does not increase linearly with concentration (Figure 1). This is a very low rate. In addition, these embryos died within the first 24 h and the number of death embryos did not increase throughout the experiment. This showed that PE microplastics only affect a small number of embryos that are very sensitive to the environment. PE microplastic particles have an impact on the embryo hatchability although the level of impact is quite small. Specifically, zebrafish embryos in concentrations of 10 and 20 μg/mL had a lower hatching rate than the control and other concentrations, only reaching an average of about 80% compared to nearly 100% in other concentrations (Figure 2). However, the special point is the hatching rate at these two concentrations was restored at 96 h.

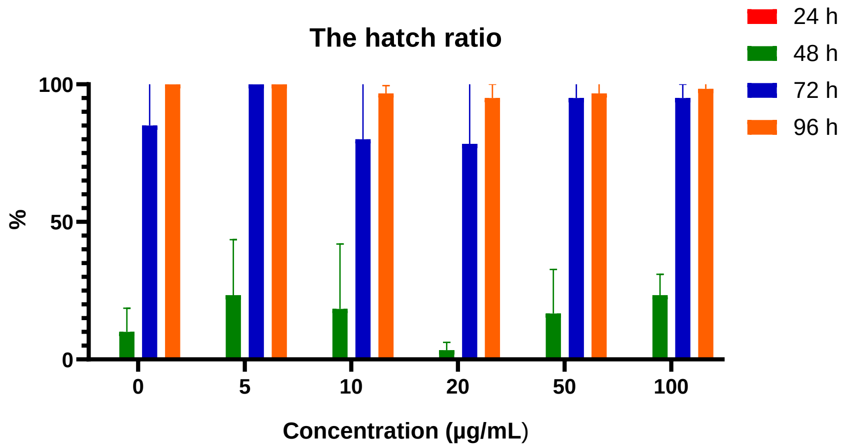

Figure 2. The hatch variation of zebrafish embryos (*Danio rerio*) after 24, 48, 72 and 96 h exposure to 0; 5; 10; 20; 50 and 100 μg/mL PE microplastic particles; values are reported as the mean ± standard deviation (SD) of three replicates (n=3).

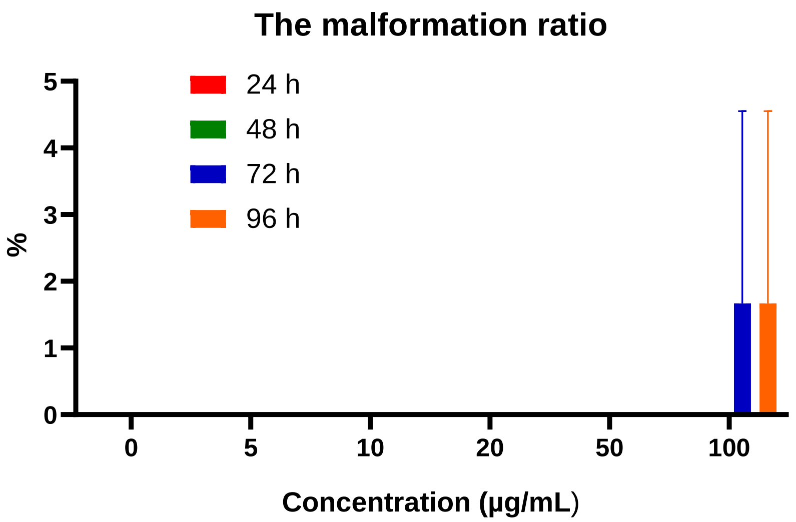

Figure 3. The malformation variation of zebrafish embryos (*Danio rerio*) after 24, 48, 72 and 96 h exposure to 0; 5; 10; 20; 50 and 100 μg/mL PE microplastic particles; values are reported as the mean ± standard deviation (SD) of three replicates (n=3).

3.2. Effect of PE microplastic on the zebrafish embryos (Danio rerio) structure and assessment of the survival/mortality ratio.

The observed results of the morphology and structure of fish embryos are shown in Figure 4. At 96 h, the embryos were noted to have morphological malformation with deformed pericardial edema and tail curvature (Figure 4). The results of assessment of the survival or mortality ratio shown in Figure 5. These results indicated that the mortality ratio did not change throughout the experiment.

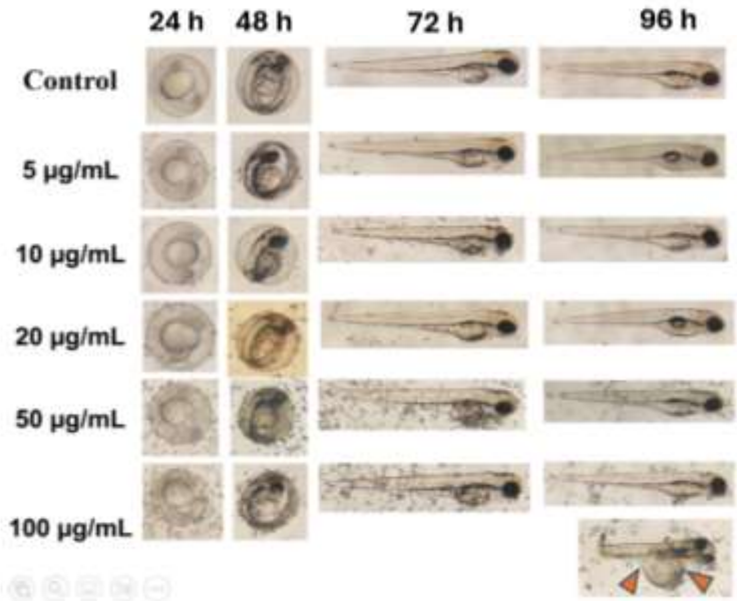

Figure 4. The morphological changes of zebrafish embryos (*Danio rerio*) (the red arrows) after 24, 48, 72 and 96 h exposure to 0; 5; 10; 20; 50 and 100 µg/mL PE microplastic particles (Bars, 20µm, Magnification 40×).

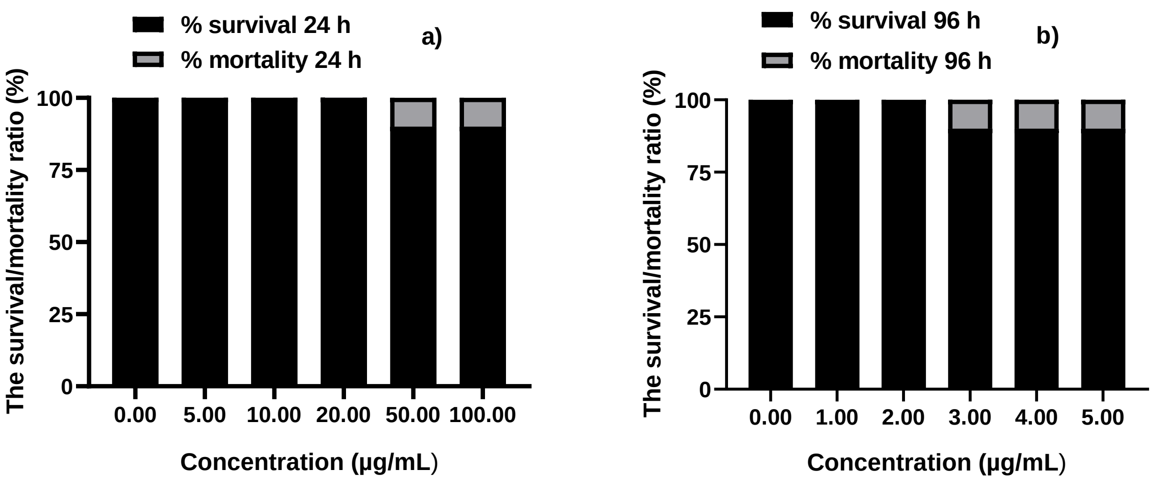

Figure 5. The mortality variation of zebrafish embryos (*Danio rerio*) after 24 and 96 h exposure to 0; 5; 10; 20; 50 and 100 µg/mL Polyethylene microplastic particles; values are reported as the mean of three replicates ± SD (n = 3).

The results of this study are similar to studies on the ecotoxicity of some other polymers such as polymethylmethacrylate (PMMA), PE-PM, PS etc [11, 12]. Different polymers exhibit different effects on the growth and development of *Danio rerio* embryos. Freire et al. (2023) found neither genotoxicity after acute exposure to PE-MPs concentrations of 0.0, 12.5, 50 and 100 mg.L^{-1} at 96 h, nor cytotoxicity through the nuclear abnormalities test. These PE-MP spheres did not cause serious toxic effects in zebrafish because they did not cross the chorion barrier. There was no internalization and no embryotoxic effects after exposures at 0.0, 6.25, 12.5, 50.0, 100.0 mg.L^{-1}. Manuel et al., (2022) also indicated that organisms exposed to PMMA nanoparticles exhibited higher mortality and pericardial edema than those exposed to PS nanoparticles but displayed less effects on swimming behavior after 96 and 120h exposure. In their study, both the impact of polystyrene (PS; 22 nm) and polymethylmethacrylate (PMMA; 32 nm) nanoparticles on zebrafish embryo-larval stages were assessed by studying mortality, hatching, morphological features and have no observed effects. In contrast, other studies [13, 14] found that plastic nano/microparticles (NPs/MPs) did induce significant morphological alterations in zebrafish embryos such as organic impairments and tissue damage, developmental retardation, chronic inflammation, oxidative stress (OS) [13] or PS-MPs 5 μm and 70 nm caused inflammation and lipid accumulation in fish liver [14], different was observed in our study.

These results could be explained by the fact that microplastic particles caused physiological disturbances in zebrafish after short-time exposures, mainly activity changes but it is not enough to die. It may be associated with oxidative stress, but it was not effective enough to induce genotoxicity and cytotoxicity [11, 12]. The size of microplastics is also related to intestinal transit, if the size is bigger, they will not be absorbed, and it are not found in different tissues. Moreover, when the high microplastic concentrations were seen in the intestine, the zebrafish still managed to eliminate this type of microparticle due to their behavior [11].

4. Conclusion

Until now, the accumulation potential and impact of microplastics on fish and bivalve molluscs is still controversial. PE microplastics in this study have little effect on the survival and hatchability of *Danio rerio* zebrafish embryos at concentration range 5, 10, 20, 50 and 100 µg/mL. Even at the highest concentration of 100 µg/mL, microplastics can only cause abnormal pericardial edema and tail curvature in embryos, but at a quite small ratio. The experimental results do not fully reflect the impact of microplastics on aquatic ecosystems so that it needs to be tested with a longer period, with more polymers, higher microplastic concentrations, and at different growth stages of fish.

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