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Investigation of Anxiety and Depression Patterns in an In Vivo Model Following Chronic Exposure to Polyethylene Microplastic

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Abstract

Microplastic pollution has emerged as a critical environmental issue with growing evidence of its biological toxicity in aquatic organisms. This study investigated the neurobehavioral and biochemical effects of chronic polyethylene (PE) microplastic exposure in zebrafish larvae. Behavioral assays, including locomotion tracking, light/dark preference, and T-maze tests, were conducted alongside acetylcholinesterase (AChE) enzymatic activity measurements to assess the impact of PE at two concentrations (10 µg/mL and 100 µg/mL). Locomotion analysis revealed that control larvae displayed normal exploratory swimming patterns, while PE-exposed groups exhibited dose-dependent reductions in distance travelled and average velocity, with the 100 µg/mL group showing restricted movement confined to limited zones. In the light/dark preference assay, control larvae maintained a natural preference for the dark zone, whereas PE exposure disrupted this behaviour. Larvae exposed to 10 µg/mL showed equal distribution of time in both zones, and at 100 µg/mL, they predominantly remained in the light zone, reflecting abnormal anxiety-like responses. T-maze testing further demonstrated impaired exploratory and cognitive behaviour, with significantly reduced exploration of the reward zone in PE-treated groups compared to controls. Biochemical analysis revealed marked inhibition of AChE activity in the head region of exposed larvae, suggesting impaired cholinergic neurotransmission due to excessive acetylcholine accumulation. Collectively, these findings highlight that chronic PE exposure disrupts locomotor activity, anxiety-related behaviour, cognitive function, and cholinergic signalling in zebrafish larvae in a concentration-dependent manner. This study underscores the neurotoxic potential of microplastics and emphasizes the urgent need to evaluate their ecological and health risks in aquatic environments.

1. Introduction

Plastic pollution has emerged as one of the most pressing global environmental problems in recent decades. Large plastic waste present in the environment undergoes degradation and fragmentation to form microplastics, which are plastic particles less than 5 mm in size (Zhang and Liu, 2018). These particles are widespread in aquatic and terrestrial ecosystems and have been detected in rivers, oceans, sediments, and even in drinking water. Polyethylene (PE) is one of the most widely produced plastics globally, commonly used in packaging, bottles, and bags. Because of its non-biodegradable nature and extensive use, PE is one of the dominant contributors to microplastic pollution (Lamichhane et al., 2023). Recent studies have shown that PE microplastics are not only persistent in the environment but can also accumulate in biological systems (Ribeiro et al., 2019; Valdivia et al., 2025), raising serious concerns about their potential health effects on both aquatic species and humans. Microplastics can enter aquatic organisms through ingestion, and once internalized, they may accumulate in tissues or interact with physiological systems (Al-Thawadi, 2020; Jovanović, 2017). Their small size and large surface area allow them to absorb and carry other toxic substances, such as heavy metals and persistent organic pollutants, thereby enhancing their

harmful effects (Kinigopoulou et al., 2022; Liu et al., 2022). While most studies on microplastic toxicity have focused on physical damage, oxidative stress, or general organ toxicity, emerging evidence suggests that microplastics may also interfere with neurological functions (Wang et al., 2022). The brain is highly sensitive to environmental stressors, and chronic exposure to pollutants can trigger behavioural and neurochemical alterations (Molot et al., 2022). This raises the possibility that microplastics may affect higher-order brain functions such as mood regulation, anxiety, and depression.

Anxiety and depression are complex neurobehavioral conditions that are influenced by multiple biological and environmental factors (Kalin, 2017). In aquatic models, behavioural patterns such as reduced exploratory activity, altered social interaction, and abnormal locomotor responses are considered reliable indicators of anxiety- or depression-like states. Zebrafish (*Danio rerio*) has gained prominence as a model organism in behavioural neuroscience research due to its genetic similarity to humans, well-characterized brain structure, and ease of behavioural observation (Gerlai, 2020). Zebrafish display a wide range of complex behaviours that can be quantitatively assessed, making them a valuable system for studying the neurotoxic effects of environmental contaminants. Moreover, their small size, transparency during early development, and rapid life cycle make them ideal for long-term exposure studies (Tierney, 2011). Previous studies have shown that zebrafish respond to environmental toxins with measurable changes in behaviour and neurochemistry. For example, exposure to heavy metals, pesticides, and pharmaceutical residues has been linked to increased anxiety-like and depression-like behaviours in zebrafish (Ommati et al., 2024; Sun et al., 2025). However, studies exploring the effects of chronic exposure to PE microplastics on behaviour are still limited. Given the widespread environmental occurrence of PE microplastics, it is important to investigate whether chronic exposure can lead to neurobehavioral disturbances. Such findings would not only expand our understanding of microplastic toxicity but also provide valuable insights into the potential risks for human health, since microplastics have been detected in food, water, and even human tissues. Therefore, the present study was designed to investigate the anxiety and depression patterns in zebrafish following chronic exposure to PE microplastics. By evaluating behavioural parameters associated with anxiety and depression, this study aims to determine whether long-term microplastic exposure induces measurable alterations in zebrafish neurobehavior.

2. Materials and Methods

2.1. Zebrafish Maintenance and Experimental Groups

Adult zebrafish of similar age (3–4 months) and size (3–4 cm length) were obtained from a certified breeding facility. Fish were maintained in glass aquaria under controlled conditions: temperature 26 ± 2 °C, pH 7.0 ± 0.5, with a 14:10 hour light–dark cycle. The fish were fed a commercial flake diet twice daily and kept under continuous aeration. The male and female zebrafish were kept for breeding and the embryos are collected. The 96 hours post fertilized (hpf) zebrafish larvae was used for the. For the study, zebrafish larvae were randomly divided into three groups (n = 10 per group): Control group (untreated); PE microplastic exposure (10 µg/mL); and PE microplastic exposure (100 µg/mL). Exposure was performed chronically by dissolving PE microplastic suspension in aquarium water, which was renewed every 48 hours to maintain concentration (Cruz et al., 2015; Gemmer et al., 2022).

2.2. Distance and Velocity Analysis

Behavioral tracking was conducted using the Umatracker software with a high-resolution overhead camera. Each fish was individually placed in a transparent observation tank (25 × 15 × 10 cm) filled with system water. After 5 minutes of habituation, locomotor activity was recorded for 10 minutes. The parameters were measured in Distance travelled (cm) and Average velocity (cm/s) (Haridevamuthu et al., 2025).

2.3. Light–Dark Preference Test

Anxiety-related behavior was assessed using the light–dark tank test. The tank was equally divided into a light zone (white background) and a dark zone (covered with black film). Each fish was gently introduced into the center of the tank and allowed to explore freely for 10 minutes. Umatracker software recorded the time spent (seconds) in each zone. Increased time in the light zone was interpreted as reduced anxiety, whereas prolonged time in the dark zone indicated anxiety-like behavior (Magno et al., 2015; Steenbergen et al., 2011).

2.4. T-Maze Test

Cognitive and exploratory behavior was tested using a T-shaped maze (30 × 20 × 10 cm). The maze consisted of three regions: an entry arm, a red zone (no food), and a green zone (with food reward). Each fish was placed at the entry point and observed for 10 minutes. The time spent in each zone (seconds) and the preference for the green (reward) region were recorded. This experiment was used to assess exploratory drive, learning, and depression-like responses (Ngoc Hieu et al., 2020; Wang et al., 2020).

2.5. Acetylcholinesterase (AChE) Activity Assay

Following behavioral studies, zebrafish larvae were euthanized on ice, and the head regions were carefully dissected under a stereomicroscope. The zebrafish larvae head were homogenized in 0.1 M phosphate buffer (pH 7.4)

at a ratio of 1:10 (w/v) using a glass–Teflon homogenizer. The homogenates were centrifuged at 10,000 × g for 15 minutes at 4 °C, and the supernatant was collected for enzymatic assay. AChE activity was determined using Ellman's method with acetylthiocholine iodide as the substrate and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) as the chromogen. Briefly, 100 µL of supernatant was added to a cuvette containing 2.6 mL of 0.1 M phosphate buffer (pH 7.4), 100 µL of DTNB (10 mM), and 20 µL of acetylthiocholine iodide (75 mM). The reaction was monitored at 412 nm for 5 minutes using a UV–Vis spectrophotometer. The increase in absorbance per minute was recorded, and AChE activity was expressed as nmol of acetylthiocholine hydrolyzed (ACTI) per minute per mg protein (Haverroth et al., 2015; Richetti et al., 2011).

2.6. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Changes in Locomotion Pattern of Zebrafish larvae

The behavioural tracking patterns of zebrafish revealed clear differences between the control and PE exposed groups. In the control group (Figure 1a), zebrafish exhibited normal exploratory swimming activity, covering a large area of the observation tank with frequent movement across different zones. In contrast, zebrafish exposed to 10 µg/mL PE (Figure 1b) displayed reduced swimming activity, with less exploratory movement compared to the control. The reduction was more significant in the 100 µg/mL PE group (Figure 1c), where zebrafish showed restricted swimming confined to limited zones of the tank, indicating impaired locomotor behavior. Quantitative analysis further supported these observations. The distance travelled (Figure 1d) was significantly reduced in both PE-treated groups compared to the control, with the 100 µg/mL group showing the lowest values. Similarly, average velocity (Figure 1e) was markedly decreased in the PE-exposed zebrafish, with a dose-dependent decline observed. The statistical analysis showed that both 10 µg/mL and 100 µg/mL groups had significantly lower values ($p < 0.05$) compared to the untreated control.

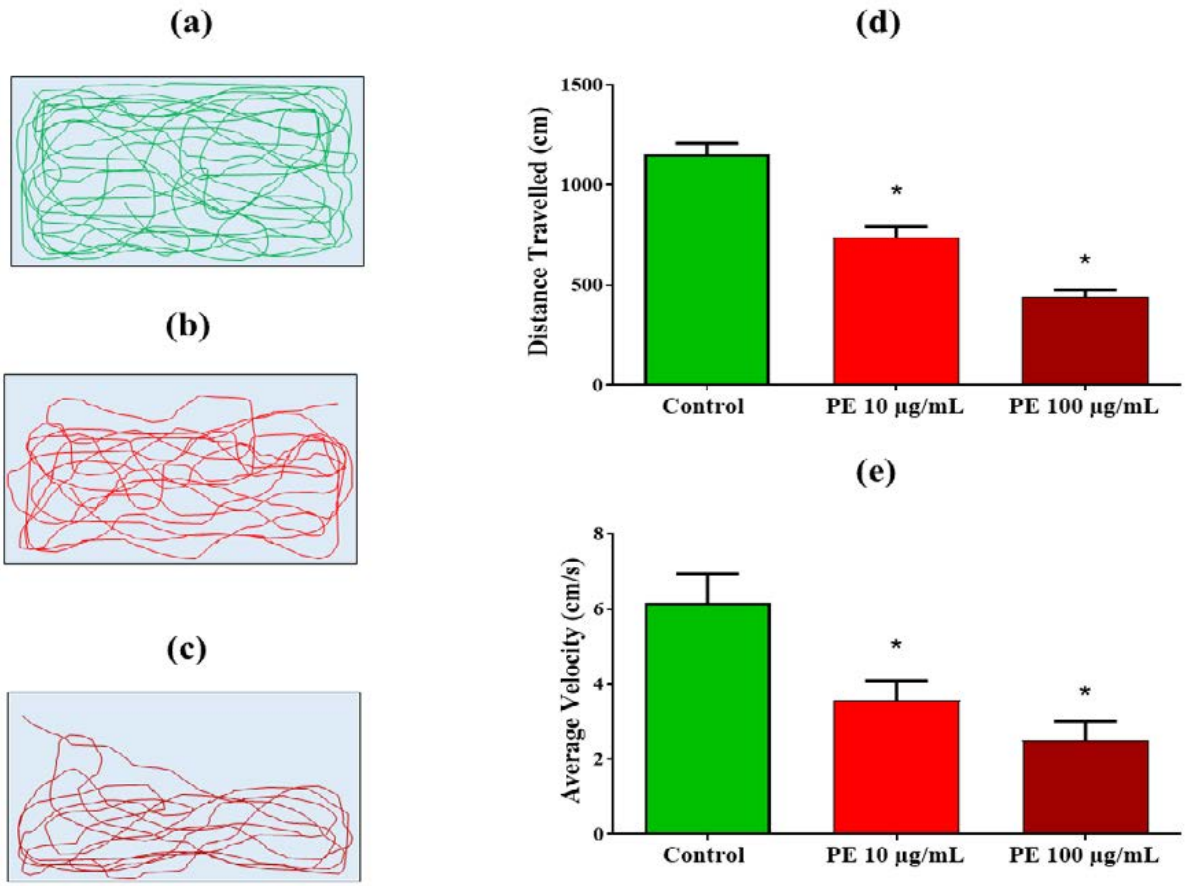

Figure 1: Behavioral analysis of zebrafish following chronic exposure to PE. (a) Control group (untreated); (b) Zebrafish exposed to PE 10 µg/mL; (c) Zebrafish exposed to PE 100 µg/mL; (d) Quantitative representation of total distance travelled (cm); and (e) Quantitative representation of average velocity (cm/s)

3.2. Changes in Behavioural Pattern of Zebrafish larvae

The light and dark preference test revealed significant alterations in zebrafish behaviour following chronic exposure to PE. In the control group (Figure 2a), zebrafish showed a natural preference for the dark zone, spending more time in it while making occasional transitions to the light zone, which is consistent with normal anxiety-related behavior in zebrafish. In contrast, zebrafish exposed to PE at 10 µg/mL (Figure 2b) displayed a more balanced distribution of time between the light and dark zones, indicating reduced anxiety-like behavior. At the higher concentration of 100 µg/mL (Figure 2c), zebrafish predominantly remained in the light zone, avoiding the dark region, which reflects a marked reduction in natural dark preference and abnormal behavioral patterns. Quantitative analysis (Figure 2d) confirmed these observations. The control group spent significantly more time in the light zone (158 seconds) compared to the dark zone (43 seconds), reflecting normal behavioral preference. In the 10 µg/mL PE group, the time spent in the light zone (97 seconds) and dark zone (110 seconds) became nearly equal, indicating a shift in normal preference. At 100 µg/mL PE, zebrafish spent the majority of time in the light zone (165 seconds) with very little time in the dark (26 seconds).

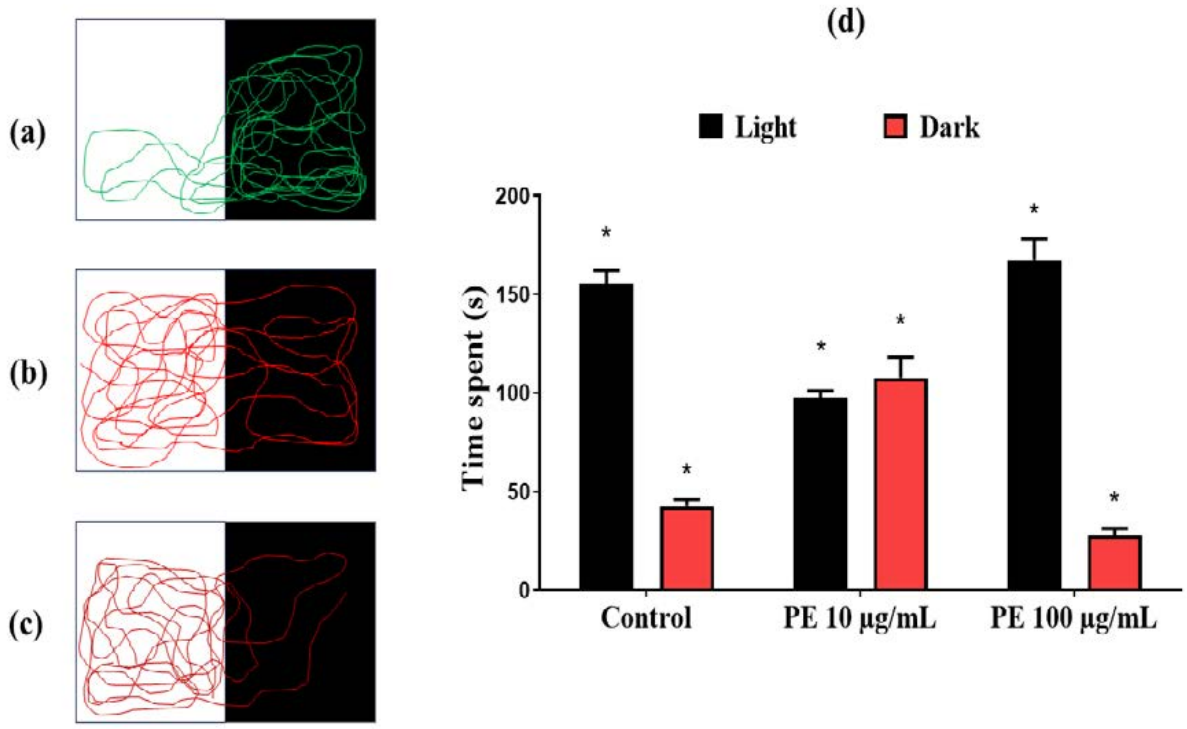

Figure 3: Light and dark preference behavior of zebrafish following chronic exposure to PE. (a) Control group; (b) Zebrafish exposed to PE 10 µg/mL; (c) Zebrafish exposed to PE 100 µg/mL; and (d) Quantitative representation of time spent (seconds) in light and dark zones across groups.

3.3. Exploratory and Cognitive behaviour changes

The T-maze test demonstrated significant changes in exploratory and cognitive behaviour of zebrafish following chronic exposure to PE. In the control group (Figure 3a), zebrafish actively explored the entire maze, including the entry zone, the red zone (no food), and the green zone (food reward), spending a longer duration in the green zone, which reflects normal exploratory drive and motivation toward the reward. In contrast, zebrafish exposed to PE at 10 µg/mL (Figure 3b) showed a noticeable reduction in exploration, with limited movement toward the green zone and reduced overall exploration time. This decline in exploratory behaviour became more pronounced in the 100 µg/mL PE group (Figure 3c), where zebrafish displayed highly restricted movement and minimal time spent in the green (reward) zone, indicating impaired motivation and reduced cognitive activity. Quantitative analysis (Figure 4d) further supported these findings. The control group showed the highest exploration time (55 seconds), while the 10 µg/mL group exhibited a significant reduction (41 seconds). The 100 µg/mL group had the lowest exploration time (23 seconds), demonstrating a dose-dependent decline. Statistical analysis indicated that both exposure groups showed a significant decrease ($p < 0.05$) in exploration compared to the control.

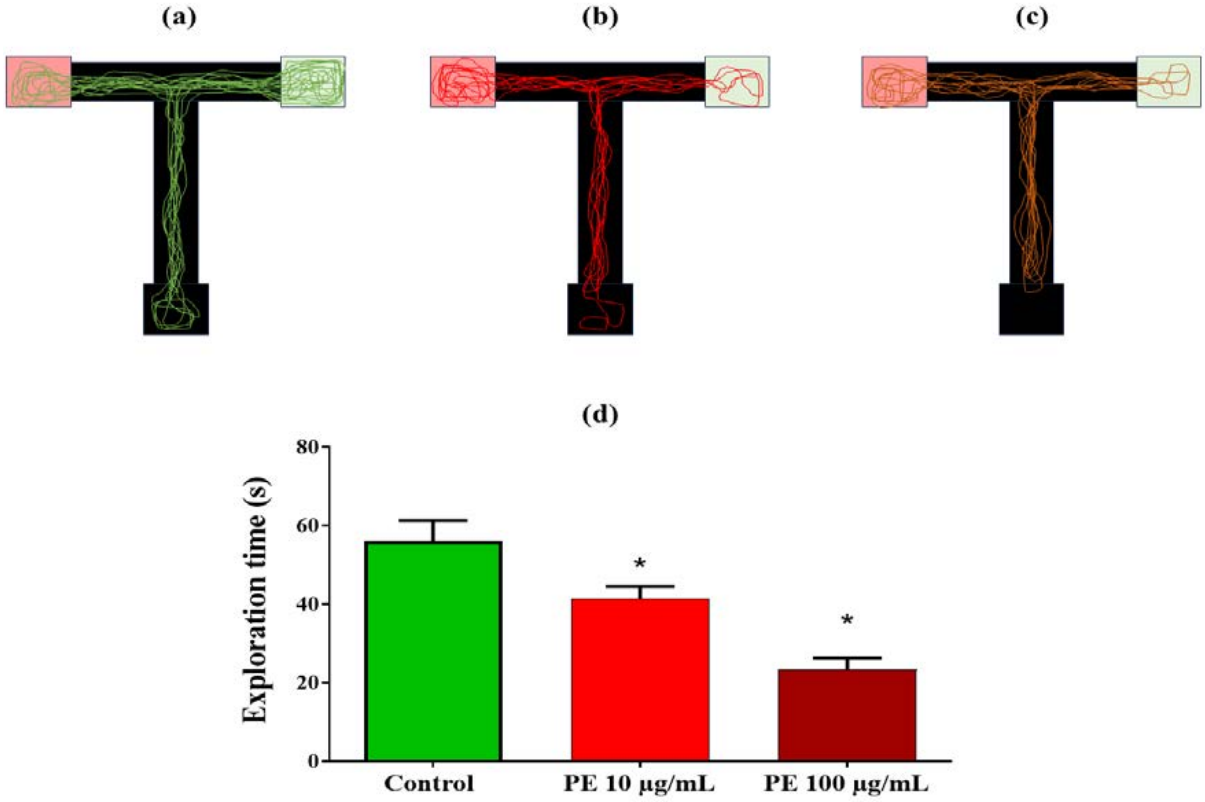

Figure 3. T-maze behavioral assessment of zebrafish following chronic PE. (a) Representative movement of the control; (b) Movement of zebrafish exposed to 10 µg/mL; (c) Movement of zebrafish exposed to 100 µg/mL PE; and (d) Quantitative analysis of exploration time across different treatment groups.

3.4. AChE Enzymatic changes in Zebrafish larvae head region

The concentration-dependent inhibition of AChE activity in the head region of zebrafish larvae exposed to PE. This would be evidenced by the PE 10 µg/mL (107 nmol/ATCI/min/mg of protein) group showing a statistically significant decrease in enzyme activity compared to the untreated Control group, with the PE 100 µg/mL (175 nmol/ATCI/min/mg of protein) group exhibiting an even more significant reduction (Figure 4). Biologically, this inhibition means the enzyme is less effective at breaking down the neurotransmitter acetylcholine, leading to its accumulation and subsequent overstimulation of nerve cells.

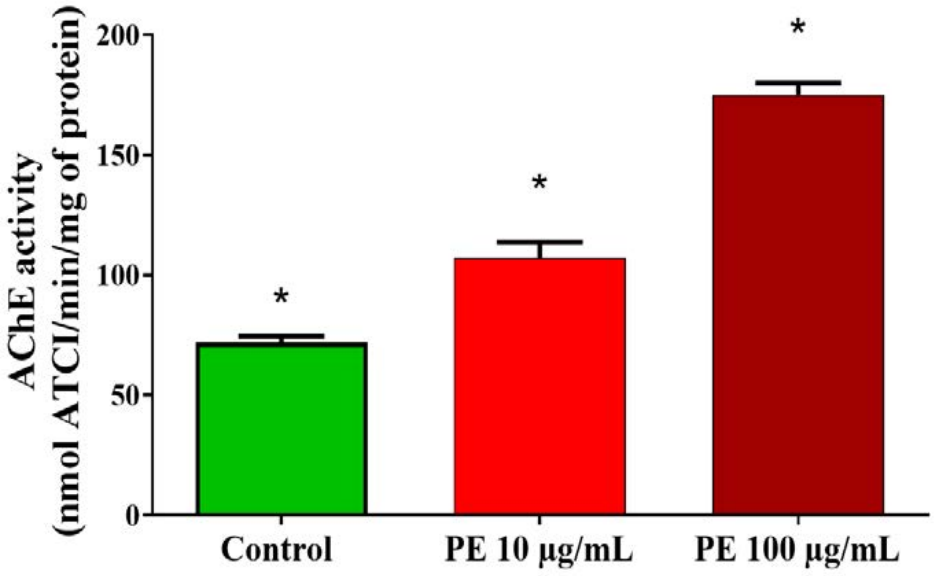

Figure 4: Inhibition of AChE activity in zebrafish larvae after PE at different concentration 10 µg/mL and 100 µg/mL.

4. Discussion

The findings from this study provide compelling evidence that chronic exposure to PE adversely affects zebrafish larvae, leading to significant alterations in locomotor activity, anxiety-related behaviour, cognitive function, and AChE enzymatic activity. These results align with a growing body of literature highlighting the neurotoxic potential of microplastics in aquatic organisms (Ding et al., 2023). The observed reduction in swimming activity and exploratory behaviour in PE-exposed zebrafish larvae suggests a disruption of normal neurodevelopment. Similar behavioural deficits have been reported in zebrafish exposed to various microplastics, including polystyrene and polyethylene terephthalate, indicating that these particles can interfere with neural circuits responsible for movement and exploration. For instance, exposure to polystyrene microplastics has been shown to decrease locomotor activity and alter anxiety-like responses in zebrafish larvae (Luan et al., 2023; Sarasamma et al., 2020). The light/dark preference test further revealed that PE exposure diminishes the natural preference for the dark zone, a typical anxiety-related behaviour in zebrafish. At higher concentrations, larvae exhibited a preference for the light zone, indicating reduced anxiety-like behavior. This alteration in light/dark preference has been observed in zebrafish exposed to other environmental contaminants, suggesting that PE microplastics may interfere with the neural pathways governing anxiety responses.

The T-maze test demonstrated that PE exposure impairs the cognitive abilities of zebrafish larvae, as evidenced by reduced exploration time in the reward zone. This decline in exploratory behaviour is consistent with findings from studies investigating the effects of microplastics on cognitive function in zebrafish. For example, exposure to polystyrene nanoplastics has been shown to inhibit acetylcholinesterase activity and reduce locomotor activity (Lei et al., 2018; Saputra et al., 2025), which may be linked to alterations in cognitive function. The significant inhibition of AChE activity in the head region of PE-exposed zebrafish larvae provides a mechanistic insight into the neurotoxic effects observed. AChE is crucial for terminating neurotransmission by hydrolyzing acetylcholine (Greenfield, 1991); its inhibition leads to the accumulation of acetylcholine, resulting in overstimulation of cholinergic neurons. This can disrupt normal neural signalling and contribute to behavioural deficits (Aroniadou-Anderjaska et al., 2023; Grasshoff et al., 2007). Similar reductions in AChE activity have been reported in zebrafish exposed to other microplastics, such as polystyrene nanoparticle and plasticizer metabolite mono-(2-ethylhexyl) phthalate (Liu et al., 2025). The neurotoxic effects of PE microplastics may be mediated through the induction of oxidative stress and neuroinflammation. Microplastics have been shown to generate reactive oxygen species (ROS) and activate inflammatory pathways in aquatic organisms (Cao et al., 2023), leading to neuronal damage and dysfunction. For instance, exposure to photoaged polystyrene microplastics has been associated with increased ROS levels and altered antioxidant enzyme activities in zebrafish (Ding et al., 2024). These findings suggest that PE microplastics may exert neurotoxic effects through similar mechanisms. The results of this study underscore the potential risks posed by PE microplastics to aquatic organisms, particularly during early developmental stages. Given the ubiquity of microplastics in aquatic environments, understanding their neurotoxic effects is crucial for assessing the ecological risks they pose. The use of zebrafish larvae as a model organism provides valuable insights into the behavioural and biochemical alterations induced by microplastics and can inform regulatory decisions regarding plastic pollution.

5. Conclusion

In conclusion, chronic exposure to PE microplastics impairs locomotor activity, alters anxiety-related behaviour, diminishes cognitive function, and inhibits AChE activity in zebrafish larvae. These findings contribute to the growing body of evidence linking microplastic pollution to neurotoxic effects in aquatic organisms. Further research is needed to elucidate the underlying mechanisms and to assess the long-term consequences of microplastic exposure on aquatic ecosystems.

Declarations

Ethics approval statement

Not applicable

Consent to participate

Not applicable

Consent to publish

Not applicable

Data Availability Statement

The data are available from the corresponding author upon reasonable request

Competing Interests

The authors declare that they have no conflict of interest

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Author contribution

Conceptualization, Data curation: A.S.N.N Investigation, & Writing: S.R.S. Formal analysis and Editing: M.J.M. All authors have read and agreed to the published version of the manuscript`

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