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Photoaged polystyrene microplastics result in neurotoxicity associated with neurotransmission and neurodevelopment in zebrafish larvae (*Danio rerio*)

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ABSTRACT

Microplastics (MPs) are emerging pollutants widely distributed in the environment, inducing toxic effects in various organisms. However, the neurotoxicity and underlying mechanisms of simulated sunlight-aged MPs have rarely been investigated. In this study, zebrafish (*Danio rerio*) were exposed to environmentally relevant concentrations (0, 0.1, 1, 10, and 100 μg/L) of virgin polystyrene (V-PS) and aged polystyrene (A-PS) for 120 hpf to evaluate the neurotoxicity. The results demonstrated that simulated sunlight irradiation altered the physicochemical properties (morphology, functional groups, and chemical composition) of V-PS. Exposure to A-PS causes greater toxicity on locomotor ability in larval zebrafish than V-PS. Motor neuron development was disrupted by transgenic (*hb9-GFP*) zebrafish larvae exposed to A-PS, with significant alterations in neurotransmitter levels (ACh, DA, 5-HT, and GABA) and enzyme activity (AChE, ChAT, and ChE). Further investigation found that exposure to A-PS had a significantly impact on the expression of neurotransmission and neurodevelopment-related genes in zebrafish. These findings suggest that A-PS induces neurotoxicity by its effects on neurotransmission and neurodevelopment. This study highlights the neurotoxic effects and mechanisms of simulated sunlight irradiation of MPs, providing new insights for assessing the ecological risks of photoaged MPs in the environment.

1. Introduction

Microplastics (MPs) have received global attention as an important class of environmental pollutants. MPs are widely distributed in environments, such as soils, sediments, freshwater, oceans, and in the atmosphere (Wang et al., 2021). MPs in the water column can be rapidly ingested and accumulated by aquatic organisms, posing a potential hazard to these organisms (Elizalde-Velázquez and Gómez-Oliván, 2021). Furthermore, MPs can enter the human body through diet and respiration, posing considerable risk to human health (Kutralam-Muniasamy et al., 2023; Malafaia and Barceló, 2023). MPs have been shown to produce neurotoxicity in organisms, including Common Ragworm (*Hediste diversicolor*), Javanese Medaka Fish (*Oryzias javanicus*), and Red Tilapia (*Oreochromis niloticusas*) (Ding et al., 2020; Urban-Malinga et al., 2022; Usman et al., 2021). However, these studies have focused on the assessment of virgin commercial microbeads.

MPs in the environment are exposed to solar radiation, which causes photo-oxidation. Photo-irradiation is regarded as one of the most important environmental elements influencing the aging of MPs (Zhu et al., 2019). As xenon lamps emit a spectrum extremely proximate to that of natural light, they can be utilized as solar simulators (Dibowski and Esser, 2017). Polystyrene (PS) is one of the most widely utilized and

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common MPs, and studies have shown that photodegrading processes could alter their environmental fate and biotoxicity. Research by Liu et al. demonstrated that aged polystyrene (A-PS) is expected to have a different toxicity than that of virgin polystyrene (V-PS) due to particle density, changes in size, oxygen functional groups, and biofilm formation (Liu et al., 2021). In Grouper (*Epinephelus moara*), A-PS caused more severe hepatotoxicity and growth toxicity than in V-PS (Wang et al., 2020). Numerous studies have been conducted to investigate the negative effects of UV-aging MPs on organisms, whereas few studies use simulated natural light aging (Chen et al., 2021; Wang et al., 2020).

Zebrafish are an excellent model for assessing environmental toxins owing to their tiny size, year-round spawning, sensitivity to environmental changes, and simplicity of culture (Brion et al., 2019). Due to the high degree of conservation within the neural systems of zebrafish and mammals, zebrafish are increasingly employed in developmental neurotoxicity research (Zhao and Yang, 2022). Jeong et al. found that PS impairs the swimming abilities of zebrafish larvae and alters neurotransmitter concentrations (Jeong et al., 2022). Guimarães et al. demonstrated that short-term exposure to environmental MPs causes neurotoxic alterations in zebrafish and that this exposure was sufficient to change the neurotoxicity biomarkers studied (Guimarães et al., 2021). Nevertheless, there are fewer reports on the neurotoxic effects of aging on microplastics and their intrinsic mechanisms. Therefore, research is required into the neurotoxicity of organisms caused by aging MPs and the underlying mechanisms.

The nervous system is the dominant system controlling physiological functioning activities in animals, and its malfunction can result in abnormal behavior and poor survival. To date, few researchers have attempted to review the toxic effects of simulated sunlight irradiation of MPs. This study aimed to investigate the neurotoxic effects of photoaging MPs by comparing the changes in motor behavior of the zebrafish larvae when exposed to V-PS and A-PS. The concentrations of V-PS and A-PS were selected based on environmentally relevant concentrations (Green et al., 2017). The disruptive effect of simulated sunlight irradiation of MPs on neurotransmission and its mechanism were investigated by measuring neurotransmitter changes, neuronal development, and the expression of related genes. The novelty of this work are as follows: (1) Exposure to A-PS at environmentally relevant concentrations induced more severe neurotoxicity in zebrafish than V-PS. (2) The critical role of neuronal damage and abnormal neurotransmission in neurotoxicity induced by A-PS were found in zebrafish. Our results revealed the adverse hazard of photoaged MPs to aquatic organisms, providing evidence for the potential risk of MPs infiltrating the environment.

2. Materials and methods

2.1. Xenon irradiation experiments

Janus New-Materials Co. (Nanjing, China) supplied the V-PS, which was 1 μm in diameter. V-PS placed on a Petri dish glass surface were continuously irradiated with the 60 W Xenon lamp for 6 months at 25 °C to produce A-PS. To eliminate any impure residues, A-PS samples were washed with ultrapure water and heated to 50 °C in an oven. PS characteristics were investigated using dynamic light scattering, scanning electron microscopy (SEM), gel permeation chromatography (GPC), Fourier transform infrared (FTIR), water contact angle (WCA), and X-ray photoelectron spectroscopy (XPS). Additional details are given in Text S1.

2.2. Non-transgenic embryo exposure design

Adult wild-type (AB strain) zebrafish were purchased from Nanjing EzeRinka Biotechnology Co., Ltd. (China). Healthy embryos were collected by natural mating under a 14/10 h light-dark cycle at 28 °C. Embryos from 2 h (hpf) to 5 d (dpf) after fertilization were placed in V-PS and A-PS solutions at concentrations of 0, 0.1, 1, 10, and 100 μg/L. The exposure solution was changed every 24 h and embryo development and mortality were observed. Each experimental concentration was performed concurrently in triplicate.

2.3. Transgenic zebrafish embryos exposure design

Transgenic (*hb9-GFP*) zebrafish were purchased from Nanjing EzeRinka Biotechnology Co., Ltd. (China). The larvae were grown and exposed to V-PS and A-PS for the non-transgenic zebrafish to study the development of motor neurons. We used a fluorescence microscope (Nikon, DS-Qi2, Japan) for photographing larvae to obtain motor nerve images of *hb9-GFP* transgenic larvae at 120 hpf. The green fluorescent protein (GFP) fluorescence intensity was calculated using Image J (ImageJ, U.S. National Institutes of Health, Bethesda, Maryland) software.

2.4. Locomotor activity

The Danio Vision video tracking system was used to monitor the locomotor behavior of zebrafish following 120 hpf exposure to V-PS and A-PS. Each experiment was conducted on a 24-well plate, with one plate per treatment and one young fish per well (Yuan et al., 2022). Before testing, the fish were dark-adapted in a soundproof environment for 40 min, and data on the distance and duration traveled by zebrafish in different swimming stages over the 40 min were recorded, and swimming speed was estimated.

2.5. Biochemical analysis

The zebrafish larvae were collected after being washed 3–5 times with ultrapure water (Watsons, China). Neurotransmitter (ACh, DA, 5-HT, and GABA), and neurotransmitter-related enzyme (AChE, ChAT, and ChE) levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Meimian Industrial Co., Ltd., China) following the manufacturer's instructions (Hu et al., 2022). The detailed descriptions are in Text S2.

2.6. Quantitative real-time polymerase chain reaction

The 5 dpf A-PS exposed zebrafish larvae were collected for quantitative real-time polymerase chain reaction (qRT-PCR) analysis. Total RNA was extracted using the Trizol ® reagent (Invitrogen, USA), and RNA purity was determined using a NanoPhotometer (Implen, CA). The extracted RNA samples were analyzed using qRT-qPCR (Chen et al., 2024; Li et al., 2019; Qin et al., 2022), and gene expression was determined using the StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA). Primers for the associated genes were utilized in the same manner as previously reported. Table S1 contains the primers.

2.7. Statistical analyses

In this study, each treatment was repeated three times. Pearson correlation analysis was performed using IBM SPSS 26 and data were expressed as mean ± standard deviation (SD). We determined statistical significance by one-way Analysis of Variance (ANOVA) and Tukey post hoc testing (IBM SPSS 26, USA). All graphs were created using Origin 2022 software.

3. Results and discussion

3.1. Impact of photoirradiation on the physicochemical characteristics of PS

The effect of xenon arc lamp irradiation on the surface morphology of PS was studied using SEM. The surface of V-PS (Fig. 1A) was smooth, whereas A-PS (Fig. 1B) exhibited a rough, honeycomb structure with

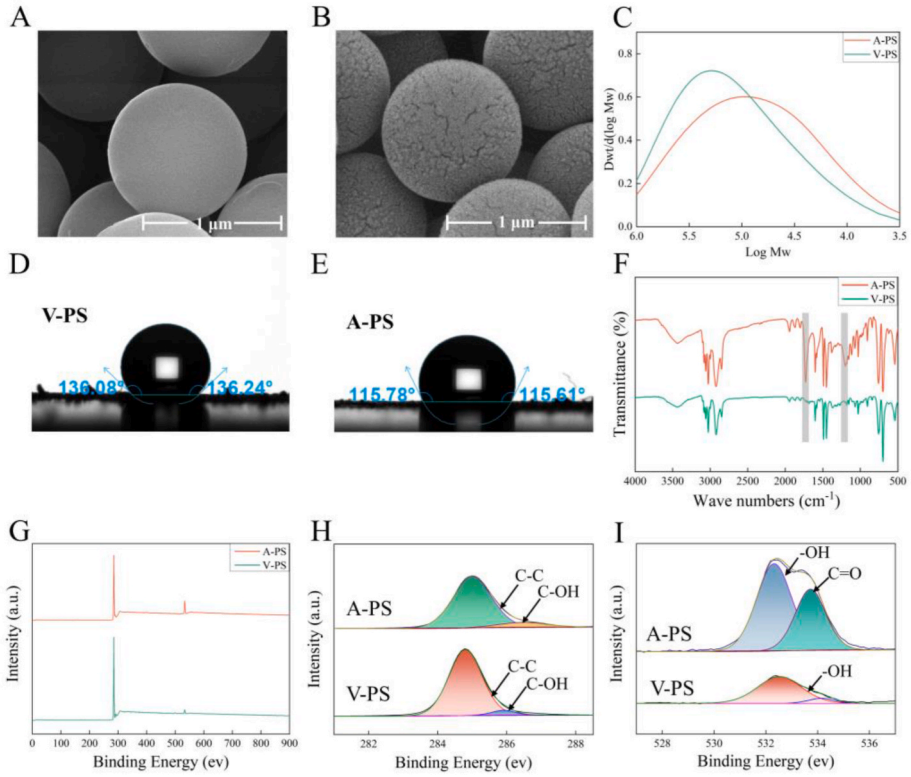

Fig. 1. Characterization of V-PS and A-PS. The morphology obtained from SEM of V-PS (A) and A-PS (B), GPC spectra (C), Water contact angle (D–E), ATR–FTIR spectra (F), XPS survey spectra (G), C 1s XPS spectra (H), O 1 XPS spectra (I).

cracks and pits, which may be due to partial breakage caused by light degradation (Xiong et al., 2020). There were no significant no significant differences in size between V-PS (1.07 ± 0.04 μm) and A-PS (0.96 ± 0.07 μm). According to the GPC spectra of V-PS and A-PS (Fig. 1C), the GPC peak of A-PS shifted to a greater retention volume and had lower detector response or refractive indices (RI) than those in V-PS, indicating that the aging process produced a lower molecular weight than that in the V-PS (Liu et al., 2021). To further study the effect of Xenon arc lamp photoaging on the surface characteristics, we analyzed the WCA measurement. The WCA values reveal a significant decrease from 143.86° for V-PS to 121.80° for A-PS, as shown in Fig. 1D and E. This result indicates that the surface of A-PS becomes more hydrophilic as a result of photoaging. Similar results were observed in previous studies (Liu et al., 2021; Xiong et al., 2020).

As shown in Fig. 1F, the FTIR spectra of the A-PS were distinct and displayed new peaks at 1729.38 cm^{-1} and 1198.58 cm^{-1}, indicating the existence of C═O and –OH bonds, respectively. The carbonyl index (CI) is often used in the analysis of polymer photoaging (Liu et al., 2019). The CI value of A-PS (0.770) increased considerably in comparison with that of V-PS (0.075). Furthermore, XPS survey spectra were employed to investigate the surface chemical compositions of V-PS and A-PS (Fig. 1G). The proportion of O 1s in PS increased from 3.03% to 11.16% by light degradation, whereas the proportion of C 1s decreased from 96.97% to 88.84%. Fig. 1H and I shows that the peaks of partial oxygen-containing functional groups (C═O and –OH) were higher in the C 1s and O 1s regions of A-PS compared with those of V-PS. This result is consistent with those obtained by FTIR. Previous investigations on the artificial aging of PS showed similar results (Mao et al., 2020). Overall, light irradiation plays a critical role in the natural aging process of MPs.

3.2. Impact of A-PS on the locomotor behavior in zebrafish

This study used average speed as a quantitative indicator to analyze zebrafish movements and assess the capacity of A-PS and V-PS to damage brain development and ultimately behavior. Larval locomotor activity was measured during an alternating light/dark test over 40 min (10 min light/10 min dark) at 120 hpf. In all PS treatment groups, larval zebrafish showed a concentration-dependent decrease in locomotor indicators. The results of the light/dark alternation experiment showed the effects of the V-PS and A-PS treatments on the swimming speed of the larvae. The swimming speed was significantly higher in the dark than that of each treatment group in the light, which is consistent with the phenomena of enhanced zebrafish activity in the dark (Fig. 2A and B). As indicated in Fig. 2C, V-PS and A-PS exposure lowered the mean distance traveled per minute in the exposed group as the exposure concentration increased ($P < 0.05$). The results of a one-way ANOVA revealed that exposure to A-PS and V-PS (at 0.1 μg/L each) significantly affected the locomotor behavior of the larvae ($P < 0.05$) and that the effect was highly significant at concentrations greater than 1 μg/L ($P < 0.01$). A-PS had a more significant impact on decreasing movement speed than V-PS when exposure concentrations reached 100 μg/L ($P < 0.05$).

The locomotor behavior of zebrafish is an indication of neurotoxic impacts and can reflect neurological damage to some degree (Xu et al., 2022). The average zebrafish speed decreased as the levels of A-PS and V-PS increased, indicating that zebrafish were less active after being exposed to MPs. Similarly, some researchers have found that MPs reduce the swimming ability of zebrafish larvae (Jeong et al., 2022; Qiang and Cheng, 2019). Consistent with the findings of this study, Pitt et al. found that light conditions had a substantial influence on the locomotor activity of zebrafish, with the average speed of larvae in all exposed groups being greater in the dark than in the light (Pitt et al., 2018). More crucially, PS aging changed the locomotor behavior of zebrafish larvae in this experiment, with A-PS suppressing larval behavior at various degrees when compared with that in V-PS. Similar to this study, researchers found that photoaging increases the toxicity of MPs to zebrafish (Ding et al., 2023; Zhang et al., 2022; Zhong et al., 2023). Swimming ability is an essential part of the predator avoidance reaction

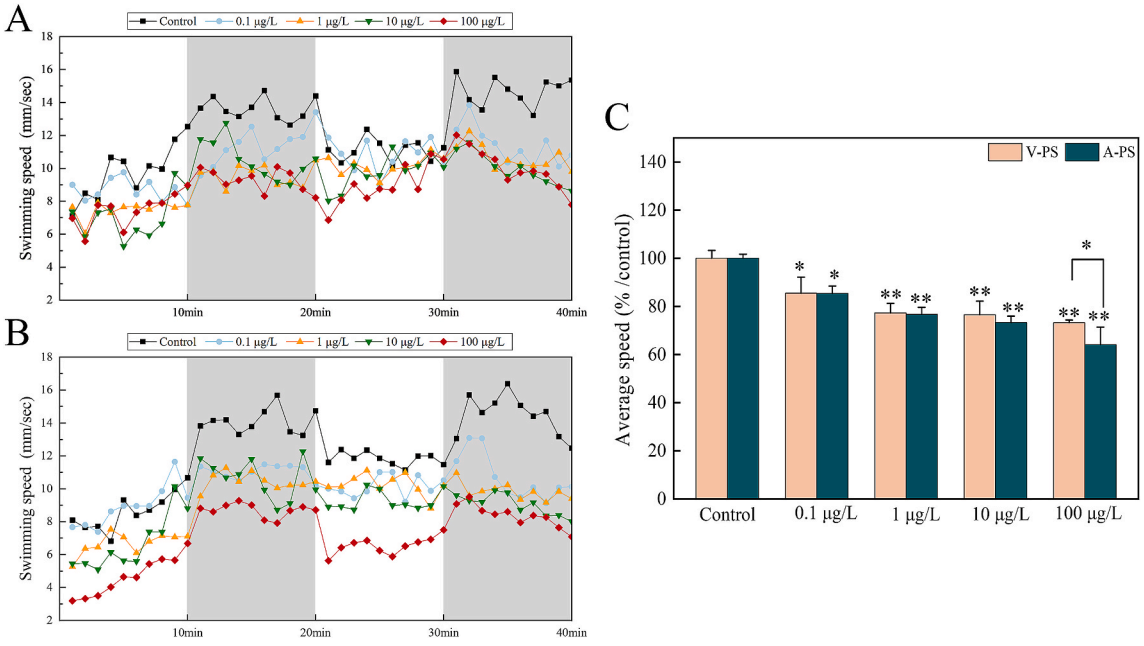

Fig. 2. Effects of V-PS and A-PS on the locomotor behavior in zebrafish larvae. Trends in locomotor behavior of zebrafish exposed to various concentrations of V-PS (A), and A-PS (B). Comparison of the average speed of the larvae exposed to different concentrations of V-PS and A-PS (C). Values are presented as the mean ± SD. Significant differences between the exposed and control groups of larvae: *$P < 0.05$, **$P < 0.01$.

(Qian et al., 2021). Because the average speed of larvae decreases after exposure to A-PS, larvae have a reduced ability to forage and escape from predators after exposure to A-PS, and they may struggle to survive in the natural environment. As a result, to investigate the neurotoxicity of A-PS, the average swimming speed must be used as one of the significant indicators. The average swimming speed of juvenile zebrafish is thus a proxy for their capacity to survive in their environment and may be an important indicator of neurotoxicity.

3.3. Impact of A-PS on motor neuron development in zebrafish larvae

We used transgenic Tg (*hb9-GFP*) larvae to study the impact of various A-PS concentrations on motor neurons and explore the structural and functional alterations in neurons throughout early development. Fig. 3A–E shows the GFP fluorescence of motor neurons in the brain and spinal cord in 120 hpf zebrafish larvae exposed to various concentrations of A-PS. The motor neurons of Tg (*hb9-GFP*) larvae were noticeably reduced compared with that in the control group after A-PS exposure. As shown in Fig. 3F, the GFP fluorescence intensity decreased in zebrafish larvae from 238 ± 2.58 AU in the control group to 234 ± 2.88 AU, 232 ± 4.97 AU, 230 ± 3.95 AU, and 229 ± 2.53 AU in the 0.1 μg/L A-PS, 1 μg/L A-PS, 10 μg/L A-PS, and 100 μg/L A-PS exposure groups, respectively ($P < 0.05$, Fig. 3F).

Individual neurons in zebrafish may be visualized throughout embryonic development due to the transparency of embryos (Wu et al., 2019); therefore, embryos of *Tg* (*hb9-GFP*) zebrafish were used to evaluate the development of motor neurons (Yu et al., 2022a). In our investigation, all A-PS exposure reduced motor neuron proliferation in the brain and spinal cord of Tg (*hb9-GFP*) zebrafish larvae, indicating the sensitivity of the central nervous system and motor neurons to A-PS. The reduction in GFP fluorescence following A-PS exposure suggests that A-PS may damage axonal development in zebrafish larval motor neurons. MPs can adhere to the surface of the chorionic surface, resulting in the establishment of a hypoxic microenvironment within the embryo

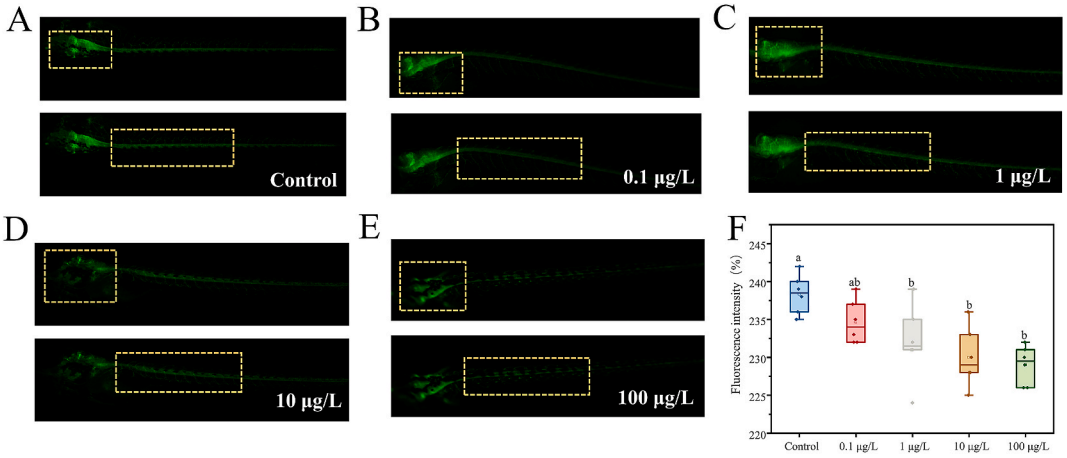

Fig. 3. Effects of A-PS on motor neuron development in zebrafish larvae. Development of motor neurons in 120 hpf larvae exposed to different concentrations of A-PS (A–E). Fluorescence intensity of motor neurons in 120 hpf larvae treated with different A-PS doses (F). Different letters at the top of the box plot ($P < 0.05$) indicate significant differences between treatment groups.

(Duan et al., 2020), which may be responsible for the aberrant neuronal development in the brains of zebrafish larvae following A-PS exposure. Zhou et al. reported similar findings that the 1 μm V-PS exposure caused a decrease in the neuron number of Tg (*hb9-GFP*) zebrafish larvae (Zhou et al., 2023). However, the precise location of damage and possible mechanisms of A-PS exposure remain unclear and require further investigation.

3.4. Impacts of A-PS on neurotransmitter conduction

The function of the neurotransmitter system in regulating the locomotor behavior of zebrafish larvae exposed to A-PS was explored in this study. As shown in Fig. 4, we measured neurotransmitter levels, including ACh, DA, 5-HT, and GABA, as well as enzyme activities, such as AChE, ChAT, and ChE. Quantitative analysis showed that an increase in the concentration of A-PS, significantly increased the content of neurotransmitters ACh, DA, 5-HT, and GABA, in zebrafish larvae; these neurotransmitters are members of the cholinergic, dopaminergic, serotonergic, and cholinergic systems, respectively (Fig. 4A–D). Similarly, substantially higher activities of AChE, ChAT, and ChE were observed compared with that in the control after A-PS exposure at dosage ranging from 0.1 to 100 μg/L (Fig. 4E–G).

Neurotransmitters are substances that transfer information between neurons or between neurons and effector cells (Xia et al., 2021), which are highly correlated with neurobehavior. ACh and DA are excitatory neurotransmitters, whereas 5-HT and GABA are inhibitory neurotransmitters (Rosa et al., 2022). All four neurotransmitter activities were considerably elevated in A-PS exposed larvae at all concentrations compared with that in the control group in this present examination. The researchers discovered that zebrafish larvae exposed to PS had significantly higher DA and GABA levels than that in the control neurotransmitter content ($P < 0.05$) (Jeong et al., 2022). ACh is rapidly destroyed by ChE after being synthesized by ChAT in the presynaptic neuron, and it is released into the synaptic cleft (Agostini et al., 2018). The two forms of ChE enzymes that are commonly recognized are AChE and butyrylcholinesterase (BuChE), with AChE enzymes being the most active in the hydrolysis of physiological quantities of ACh (Halder and Lal, 2021). In this study, both ChAT and AChE were enhanced, and ACh was elevated, most likely because the synthesis of ChAT was stronger than the hydrolysis of ACh by ChE. A previous study reported that AChE activity was considerably higher in zebrafish exposed to naturally aged MPs than in the controls (Guimarães et al., 2021). Similarly, Hairui Yu et al. found that the MPs exposure enhanced AChE, ChE, and ChAT activity in zebrafish brains compared with that in the controls, with activity rising gradually with a rise in MPs concentration (Hairui Yu et al., 2022a). Consequently, differing A-PS dosages may lead to behavioral abnormalities and developmental neurotoxicity in zebrafish by interacting with the cholinergic, dopaminergic, 5-HT, and GABAergic systems. Moreover, the swimming behavioral alterations were most likely caused by neurotoxicity.

3.5. Correlation analysis in zebrafish larvae exposed to A-PS

Motor neuron abnormalities have been linked to altered motor behavior in zebrafish at early stages of development (Fan et al., 2022). The locomotor abnormalities observed in A-PS-exposed zebrafish larvae prompted us to investigate the relationship between locomotor behavior and neuronal damage in larvae following A-PS exposure. A reduction in both locomotor activity and neuron numbers was observed in larvae exposed to various A-PS concentrations; thus, the relationship between these two factors was investigated. Pearson correlation analysis revealed a positive correlation between locomotor activity and neuron numbers in larvae (Fig. 5A), which may explain the lower swimming activity of 120 hpf larvae exposed to all doses of A-PS in our experiments.

Neurotransmitter levels are strongly associated with neurobehavioral changes, and DA, 5-HT, GABA, and ACh have been determined as essential neurotransmitters regulating zebrafish larval motor behavior (Wu et al., 2016; Yu et al., 2022a,b). As a result, we investigated the relationship between locomotor behavior and neurotransmitter content in larvae (Fig. 5B). The association between 5-HT and GABA and behavior was extremely significant, indicating that the neurotransmitters with the greatest influence on speed in this investigation were 5-HT and GABA. Furthermore, researchers have proposed that ACh accumulation may terminate neural signaling by causing sustained depolarization of the postsynaptic membrane, resulting in abnormal motor behavior (Lin et al., 2013), and may thus be a causal factor in the

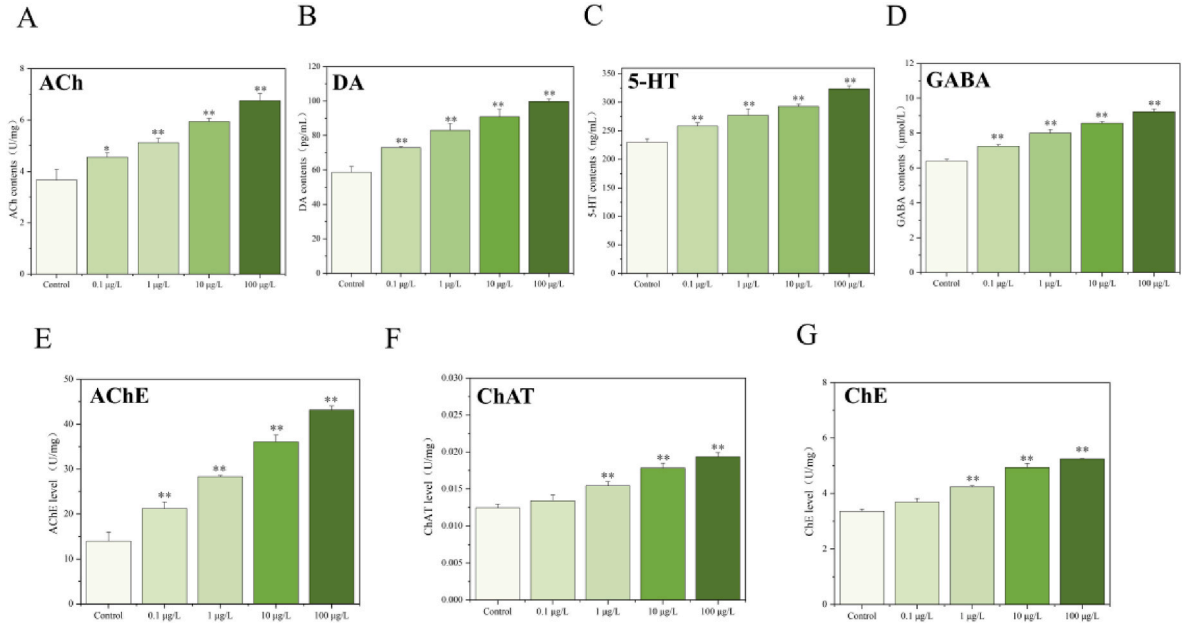

Fig. 4. Effects of A-PS on neurotransmitters content in zebrafish larvae. ACh, DA, 5-HT, and GABA content in zebrafish exposed to different concentration groups (A–D). AChE, ChAT, and CHE activity in zebrafish exposed to different concentration groups (E–G). Significant differences between the exposed larvae and the control group: *$P < 0.05$, **$P < 0.01$.

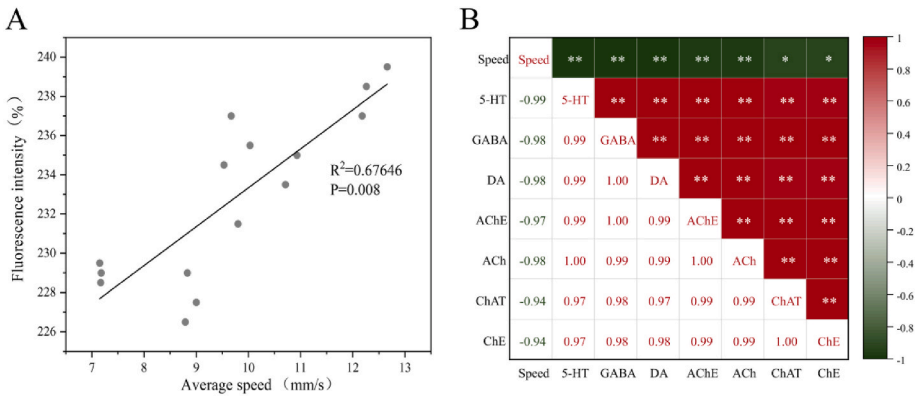

Fig. 5. Effects of A-PS on neurotransmitters content in zebrafish larvae. Pearson correlation analysis between motor behavior and number of neurons (A). Pearson's correlation coefficients between locomotor speed and biomarkers in zebrafish exposed to A-PS (B). Significant differences between the exposed and control groups of larvae: *$P < 0.05$, **$P < 0.01$.

reduced motor activity of larvae following A-PS exposure. The gene expression results are concentration-dependent. Based on the correlation analyses, disorders in locomotor behavior may relate to neurotoxicity.

3.6. Impact of A-PS on the expression of genes related to neurotransmitters

We examined the expression of neurotransmitter-related genes and nervous system development-related genes to better understand the molecular processes behind the neurotoxic effects of A-PS on 120 hpf zebrafish larvae. As shown in Fig. 6, the 0.1–100 μg/L A-PS treatment significantly affected the mRNA levels of neurotransmitter-related genes for ACh (*chat*, *chrna7*, and *ache*), 5-HT (*5ht2c*, *5ht1b,* and *5ht1aa*), DA (*drd3*, *drd2a*, *dra1*, and *dat*), and GABA (*gad1b*, *abat*, *gat1*, and *gabra1*). The expression of genes related to nervous system function or development, such as *nestin*, *gfap*, *manf*, *shha*, *alpha–tubulin*, and *mbp,* were measured. Significant alterations in the expression of these genes were observed in 120 hpf zebrafish larvae exposed to A-PS compared with that in the controls. In comparison with that in the control group, the presence of A-PS significantly inhibited the expression of *gabra1* and *manf,* whereas it significantly elevated the expression of genes, such as ACh (*chat* and *chrna7*), 5-HT (*5ht1aa*), DA (*drd2a* and *dat*), GABA (*gad1b* and *abat*), *nestin*, *gfap, alpha–tubulin*, and *mbp*.

This study determined that A-PS exposure altered the expression of genes involved in neurotransmission and neurodevelopment, suggesting that A-PS causes anomalies in the zebrafish nervous system. These genes encode proteins involved in neuroectodermal stem cell development (*nestin*), astroglial cytoskeleton regulation (*gfap*), dopaminergic neuron maintenance and regulation (*manf*), nervous system formation (*shha)*, microtubule formation (*alpha–tubulin*), and myelin formation (*mbp*) in developing zebrafish, and they are considered neurotoxic biomarkers (Chen et al., 2017; Zhu et al., 2022). A previous study found that, following tetrabromobisphenol A exposure, the expression levels of several genes were reduced (Zhu et al., 2018). Bhagat et al. found that, following MPs exposure, AChE gene expression was downregulated in all exposure groups in zebrafish (Bhagat et al., 2021). Furthermore, the transcription levels of 5-HT (*5-ht1a and 5-ht2c*), DA (*drd2a, drd1b, and drd2b*), and GABA (*gad2 and gabrg2*) genes are altered in zebrafish following contaminant exposure (Tang et al., 2022; Tu et al., 2020). Thus, alterations in the expression levels of these genes may be partially responding to the altered neurotoxicity of A-PS in *Danio rerio*.

4. Conclusion

Exposure to environmentally relevant concentrations of A-PS produced more severe neurotoxicity in zebrafish than V-PS. Exposure to A-PS induced motor neuron injury and neurotransmission problems,

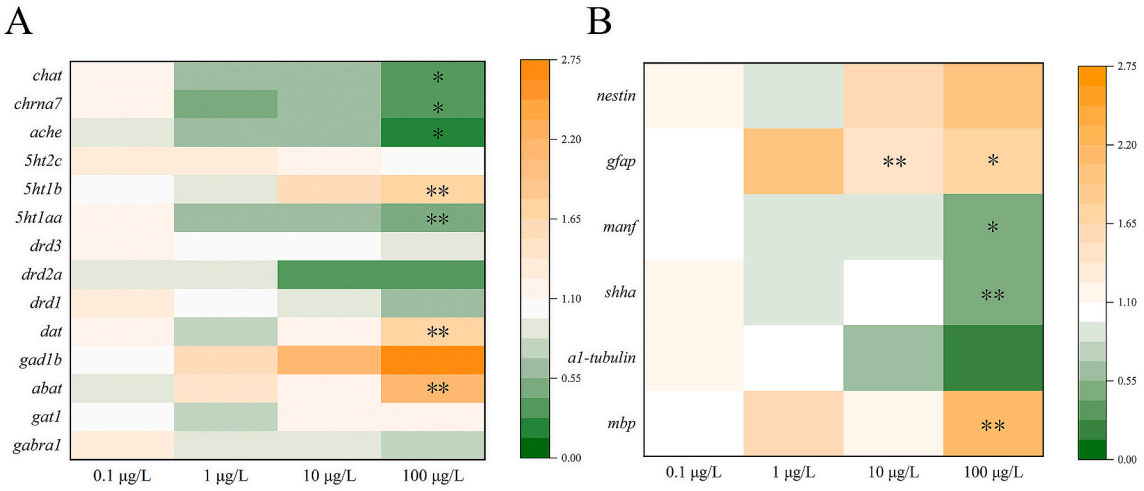

Fig. 6. Effect of A-PS exposure on expression of neurotransmitter- and nervous system development-related genes in zebrafish. Expression of ACh-, 5-HT-, DA -, and GABA-related-genes(A). Expression of nervous system development-related genes. The effects of A-PS on neurotransmitter content in zebrafish larvae were standardized to that of the -actin gene(B). Significant differences between the exposed and control groups of larvae: *$P < 0.05$, **$P < 0.01$.

which are strongly connected to nervous system function. The expression of neurotransmission-related and nervous system development-related genes was also changed in zebrafish. Thus, neuronal damage and abnormal neurotransmission were involved in the neurotoxicity induced by A-PS in zebrafish.

CRediT authorship contribution statement

Xintong Li: Writing – original draft, Investigation. **Tong Zheng:** Validation, Data curation. **Jiayi Zhang:** Investigation. **Haibo Chen:** Writing – review & editing. **Chongdan Xiang:** Investigation. **Yanan Sun:** Investigation. **Yao Dang:** Validation, Data curation. **Ping Ding:** Writing – review & editing, Visualization, Conceptualization. **Guocheng Hu:** Writing – review & editing, Resources. **Yunjiang Yu:** Resources, Conceptualization.

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

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118524.

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