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Photoaged microplastics induce neurotoxicity associated with damage to serotonergic, glutamatergic, dopaminergic, and GABAergic neuronal systems in *Caenorhabditis elegans*

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ABSTRACT

Microplastics (MPs) are ubiquitous environmental contaminants that cause neurotoxicity in various organisms. MPs are typically affected by light irradiation and undergo photoaging. However, the neurotoxic effects of photoaged polystyrene (P-PS) and its underlying mechanisms remain unclear. In this study, locomotion behaviors, neuronal development, neurotransmitter levels, and the expression of neurotransmission-related genes were investigated in *Caenorhabditis elegans* exposed to P-PS at environment-relevant concentrations (0.1–100 μg/L). The characterization results showed that photoaging accelerated the aging process and changed the physicochemical properties of the MPs. The toxicity results suggested that exposure to 1–100 μg/L P-PS caused more severe neurotoxicity than virgin polystyrene (V-PS) with endpoints of head thrashes, body bends, wavelength, and mean amplitude. Exposure to P-PS also altered the fluorescence intensity and neurodegeneration percentage of serotonergic, glutamatergic, dopaminergic, and aminobutyric acid (GABA) in transgenic nematodes. Similarly, significant reductions in the levels of these neurotransmitters were also observed. Based on Pearson's correlation, locomotion behaviors were negatively correlated with the neurotransmission of serotonin, glutamate, dopamine, and GABA. Further investigation suggested that the expression of neurotransmitter-related genes (e.g., *tph-1*, *eat-4*, and *unc-46*) was significantly altered in the nematodes. Collectively, the neurotoxic effects of P-PS were attributed to abnormal neurotransmission. This study highlights the potential toxicity of MPs photoaged under environmentally relevant conditions.

1. Introduction

Plastics have been widely used in human production and life as it has the advantage of lightweight, corrosion resistance, and chemical stability. However, nearly 80 % of this is plastic waste, buried, or accumulated in the environment (Geyer et al., 2017). These plastic wastes decompose into smaller fragments under physicochemical effects, such as erosion, corrosion, and light, for a long time. When the size of plastic is <5 mm, it can be defined as microplastics (MPs) (Smith et al., 2018). Soil is an important reservoir for MPs, and MPs may originate from sewage sludge, the weathering of plastic films, littering, and atmospheric deposition (Rillig, 2012; Blasing and Amelung, 2018; Li et al., 2018). Many studies have found diverse MPs in soils worldwide, with ranging from 0.34 to 690,000 particles/kg (Wang et al., 2020a). The particle size of the MPs in soils was mainly concentrated in 20–5000 μm, and a few research works on MPs size were at <5 μm (Zhang et al., 2021a). Polystyrene (PS), one of the most common MPs, is ubiquitous in the soil, oceans, rivers, atmosphere, and other environmental matrices (Rachman, 2018). PS is easily ingested by various organisms and cause negative effects, including slowed growth, oxidative stress, neurotoxicity, and reproductive disorders (Kim et al., 2020a; Kim et al., 2020c; Liu et al., 2020b). Neurotoxicity is one of the most significant toxicities associated with PS treatment. Recent reports have shown that it causes neurotoxic effects in various animals such as Javanese medaka,

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zebrafish, mice, and red tilapia (Ding et al., 2020; Sarasamma et al., 2020; Usman et al., 2021; Jeong et al., 2022; Wang et al., 2022). However, pristine PS were typically used in these studies, and the potential neurotoxicity of PS should be estimated under environmentally relevant conditions.

Environmental MPs are affected by light irradiation and weathering processes (Alimi et al., 2018). Ultraviolet (UV) light accounts for a smaller portion than visible light (Santos et al., 2013). Xenon lamps can better reflect the natural aging process of MPs exposed to sunlight because they can simulate the full sunlight spectrum. Several studies have shown that xenon lamp irradiation can change the surface structure through cracking, fragmentation, chain scission, and oxidation (Lin et al., 2021; Sorensen et al., 2021; Yu et al., 2022b). However, the toxic effect of PS aged by xenon lamps on organisms remains unclear, especially on soil organisms.

Caenorhabditis elegans (*C. elegans*) is a soil-dwelling nematode with the properties of short lifespan, transparent body, easy culture, and well-characterized genetics (Yu et al., 2022c). *C. elegans* has been considered a critical non-mammalian alternative model for assessing toxicity and elucidating the underlying mechanisms of environmental contaminants. *C. elegans* and mammals share similar biosynthetic and metabolic pathways, which can be used to predict mammalian toxic responses by comparing the toxicity of nematodes with data on mammalian toxicity (Aschner et al., 2010; Hunt et al., 2012; Zhao et al., 2013). The connectivity of neuronal networks has been fully elucidated, and almost all families of genes relevant to neurotransmission in mammals have been identified in nematodes (Chen et al., 2013). Owing to their neuronal lineage being thoroughly described, nematodes help detect neurotoxicity and its underlying mechanisms (Li et al., 2020a).

Multiple endpoints can be used to evaluate neurotoxicity of MPs, including head thrashes, body bends, neuronal development, neurotransmitter levels, and gene expression. Recent reports have shown that exposure to MPs would cause neurotoxicity in nematodes (Qu et al., 2019; Qu and Wang, 2020; Chen et al., 2021b). Similarly, decreased locomotion behaviors were observed in nematodes exposed to virgin PS, indicating the potential of PS to induce neurotoxicity (Lei et al., 2018; Yu et al., 2020). It has been proven that the neuronal systems (serotonergic, glutamatergic, dopaminergic, and GABAergic neurons) play an essential role in the regulation of locomotion behavior in *C. elegans* (Sawin et al., 2000; Mano et al., 2007; Li et al., 2017; Ishita et al., 2020). Recently, this neuronal damage was involved in decreased locomotion behaviors in nematodes exposed to carboxyl-modified PS (Yu et al., 2023). Previous research has demonstrated that exposure to PS adversely affects on locomotion behaviors and damages the neuronal systems of dopaminergic and GABAergic nematodes (Lei et al., 2018; Qu and Wang, 2020). However, the neurotoxicity and underlying mechanisms of photoaged PS remain unclear and require further investigation.

To evaluate the neurotoxicity and mechanisms of xenon lamp-aged PS, the locomotion behavior, neuronal damage, and neurotransmitter levels were examined using *C. elegans* as an animal model. In addition, the expression of neurotransmission-related genes was investigated to explore the underlying mechanisms. This study highlights the environmental risks posed by aged MPs to environmental organisms.

2. Materials and methods

2.1. Characterization of PS

A 500 W Xenon lamp was used to simulate aging reactions in sunlight, as the spectrum of the xenon lamp in the range of 295–800 nm coincides with the spectrum of sunlight. Virgin PS (V-PS) powder at a size of 1 μm was purchased from Janus New Materials Co. (Nanjing, China), and V-PS placed in quartz Petri dishes were continuously irradiated with xenon lamps (Sailham, Guangdong, China) for 45 d to obtain photoaged PS (P-PS). The powder was thoroughly mixed daily to ensure the particles were uniformly aged. After irradiation, P-PS was washed with ultrapure water and dried at 50 °C. SEM, FTIR, and XRD were used to analyze the morphology, functional groups, and crystallinity of V-PS and P-PS. Details are provided in Text S1.

2.2. C. elegans exposure

Nematodes were maintained on plates supplemented with *Escherichia coli* OP50 (Brenner, 1974). Clorox solution (5 % NaOCl/1 N NaOH, 2:5) was used to lyse gravid *C. elegans* to obtain eggs. After washing three times by K-medium (2.386 g KCl L^{-1}, 2.98 g NaCl L^{-1}), the collected eggs were cultured for 48 h at 20 °C (Williams and Dusenbery, 1990). Next, *C. elegans* was exposed to V-PS and P-PS suspensions at the dose of 0.1–100 μg/L for 24 h.

2.3. Experiment of locomotion behaviors

Head thrashes, body bends, wave lengths, and mean amplitudes were used to evaluate locomotion behaviors. After exposure to V-PS and P-PS, the nematodes in each concentration group were individually transferred to a plate without OP50. A head thrash was counted as a change in the direction of the body mid-region (Shang et al., 2021). A body bend was defined as a change in the direction of the posterior bulb (Jin et al., 2022). Wave lengths and mean amplitudes were detected using the Wormlab® Imaging System (MBF Bioscience, USA).

2.4. Evaluation of neuron damaged

The endpoints of dopaminergic, glutamatergic, GABAergic, and serotonergic neurons are typically used to evaluate neuronal development (Chen et al., 2021b). Levamisole was added at a dose of 1 mM to anesthetize the nematodes, and then nematodes were captured using a fluorescence microscope (Olympus BX51, Japan). The intensity of the fluorescent signals and the percentage of neurodegeneration were examined to determine neuronal development. Forty nematodes were analyzed for each treatment.

2.5. Neurotransmitters content

Nematodes were collected and washed several times with the K-medium. Serotonin, glutamate, dopamine, and gammaaminobutyric acid (GABA) contents were measured using the corresponding ELISA kits. The operational details are presented in Text S2.

2.6. qRT-PCR experiment

Total RNA was isolated from the nematodes after treatment with TRIzol, and complementary DNA (cDNA) was synthesized using a kit (Accurate Biotechnology, China). The 7500 StepOnePlus Real-Tine PCR system mensurated gene expression. The primer sequences are listed in Table S1.

2.7. Statistical analyses

The data show mean ± standard error of the mean, and ANOVA (SPSS 18, USA) was performed to determine significance. $^{*}p < 0.05$ or $^{**}p < 0.01$ indicated significance.

3. Results and discussion

3.1. Characterization of V-PS and P-PS

SEM images of the V-PS and P-PS are presented in Fig. 1A and B, and there is no change between V-PS (1.043 ± 0.025 μm) and P-PS (0.986 ± 0.031 μm). V-PS was smooth on the surface and spherical, whereas the surface of P-PS was wrinkled and cracked. These data are consistent with previous analysis *results of PS aged* under *UV irradiation* (Liu et al.,

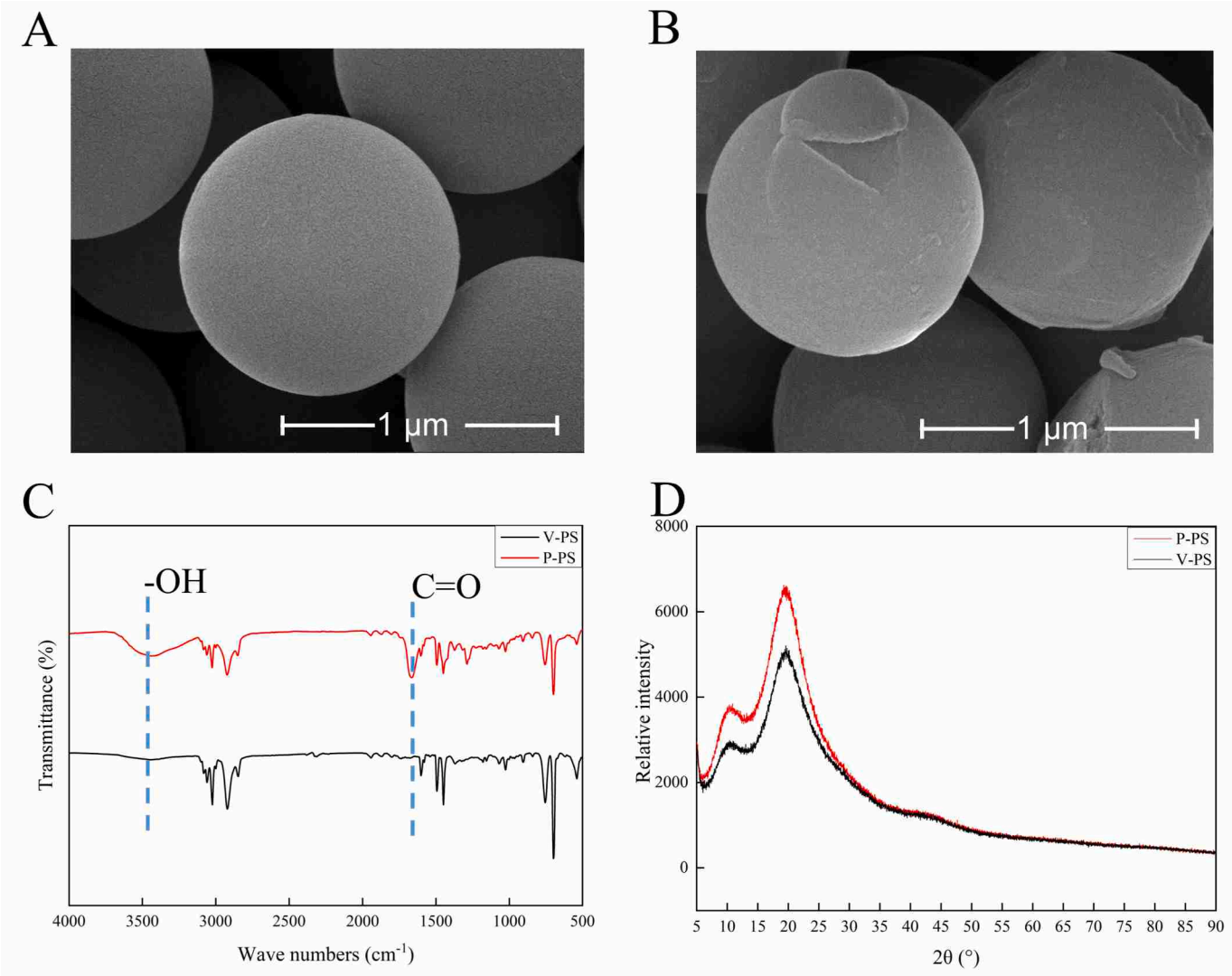

Fig. 1. Physicochemical characterization of V-PS and P-PS. (A) SEM image of V-PS. (B) SEM image of P-PS. (C) FTIR spectra. (D) XRD spectra.

2019a; Wang et al., 2020c). As shown in Fig. 1C, FTIR spectroscopy revealed that compared with V-PS, additional peaks at approximately 3433.9 and 1665.1 cm^{-1} were detected for P-PS, corresponding to the -OH and C═O stretching vibrations. The degradation degree of polymers was assessed using the carbonyl index (CI) that defined as the ratio of the intensity of carbonyl to methylene peak (Liu et al., 2019b). The CI value of P-PS was calculated to be 1.80, which was higher than 0.59 of PS. The data suggested that UV irradiation resulted in strong oxidation of P-PS, which aligns with previous studies (Hüffer et al., 2018; Xiong et al., 2020; Liu et al., 2021). Moreover, XRD showed that higher crystallinity for P-PS than V-PS (Fig. 1D), suggesting that aged PS becomes more fragile and efficiently produces smaller particles. This finding is consistent with previous studies (Mao et al., 2020; Feng et al., 2021). These results indicate that xenon lamps radiation accelerates aging and alters the physical and chemical characteristics of PS if taken together.

3.2. Effect of V-PS and P-PS exposure on locomotion behavior

Head thrashes, body bends, wavelength, and mean amplitude are commonly used to reflect locomotion behavior and evaluates the neurotoxicity of V-PS and P-PS. After exposure to P-PS at a concentration of 100 μg/L, the head thrashes and wavelength were significantly reduced compared to the control (Fig. 2A and C). Exposure to 1–100 μg/L P-PS would cause a significant decrease in wavelength and frequency of head thrashes in nematodes. Meanwhile, exposure to 10–100 μg/L of V-PS significantly reduced body bends and mean amplitude compared to the control (Fig. 2B and D), whereas a significant decrease in body bends and mean amplitude was observed in exposure groups of 1–100 μg/L P-PS.

Exposure to V-PS and P-PS reduces locomotion and induces neurotoxicity. A previous report found that PS exposure reduced locomotion behavior in nematodes, indicating that MPs induced size-dependent neurotoxicity in locomotor behaviors (Lei et al., 2018; Yu et al., 2020). Another study demonstrated that behavioral indicators, including head thrash, body bending, wavelength, and amplitude, were significantly decreased in nematodes exposed to environmental contaminants (arsenic, quantum dots, and cadmium salt), indicating that they causes significant transgenerational neurotoxicity (Contreras et al., 2013; Zhang et al., 2020). Exposure to PS causes transgenerational neurotoxicity with an endpoint of locomotion behavior (Chen et al., 2021a). In *C. fluminea, Danio rerio*, and *Carassius auratus*, neurotoxic effects also have been reported after PS exposure (Murali et al., 2015; Li et al., 2020b; Jeong et al., 2022). In addition, these data also suggested that P-PS exposure caused more severe neurotoxicity than V-PS exposure, which was in agreement with a previous observation (Huang et al., 2021). Similarly, exposure to aged PS under UV light-induced more marked chronic toxicity in *Daphnia magna* than pristine PS (Liu et al., 2022). In Caco-2 cells, photo-transformed PS had severe adverse effects

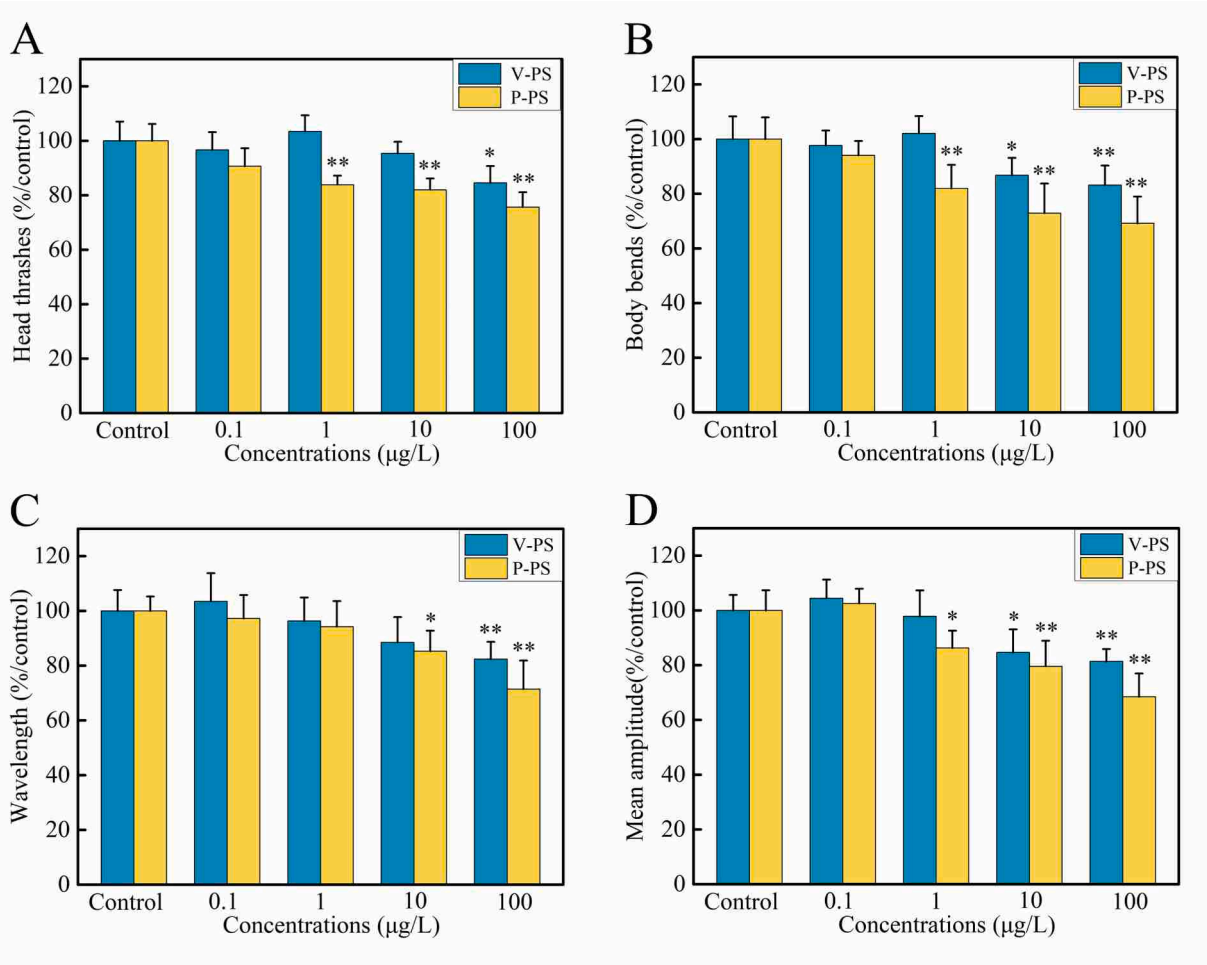

Fig. 2. Effect of V-PS and P-PS exposure on locomotion behavior in *C. elegans*. (A) Head thrashes. (B) Body bends. (C) Wavelength. (D) Mean amplitude. $^{*}p < 0.05$, $^{**}p < 0.01$. Data from three independent experiments are expressed as means ± standard error of the mean and percentage value compared to the control group. Statistical significance was analyzed using one-way ANOVA and Tukey's post hoc test.

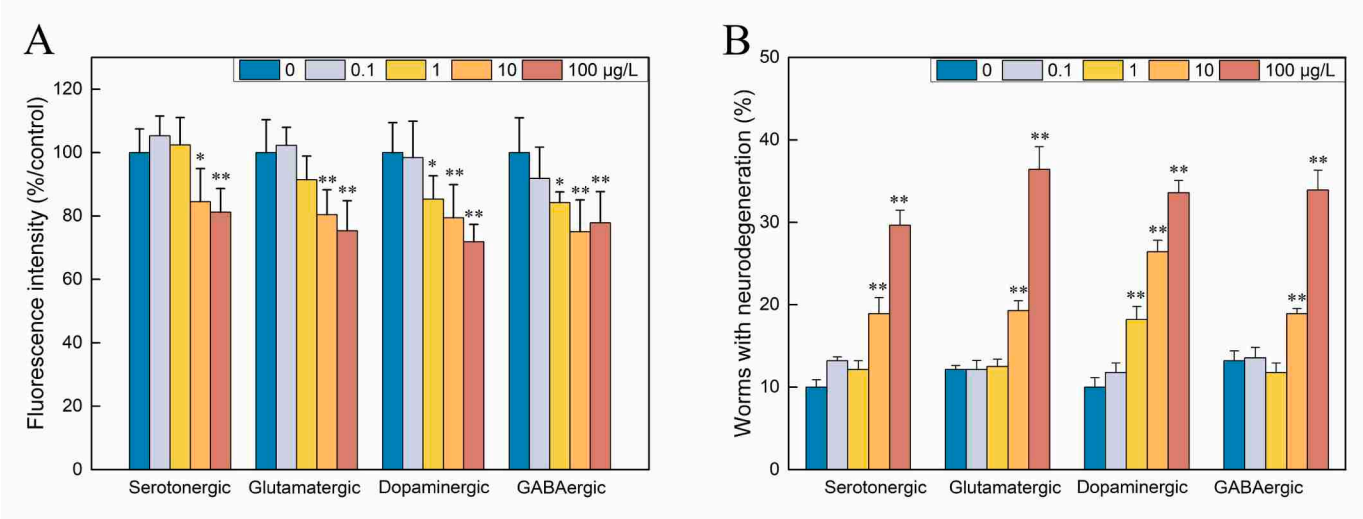

Fig. 3. Effect of P-PS exposure on neuronal neurodegeneration in *C. elegans*. (A) Comparison of relative fluorescence intensity of serotonergic and glutamatergic neurons. (B) Comparison of percentage of nematodes with neurodegeneration in transgenic nematodes. Three independent experiments and forty nematodes per treatment were conducted. Statistical significance was analyzed using one-way ANOVA and Tukey's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$.

on cell viability and lactate dehydrogenase activity compared to virgin PS (Yu et al., 2022a). Thus, exposure to V-PS and P-PS induced neurotoxicity in *C. elegans*, with P-PS showing more pronounced neurotoxicity.

3.3. Effect of P-PS exposure on neuronal development

Neuronal development is closely related to the locomotor behaviors of nematodes (Omura et al., 2012). Dopaminergic, serotonergic, glutamatergic, and GABAergic signaling are the most active neurobehavioral factors contributing to motor ability (Liu et al., 2020a). The endpoints of fluorescence intensity and neurodegeneration were examined to assess the nematode neuronal development in nematodes. As illustrated in Fig. 3A, exposure to 10–100 μg/L P-PS reduced the fluorescence intensity of serotonergic and glutamatergic neurons. Similarly, after exposure to 1–100 μg/L of P-PS, the fluorescence intensity of dopaminergic and GABAergic neurons was notably decreased compared with the control. Moreover, a significant decrease in neurodegeneration percentage of serotonergic, glutamatergic, dopaminergic, and GABAergic neurons was observed in transgenic *C. elegans* exposed to 10–100 μg/L P-PS (Fig. 3B). To reveal any possible correlation between locomotor behavior and neuronal development, Pearson correlation tests were conducted in nematodes. Locomotion behaviors correlated with damage to serotonergic, glutamatergic, dopaminergic, and GABAergic neurons (Fig. 5).

P-PS exposure damage serotonergic, glutamatergic, dopaminergic, and GABAergic neurons. These neurons have been proven to be critical in regulating the locomotion of nematodes (Sawin et al., 2000; Mano et al., 2007; Shen et al., 2016). Recently, exposure to carboxyl-modified PS resulted in adverse effects on locomotor behaviors and damage to the neuronal system of dopaminergic, serotonergic, glutamatergic, and GABAergic in nematodes (Yu et al., 2023). This neurotoxicity may be due to the injury of dopaminergic neurons in *C. elegans* exposed to the sulfonate modification PS (Qu and Wang, 2020). Similarly, exposure to PS induces neurotoxicity and the degradation of GABAergic neurons (Lei et al., 2018). Previous studies have demonstrated that decreased locomotor behaviors are associated with serotonergic, glutamatergic, dopaminergic, and GABAergic neuronal damage (Liu et al., 2020b; Wang et al., 2020b). Similarly, this neuronal damage is involved in the neurotoxic effects in nematodes after exposure to nanomaterials, nickel, and Fumonisin B1 (Li et al., 2017; Ijomone et al., 2020; Zhang et al., 2022a). Therefore, injury to dopaminergic, serotonergic, glutamatergic, and GABAergic neurons may adversely affects locomotor behaviors.

3.4. Effect of P-PS exposure on neurotransmitter content

Neurotransmission is a major components of the nervous system (Leung et al., 2008). Changes in neurotransmitter content are indicators of toxicity in the central nervous system (Yau et al., 2019). The levels of glutamate, serotonin, dopamine, and GABA were investigated to evaluate the function of the neuronal system in nematodes. Exposure to 0.1–1 μg/L P-PS did not alter neurotransmitter contents of serotonin and glutamate compared to the control (Fig. 4). However, exposure to 10–100 μg/L P-PS reduced serotonin and glutamate levels. Moreover, compared with the control group, neurotransmitter contents of dopamine and GABA were significantly decreased after exposure to P-PS at the dose of 1–100 μg/L. As shown in Fig. 5, locomotor behavior was correlated with the levels of glutamate, serotonin, dopamine, and GABA.

Neurotransmitters, including dopamine, serotonin, glutamate, and GABA, play a vital role in regulating locomotor behaviors and other motor functions in the nervous system of nematodes (Si and Song, 2018). In the present study, the glutamate, serotonin, dopamine, and GABA levels decreased after exposure to P-PS. Many studies have suggested that neurotoxicity caused by environmental pollutants may be associated with changes in neurotransmitter level. For example, a previous study revealed that exposure to thiocarbamate decreases dopamine content and causes neurotoxicity in nematodes (Caito et al., 2013). Analogously, fumonisin B1 reduces the levels of GABA and serotonin, which may be involved in neurotoxic effects (Zhang et al., 2022a). After exposure to graphene oxide (GO), the neurotransmitter contents of tryptophan, dopamine, tyrosine, GABA, and tyramine significantly decreased, indicating that GO poses a potential neurotoxic risk (Kim et al., 2020b). Moreover, another study suggested that alterations in the transmitter levels of dopamine and serotonin cause changes in locomotor behavior (Wang et al., 2019). Therefore, abnormal neurotransmission of glutamate, serotonin, dopamine, and GABA may be involved

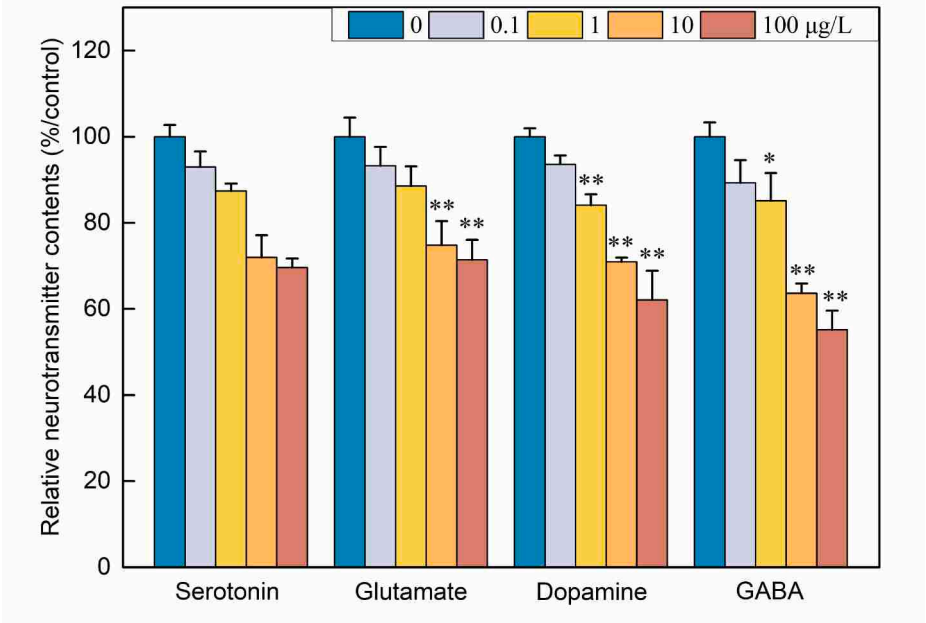

Fig. 4. Effect of P-PS exposure on neurotransmitter content in *C. elegans*. Data from three independent experiments are expressed as means ± standard error of the mean and percentage values compared to the control group. Statistical significance was analyzed using one-way ANOVA and Tukey's post hoc test. *$p < 0.05$, **$p < 0.01$.

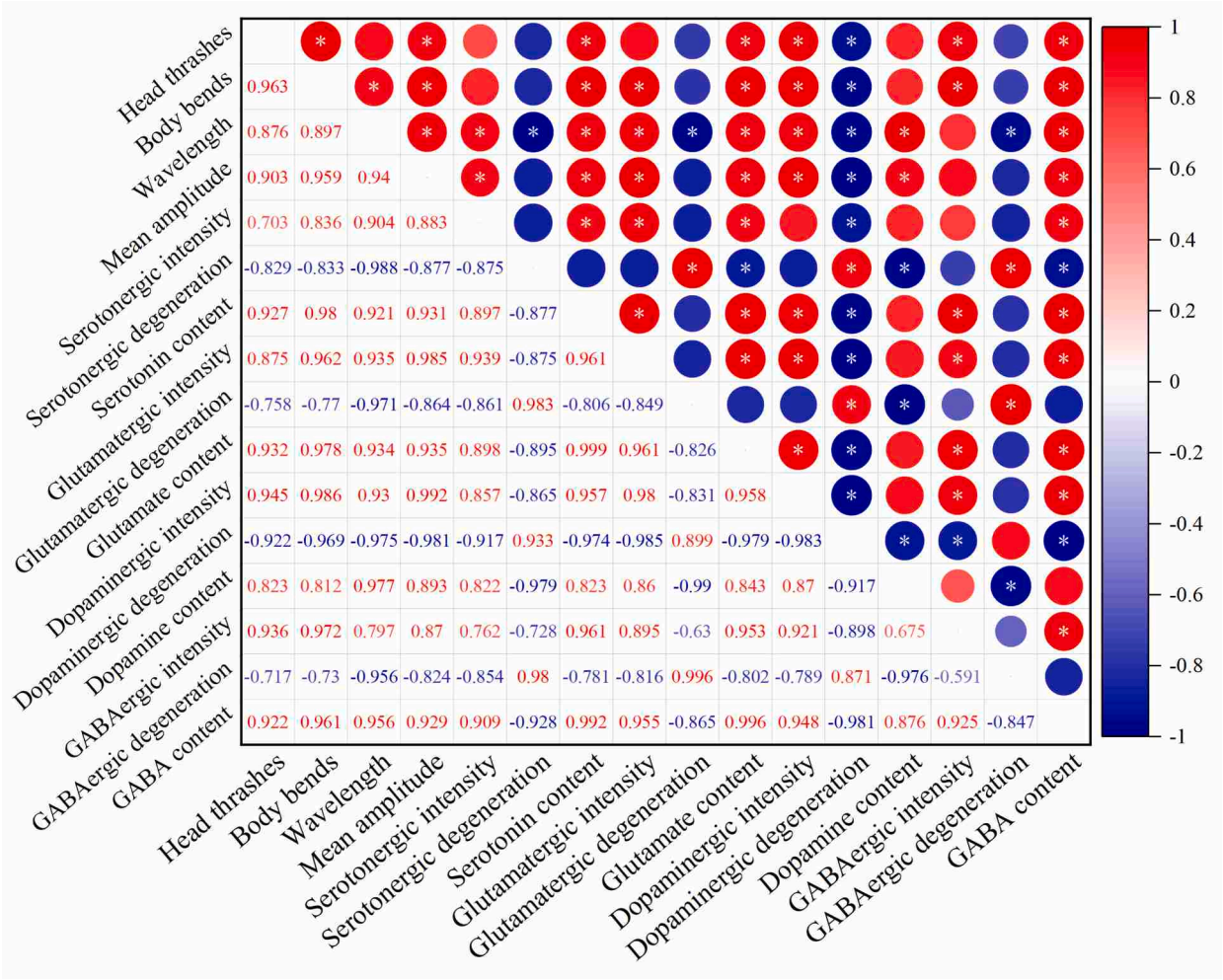

Fig. 5. Correlation between locomotion behaviors and neuronal development of nematodes exposed to P-PS. The correlation coefficients (*r* and *P* value) were obtained from Pearson correlation analysis. A relationship is represented by a red color, and a blue color indicates a negative relationship.

in the regulation of locomotor behaviors in nematodes.

3.5. Effect of P-PS exposure on neurotransmission-related gene expression

The expression of genes associated with serotonin, glutamate, dopamine, and GABA were measured further to clarify the effects of P-PS exposure on neurotransmission. As illustrated in Fig. 6, mod-*5*, *glr-1*, *dop-4*, *unc-30*, *unc-47*, and *unc-49* expression levels were not significantly altered. However, the expression of *cat-4* and *tph-1* increased compared with that in the control group. In contrast, the mRNA levels of *mod-1*, *ser-1*, *eat-4*, *glt-3*, *glt-7*, *mgl-1*, *dat-1*, *dop-1*, *dop-2*, *dop-3*, *unc-25*, and *unc-46* significantly decreased. Moreover, the mRNA levels of *dop-1*

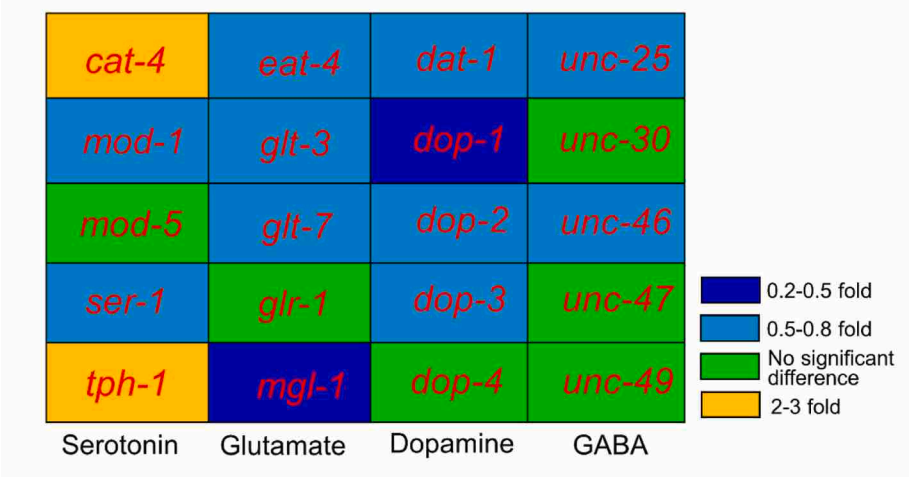

Fig. 6. Effect of P-PS exposure on neurotransmission-related gene expression in nematodes. Relative gene expressions were normalized to *tba-1* gene.

and *mgl-1* genes only 0.2–0.5 folds.

The *tph-1*, *mod-1*, *cat-4*, and *ser-1* genes are related to serotonin neurotransmission and mediate locomotion in nematodes (Li et al., 2013). Recently, the expression of *mod-1* and *ser-1* was decreased after exposure to PS-COOH (Yu et al., 2023). In addition, *glt-3*, *glt-7*, *mgl-1*, and *eat-4* are associated with glutamate neurotransmitters and influence synaptic function in nematodes (Mano et al., 2007). Exposure to lanthanum nitrate affects the expression of glutamate-related genes, indicating that glutamate-mediated neural signal transduction causes behavioral defects in *C. elegans* (Han et al., 2022). Moreover, *dop-1* affects the behaviors of nematodes, as it encodes a D1-like receptor (Chase et al., 2004). *dop-1*, *dop-2*, *dop-3,* and *dop-4* encode dopamine receptors, and *dat-1* modulates dopamine levels during dopaminergic neurotransmission (Nirenberg et al., 1996; Li et al., 2013). AgNPs significantly alter the expression of these genes and cause neurobehavior damage (Zhang et al., 2021b). The *unc-25* (encodes a glutamic acid decarboxylase) is specifically expressed in GABAergic neurons (Jin et al., 1999). The *unc-46* gene localizes to the vesicular GABA transporter in synaptic vesicles (Schuske et al., 2007). Recently, GABAergic impairment and the expression of mRNA levels *unc-46* decreased after exposure to fumonisin B1 for 48 h at a dose of 200 μg/mL (Zhang et al., 2022b). Taken together, the abnormal expression of genes related to serotonin, glutamate, dopamine, and GABA may be the main cause of changes in neurotransmitter content.

4. Conclusion

Adverse effects of V-PS and P-PS were observed by evaluating the locomotion behaviors in nematodes, and the neurotoxicity of P-PS was more severe than that of V-PS. Exposure to P-PS notably impaired the serotonergic, glutamatergic, dopaminergic, and GABAergic neuronal systems. Further investigations showed that the levels of neurotransmitter (serotonin, glutamate, dopamine, and GABA) and the expression of neurotransmission-related genes were significantly influenced in nematodes. Thus, the neurotoxicity induced by P-PS was due to the abnormal neurotransmission of serotonin, glutamate, dopamine, and GABA. These results provide new perspectives regarding the severe neurotoxicity of photoaged MPs. Because *C. elegans* plays a crucial role in terrestrial environments, the results of our study indicated that photoaged MPs at environmentally relevant concentrations can poses a potential hazard to soil ecosystems. These results also highlight the potential for the risk assessment of MPs in the environment, especially photoaged MPs.

CRediT authorship contribution statement

Yunjiang Yu: Conceptualization, Resources, Writing - Reviewing and Editing.

Shihui Tan: Investigation, Writing - Original draft preparation, Data Curation.

Dongli Xie: Investigation.

Hongyan Li: Validation.

Haibo Chen: Writing - Original draft preparation, Formal analysis, Writing - Reviewing and Editing.

Yao Dang: Writing - Reviewing and Editing.

Mingdeng Xiang: Validation.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165874.

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