Journal of Hazardous Materials 419 (2021) 126482

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research Paper

Chronic exposure to UV-aged microplastics induces neurotoxicity by affecting dopamine, glutamate, and serotonin neurotransmission in *Caenorhabditis elegans*

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ARTICLE INFO

Editor: Dr. R. Teresa

Keywords:
Aged microplastics
Caenorhabditis elegans
Neurotoxicity
Neuron damage
Neurotransmission

ABSTRACT

Microplastics are ubiquitous in all environments and exert toxic effects in various organisms. However, the neurotoxicity and underlying mechanisms of long-term exposure to MPs aged under UV radiation remain largely unclear. In this study, *Caenorhabditis elegans* was treated with 0.1–100 μg/L virgin and aged polystyrene microplastics (PS-MPs) for 10 d, with locomotion behavior, neuronal development, neurotransmitter content, and neurotransmission-related to gene expression as endpoints. Using locomotion behavior as an endpoint, chronic exposure to aged PS-MPs at low concentrations (1 μg/L) caused more severe neurotoxicity than that to virgin PS-MPs. In transgenic nematodes, exposure to 10–100 μg/L aged PS-MPs significantly influenced the fluorescence intensity and percentage of worms with neurodegeneration of dopaminergic, glutamatergic, and serotonergic neurons compared with control. Further investigations showed that the content of glutamate, serotonin, and dopamine was significantly influenced in nematodes chronically exposed to 100 μg/L of aged PS-MPs. Similarly, neurotransmission-related gene (e.g., *eat-4, dat-1*, and *tph-1*) expression was also altered in nematodes. These results indicate that aged PS-MPs exert neurotoxicity owing to their effects on dopamine, glutamate, and serotonin neurotransmission. This study provides insights into the underlying mechanisms and potential risks of PS-MPs after UV radiation.

1. Introduction

Microplastics (MPs) are plastic debris smaller than 5 mm that are distributed throughout various environmental media, such as seawater, freshwater, sediment, and soil (Wright and Kelly, 2017; Wu et al., 2019). The concentrations of MPs are normally ranged from ng/L to μg/L in the environment (Lenz et al., 2016). MPs persist in the environment as they are difficultly biodegradable, and, consequently, are ingested by various organisms and cause toxicity (Chang et al., 2020; Strungaru et al., 2019). For example, exposure to MPs causes neurotoxic effects in various organisms, including zebrafish (*Danio rerio*), wedge clams (*Donax trunculus*), earthworms (*Eisenia fetida*), and mice (*Mus musculus*) (Chen et al., 2020; Deng et al., 2018; Santos et al., 2020; Tlili et al., 2020). However, commercial microbeads are usually used as model MPs in these studies, which lack environmental relevance. Thus, the potential toxicity of MPs must be evaluated under environmentally relevant conditions.

UV irradiation is a key factor in natural aging and alters the physicochemical properties of MPs (Liu et al., 2019b). Studies have reported that UV treatment accelerates the aging process of MPs, and the characteristics and adsorption abilities of MPs are influenced by UV oxidation (Liu et al., 2019a; Xiong et al., 2020). Recently, the aging process by UV oxidation was shown to affect the structural properties and environmental behaviors of MPs (Liu et al., 2021). Some studies have investigated the toxicity of MPs aged under UV radiation in aquatic organisms (Fu et al., 2019; Wang et al., 2020a, 2020b; Zou et al., 2020). For example, MPs aged under UV light inhibit the growth of *Chlorella vulgaris* to a greater extent than virgin MPs (Wang et al., 2020a). Moreover, UV-photodegraded MPs cause more severe toxicity in

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https://doi.org/10.1016/j.jhazmat.2021.126482
Received 22 April 2021; Received in revised form 6 June 2021; Accepted 22 June 2021
Available online 24 June 2021

groupers (*Epinephelus moara*) than virgin MPs owing to the fragmentation and leaching of MPs (Wang et al., 2020b). However, the effect of UV irradiation on the toxicity of MPs in soil organisms is relatively unknown.

Caenorhabditis elegans is sensitive to various environmental toxicants and has the advantages of ease of cultivation, transparent body, and short life cycle (Leung et al., 2008). Additionally, *C. elegans* is an important model organism for studies in neurotoxicology owing to the complete description of its neuronal lineage and lack of a blood-brain barrier; thus, once environmental toxicants are ingested by nematodes, they quickly accumulate in the nervous system. To date, *C. elegans* has been widely used to assess the neurotoxicity of MPs, with endpoints of locomotion behavior, neuronal development, and gene expression (Lei et al., 2018a; Qu et al., 2019; Qu and Wang, 2019; Zhao et al., 2019). For example, short-term exposure to nanopolystyrene damages developing dopaminergic and gammaaminobutyric acid (GABA)ergic neurons in nematodes (Qu et al., 2019; Qu and Wang, 2019). These studies have focused on the neurotoxicity of short-term exposure to virgin MPs in *C. elegans*; however, the neurotoxicity and underlying mechanisms of long-term exposure to aged MPs under UV treatment remain largely unclear.

Considering that environmentally relevant concentration of MPs ranged from ng/L to μg/L (Al-Sid-Cheikh et al., 2018; Lenz et al., 2016), the exposure concentrations of 0.1–100 μg/L were selected in this study. Polystyrene microplastics (PS-MPs), which are abundant and ubiquitous in the environment, were selected to evaluate the neurotoxicity of MPs in nematodes. To evaluate the long-term neurotoxicity of virgin and aged PS-MPs, the frequency body bends and head thrashes was measured. The endpoint of neuronal development was investigated by examining the effects of PS-MPs on dopaminergic, glutamatergic, and serotonergic neurons in transgenic strains. Moreover, the contents of glutamate, serotonin, and dopamine were quantified in *C. elegans*. To investigate the underlying mechanisms, the expression of neurotransmission-related genes was analyzed. These results can be used to understand the long-term neurotoxicity and potential risks of aged PS-MPs.

2. Materials and methods

2.1. Aging and physicochemical characterization of PS-MPs

The aging process of PS-MPs referred to previous literature (Hüffer et al., 2018; Mao et al., 2020). Virgin PS-MPs were placed in quartz glass Petri dishes. The sample was continuously irradiated UV light (4 × 15 W bulbs, 254 nm) for 1 month and shaken every 24 h to ensure uniform exposure. After UV irradiation, the aged PS-MPs were washed with ultra-pure water for three times, dried at 60 °C in oven, and then stored in glass containers for analysis. Dynamic light scattering, scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and Fourier-transform infrared spectroscopy (FTIR) were employed to characterize the properties of the PS-MPs. The detailed method of PS-MPs characterization is provided in Text S1.

2.2. C. elegans preparation and exposure

The following strains, which acquired from the *Caenorhabditis* Genetics Center, were used in this study: wild-type (Bristol, N2), BZ555 [*dat-1p::GFP*], GR1366 [*tph-1::GFP + rol-6(su1006)*], and DA1240 [*eat-4::sGFP + lin-15(+)*]. The nematodes were lysed with bleaching solution, and eggs were collected followed by centrifugation. To obtain synchronized L4 larvae, the eggs were hatched on nematode growth medium plates containing *Escherichia coli* OP50 for 48 h (Williams, 1990). Additionally, PS-MPs were diluted in K medium for different concentrations of 0.1, 1, 10, and 100 μg/L. The method of chronic exposure (10-d exposure) was reported previously (Shen et al., 2009). L4 larvae were exposed to 0.1–100 μg/L virgin and aged PS-MPs for 10 days. During the exposure period, a mitotic inhibitor (5-FUDR) was added to the culture to suppress offspring production. After chronic exposure, nematodes were prepared for toxicity assessments. Three independent experiments were conducted.

2.3. Lethality and locomotion behaviors

The percentage of survival worms was used to evaluate lethality, and locomotion behaviors were analyzed by dissecting a microscope to count head thrashes and body bends (Chen et al., 2019). The details are provided in Text S2.

2.4. Evaluation of neurodegeneration

The dopaminergic, glutamatergic, and serotonergic neurons were examined in transgenic strains BZ555, DA1240, and GR1366, respectively (Li et al., 2017; Sammi et al., 2019). After chronic exposure, nematodes were anesthetized with 1 mM levamisole and placed on 2% agar pads. Fluorescent images were captured using a fluorescence microscope (Olympus BX51, Japan). Worms were scored for the absence or presence of any neuropathological alterations, including loss or breakage of dendrites, loss of soma, and shrunken soma. Forty worms per treatment were analyzed.

2.5. RNA extraction and qRT-PCR

Total RNA was isolated by TRIzol reagent, and cDNA was synthesized using a reverse transcription kit (Accurate Biotechnology, China). The gene expression was measured using the StepOnePlus System (Applied Biosystems). Three replicates were performed. The primers used are listed in Supplementary Material (Table S1).

2.6. Neurotransmitters content

After exposure, nematodes were washed and collected. The contents of dopamine, serotonin, and glutamate were determined using an ELISA kit (Meimian Institute, China). The details are provided in Text S3.

2.7. Statistical analyses

The data are expressed as mean ± S.E.M, and significance analysis was determined by ANOVA (SPSS 18, USA) with Tukey's test as a post-hoc test. $^{*}p < 0.05$ or $^{**}p < 0.01$.

3. Results and discussion

3.1. Characterization of virgin and aged PS-MPs

The average size of aged PS-MPs (1.014 ± 0.130 μm) was similar to that of virgin PS-MPs (1.005 ± 0.009 μm), as shown by dynamic light scattering. There were no changes in sizes of aged and virgin PS-MPs. SEM images showed that the virgin PS-MPs were spherical with a smooth surface, whereas the surface of aged PS-MPs was wrinkled and pitted (Fig. 1A and B), which is consistent with the observations of previous studies (Liu et al., 2019a, 2020). After UV photodegradation, the surface color of the PS-MPs changed from white to yellow, which suggested the presence of oxygen-containing moieties in aged PS-MPs (Lv et al., 2017). The polymeric composition was confirmed by FTIR spectroscopy. Compared with virgin PS-MPs, stronger and additional peaks characteristic of -OH and C═O were observed at wavenumbers of 3437.92 and 1726.26 cm^{-1} in aged PS-MPs (Fig. 1C). The carbonyl index (CI) is usually used to assess the degree of polymer aging, defined as the ratio of the intensity of carbonyl at 1726 cm^{-1} to the methylene peak at 1452 cm^{-1} (Liu et al., 2019c). The CI value of aged PS-MPs (0.442) was significantly higher than that of virgin PS-MPs (0.085), which suggests the oxidation of PS-MPs under UV treatment. The XPS

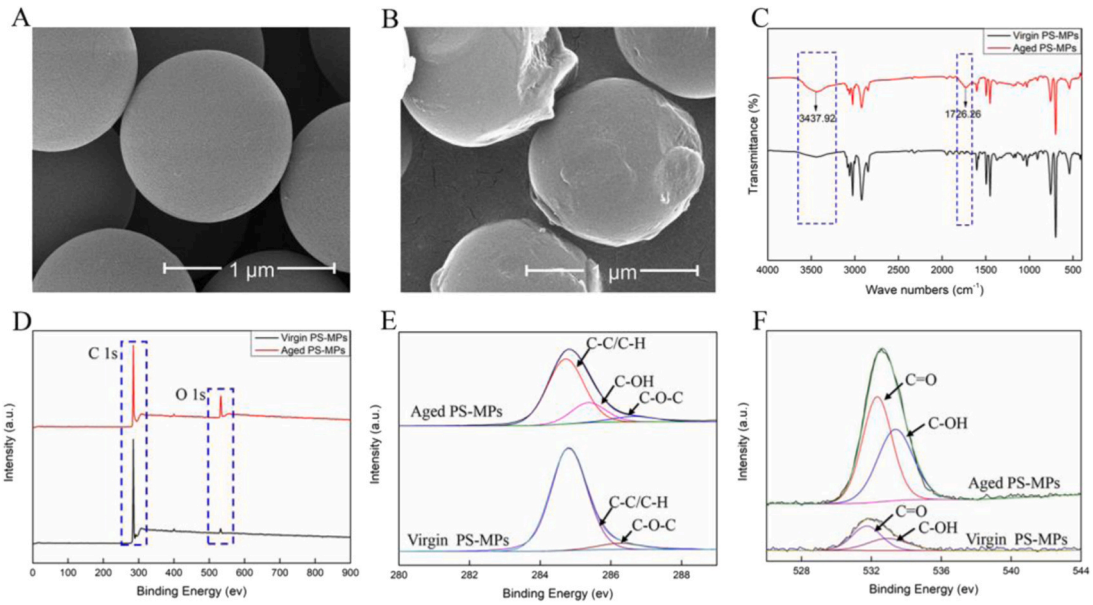

Fig. 1. Physicochemical characterization of pristine and aged PS-MPs. (A) SEM image of pristine PS-MPs. (B) SEM image of aged PS-MPs. (C) FTIR spectra. (D) XPS survey spectra. (E) C 1s XPS spectra. (F) O 1 XPS spectra.

survey spectra of the virgin and aged PS-MPs are shown in Fig. 1D. The O 1s proportion of aged PS-MPs and pristine samples increased from 3.03% to 12.85%, respectively, and the C 1s proportion of aged PS-MPs and pristine samples decreased from 96.97% to 87.15%, respectively. The oxygen-to-carbon (O/C) ratio of aged PS-MPs (0.147) was greater than that of pristine PS-MPs (0.031), which is consistent with the previous results (Liu et al., 2020; Wu et al., 2020). As shown as Fig. 1E and F, stronger peaks of C═O and -OH groups were detected on aged PS-MPs compared to virgin PS-MPs. In general, UV radiation accelerates the aging of PS-MPs, which have altered physical and chemical characteristics compared with pristine PS-MPs.

3.2. *Effect of virgin and aged PS-MPs exposure on locomotion behavior in nematodes*

In this study, exposure to virgin and aged PS-MPs (0.1–100 μg/L) did not significantly alter the survival of nematodes compared with control (Fig. S1). The locomotion behavior, measured by the frequency of body bends and head thrashes, was investigated to assess the neurotoxicity of virgin and aged PS-MPs in *C. elegans*. After chronic exposure to 10–100 μg/L virgin PS-MPs, the frequency of head thrashes was lower than that in control nematodes; moreover, chronic exposure to aged PS-MPs (1 μg/L) significantly decreased the frequency of head thrashes compared to control (Fig. 2A). Similarly, significantly fewer of body bends were observed in *C. elegans* exposed to 1 μg/L aged PS-MPs than in those exposed to virgin PS-MPs (Fig. 2B). Additionally, chronic exposure to leachate from 0.1 to 100 μg/L virgin and aged PS-MPs did not significantly influence locomotion behaviors in nematodes (Fig. S2). After chronic exposure, fluorescence-labeled PS-MPs at the dose of 1–100 μg/L were clearly visible in *C. elegans*, which suggests that the PS-MPs at the size of 1 μm were ingested and accumulated (Fig. S3).

A series of studies reported that the ingestion of MPs leads to toxic effects in nematodes (Lei et al., 2018b; Qu et al., 2018; Shang et al., 2020). Other studies suggested that PS-MPs also accumulate and result in toxicity in various organisms such as *Daphnia magna*, *Tigriopus japonicus*, and *Oryzias melastigma* (De Felice et al., 2019; Wang et al., 2019; Zhang et al., 2019). In this study, chronic exposure to virgin and aged PS-MPs significantly decreased the locomotion behavior of nematodes; however, leachate of virgin and aged PS-MPs did not cause a decrease in locomotion behavior. Thus, adverse effects on the locomotion behavior may be due to the ingestion of PS-MPs and not due to leachate of PS-MPs in *C. elegans*. Previous studies have shown that exposure to 10 μg/L or 1000 μg/L virgin PS-MPs (1 μm) significantly decreases locomotion behavior, which suggests the occurrence of neurotoxicity in nematodes (Lei et al., 2018a; Yu et al., 2020). Similarly, exposure to virgin nano-polystyrene at a dose above 10 μg/L causes neurotoxicity using the endpoint of locomotion behaviors (Qu et al., 2019). Therefore, chronic exposure to aged PS-MPs at an environmental concentration (1 μg/L) may cause more severe neurotoxicity than that to virgin PS-MPs in nematodes. In *E. moara*, PS-MPs aged under UV treatment inhibit growth and cause hepatic lipidosis (Wang et al., 2020b). Similarly, aged polyvinyl chloride (PVC) causes more severe developmental toxicity than virgin PVC in *Chlamydomonas reinhardtii* (Wang et al., 2020a); PVC aged under UVC radiation causes more severe toxicity than primary PVC in

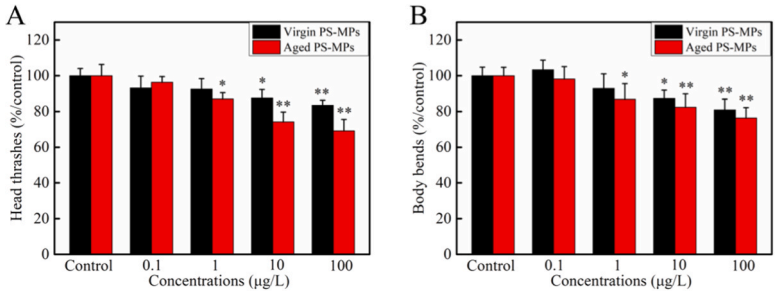

Fig. 2. Effect of virgin and aged PS-MPs exposure on locomotion behavior in *C. elegans*. (A) Head thrashes. (B) Body bends. $*p < 0.05$, $**p < 0.01$.

C. vulgaris (Fu et al., 2019). Thus, the long-term toxicity and risk assessment of MPs aged under UV oxidation require more research.

3.3. Effect of aged PS-MPs exposure on neuronal development in nematodes

The dopaminergic, glutamatergic, and serotonergic neurons mediate the locomotion behavior of *C. elegans*. Thus, the damage to these neurons by aged PS-MPs was investigated using the relative fluorescence intensity and percentage of worms with neurodegeneration in transgenic strains. Fig. 3A shows the morphological changes of these neurons in different transgenic strains. After chronic exposure to 0.1–1 μg/L aged PS-MPs, there was no significant change in the fluorescence intensity of dopaminergic and glutamatergic neurons; however, chronic exposure to 10–100 μg/L of aged PS-MPs significantly decreased the fluorescence intensity of dopaminergic and glutamatergic neurons (Fig. 3B). Additionally, chronic exposure to 1–100 μg/L aged PS-MPs influenced the fluorescence intensity of serotonergic neurons compared with control. Similarly, aged PS-MPs treatment (10–100 μg/L) significantly increased the percentage of worms with neurodegeneration of dopaminergic, glutamatergic, and serotonergic neurons relative to control (Fig. 3C).

These data indicate that exposure to aged PS-MPs cause significant damage to dopaminergic, glutamatergic, and serotonergic neurons. Exposure to PS-MPs results in significant damage to cholinergic and GABAergic neurons in *C. elegans* (Lei et al., 2018a). Similarly, exposure to nanopolystyrene influences the function of GABAergic motor neurons of nematodes (Qu et al., 2019). The neurotransmission systems are known to be phylogenetically conserved from nematodes to vertebrates, including dopaminergic, glutamatergic, and serotonergic systems (Leung et al., 2008). These neuronal systems play key roles in the regulation of locomotion (e.g., body bends and head thrashes), learning (e.g., chemotaxis and thermotaxis), and feeding (e.g., pharynx pumping) behavior in *C. elegans* (Ishita et al., 2020; Li et al., 2017; Mano et al., 2007; Sawin et al., 2000). In this study, chronic exposure to aged PS-MPs significantly decreased the locomotion behavior of *C. elegans*. After exposure to nanopolystyrene, the alterations in locomotion behaviors may be ascribed to dopaminergic neuron damage (Qu and Wang, 2019). Moreover, the locomotion behavior deficits induced by graphene-based nanomaterials may be associated with damage to dopaminergic and glutamatergic neurons (Li et al., 2017). Another study suggested that impaired serotonergic neurotransmission is involved in the defects in locomotion behaviors in *C. elegans* chronically exposed to Ag-NPs (Piechulek and von Mikecz, 2018). Additionally, exposure to perfluorooctane sulfonate causes neurotoxicity and neurodegeneration in dopaminergic, serotonergic, GABAergic, and cholinergic neurons (Sammi et al., 2019). Therefore, damage to dopaminergic, glutamatergic, and serotonergic neurons may participate in the neurotoxicity caused by aged PS-MPs in nematodes.

3.4. Effect of aged PS-MPs exposure on neurotransmitter content in nematodes

As neurotransmitter systems were impaired by aged PS-MPs, we next investigated their effects on the content of glutamate, serotonin, and dopamine. After chronic exposure, neurotransmitter contents were quantified in *C. elegans* exposed to 100 μg/L aged PS-MPs. Chronic exposure to aged PS-MPs significantly decreased the levels of glutamate and dopamine compared with control (Fig. 4). However, serotonin content was significantly increased in *C. elegans*.

In nematodes, neurotransmission is mediated by neurotransmitter content in neuronal circuits. Neurotransmitter systems are the main components of the nervous system in nematodes (Leung et al., 2008); these neurotransmitters (serotonin, dopamine, and glutamate) play an important role in the mediation of locomotion behavior in nematodes (Jorgensen, 2005; Sawin et al., 2000; Segalat et al., 1995). In this study, chronic exposure to aged PS-MPs significantly altered the content of neurotransmitters, including glutamate, serotonin, and dopamine. A previous study reported a significant decrease in dopamine content in

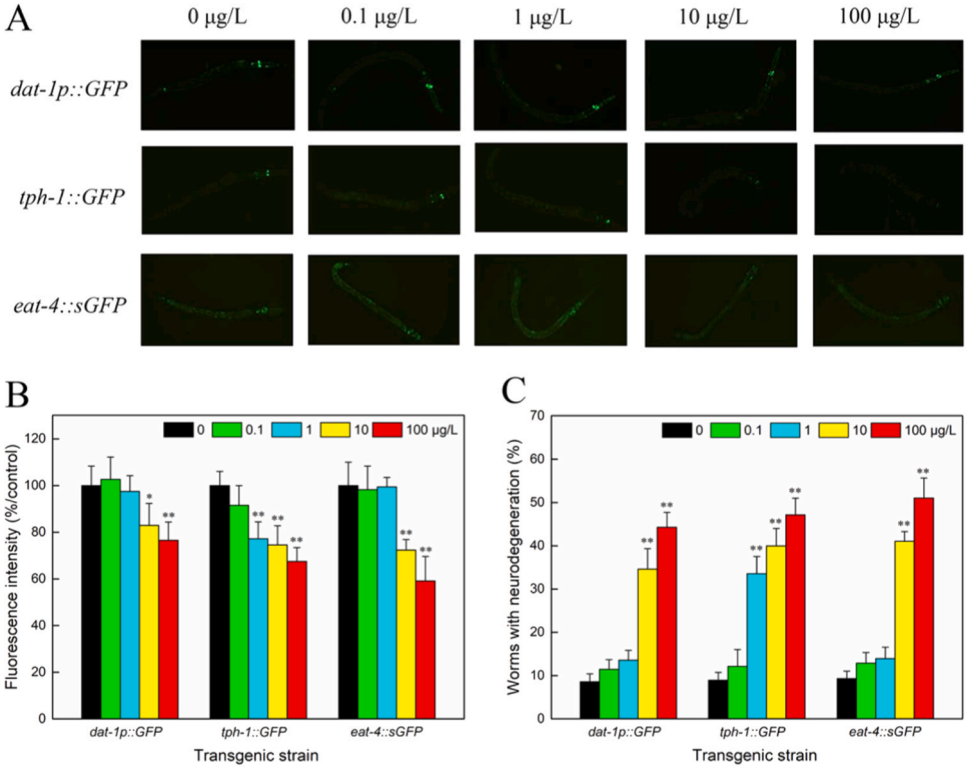

Fig. 3. Effect of aged PS-MPs exposure on neuronal neurodegeneration in *C. elegans*. (A) Representative fluorescence images in transgenic nematodes. (B) Comparison of relative fluorescent intensities in transgenic nematodes. (C) Comparison of percentage of worms with neurodegeneration. $*p < 0.05$, $**p < 0.01$.

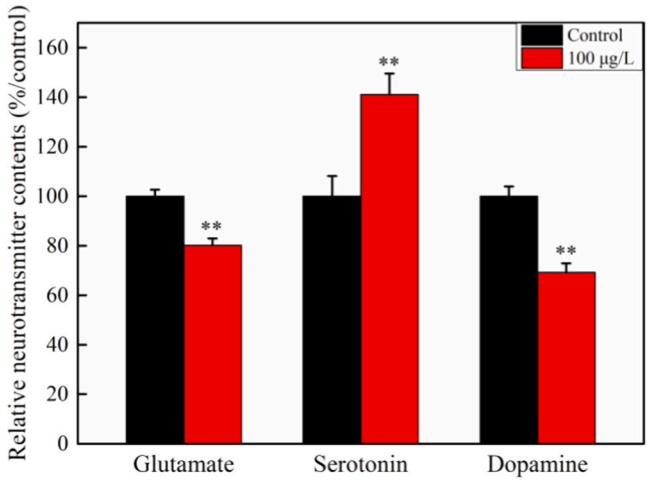

Fig. 4. Effect of aged PS-MPs exposure on neurotransmitter content in *C. elegans*. $^{**}p < 0.01$.

nematodes after Pb exposure; thus, dopamine may participate in the neurotoxicity of Pb (Akinyemi et al., 2019). Exposure to N-dipropylthiocarbamate, N-diethylthiocarbamate, and molinate also decrease dopamine content and cause neurotoxicity in *C. elegans* (Caito et al., 2013). Similar correlations between dopamine content and behavior have been observed in monocrotophos-exposed nematodes (Ali and Rajini, 2012). Recently, the neurotoxicity of graphene oxide in nematodes has been shown to be related to a decrease in the content of neurotransmitters, such as tyrosine, tryptophan, dopamine, and tyramine (Kim et al., 2020). The data suggest that chronic exposure to aged PS-MPs damages the glutamate, serotonin, and dopamine neurotransmitter systems in nematodes. These neurotransmitters content may mediate locomotion behaviors in *C. elegans*.

3.5. Effect of aged PS-MPs exposure on neurotransmission-related gene expression in nematodes

To determine the role of neurotransmission in neurotoxicity, the expression of genes required for glutamate, serotonin, and dopamine neurotransmission was investigated in *C. elegans* chronically exposed to 100 μg/L aged PS-MPs (Table S2). After chronic exposure to 100 μg/L aged PS-MPs, significant decreases in the expression of *eat-4, glt-3*, and *glt-7* were observed in nematodes (Fig. 5). Similarly, chronic exposure to aged PS-MPs significantly decreased the expression of *dat-1* and *dop-1*, which are required for dopamine neurotransmission in nematodes. Regarding the genes required for serotonin neurotransmission, the mRNA levels of *mod-1* and *tph-1* were significantly increased in nematodes.

These results indicate that chronic exposure to aged PS-MPs influences the expression of *dop-1*, *dat-1*, *eat-4, glt-3, glt-7*, *mod-1*, and *tph-1* in nematodes. In *C. elegans*, *dop-1* gene encodes a D1-like receptor that affects the behavior of nematodes (Chase et al., 2004). The *dat-1* gene, which encodes a dopamine transporter, regulates dopamine levels during dopaminergic neurotransmission (Nirenberg et al., 1996). Exposure to Pb results in dopaminergic dysfunction by altering the expression of *dat-1*, which is involved in neurological disorders in nematodes (Akinyemi et al., 2019). Similarly, the expression of *dop-3* and *dat-1* is significantly influenced in nematodes exposed to nonylphenol ethoxylate (NP-9) (De la Parra-Guerra et al., 2020). Recently, exposure to arsenic has been demonstrated to induce behavioral disorders and inhibit the expression of *cat-2* and *bas-1*, which are related to dopamine neurotransmitters (Zhang et al., 2020). Additionally, *tph-1* gene, which encodes a tryptophan hydroxylase, plays an important role in serotonin synthesis in *C. elegans* (Nuttley et al., 2002). The gene *mod-1,* which encodes a serotonin transporter, modulates locomotion defects in

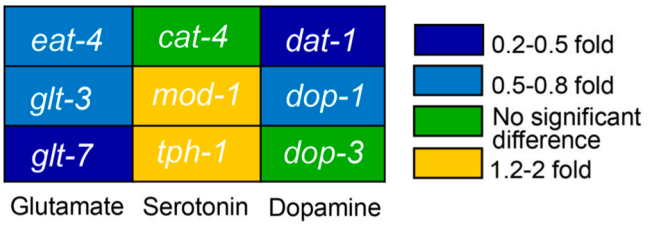

Fig. 5. Effect of aged PS-MPs exposure on neurotransmission-related gene expression in nematodes. Relative gene expressions were normalized to *tba-1* gene.

C. elegans (Ranganathan et al., 2000). A similar study showed that the expression of *tph-1* and *mod-1* is significantly decreased by nonylphenol; thus, *tph-1* and *mod-5* expression is associated with the neurotoxicity of nonylphenol (Cao et al., 2019). With regard to glutamate neurotransmission-related genes (*eat-4, glt-3*, and *glt-7* genes), which encode glutamate transporters, influence the synaptic function and behavior of *C. elegans* (Mano et al., 2007). A previous study indicated that exposure to NP-9 causes neurotoxicity and influences the mRNA level of *eat-4* in nematodes (De la Parra-Guerra et al., 2020). Moreover, long-term exposure to.

MPA-capped CdTe quantum dots influences glutamate, serotonin, and dopamine neurotransmission via alterations in the mRNA levels of *dop-1*, *dat-1*, *eat-4, glt-3*, *glt-7*, and *mod-1*, which are linked to neurological deficiencies in *C. elegans* (Wu et al., 2015). Another study showed that the expression of neurotransmitter transporters, such as those for serotonin, dopamine, and glutamate, may mediate memory formation in nematodes (Tellez et al., 2012). Overall, chronic exposure to aged PS-MPs influenced glutamate, serotonin, and dopamine neurotransmission, and neurotransmission-related genes (e.g., *eat-4, dat-1*, and *tph-1*) may be related with the neurotoxicity of aged PS-MPs in nematodes.

4. Conclusion

Chronic exposure to aged PS-MPs at low concentrations caused more severe neurotoxicity than that to virgin PS-MPs. Damaging effects of aged PS-MPs on dopaminergic, glutamatergic, and serotonergic neuronal systems were observed in nematodes. Further investigations showed that the content of glutamate, serotonin, and dopamine was significantly altered in nematodes exposed to aged PS-MPs. Moreover, the expression of neurotransmission-related genes (e.g., *eat-4, dat-1*, and *tph-1*) was altered in *C. elegans*. These results indicate that the neurotransmission of dopamine, glutamate, and serotonin is involved in the neurotoxicity induced by aged PS-MPs in nematodes.

CRediT authorship contribution statement

Haibo Chen: Writing - original draft, Investigation, Data curation, Formal analysis, Writing - review & editing. **Xin Hua:** Investigation, Data curation. **Yue Yang:** Investigation. **Chen Wang:** Writing - review & editing. **Lide Jin:** Writing - review & editing. **Chenyin Dong:** Writing - review & editing. **Zhaofeng Chang:** Validation. **Ping Ding:** Visualization. **Mingdeng Xiang:** Validation. **Hui Li:** Conceptualization, Methodology, Writing - review & editing. **Yunjiang Yu:** Conceptualization, Resources, Writing - review & editing.

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.

Acknowledgements

Financial support was received from the National Natural Science Foundation of China (21876059), Key Program of National Natural Science Foundation of China (41931298), and National Key R&D Program of China (2019YFC1803403).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126482.

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