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RESEARCH

$PM_{2.5}$-Bound Polycyclic Aromatic Hydrocarbons and Elements in Different Environments in Southern Taiwan and Their Potential Toxic Effects on *Caenorhabditis elegans* Models

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Abstract

Fine particulate matter ($PM_{2.5}$) has been demonstrated to disrupt developmental, neurological, and reproductive functions in animal models including *Caenorhabditis elegans* (*C. elegans*). In the present study, $PM_{2.5}$-bound polycyclic aromatic hydrocarbons (PAHs) and elements were studied in urban, traffic-related air pollution (TRAP), and industrial areas to examine whether $PM_{2.5}$-bound PAHs or elements caused toxic effects in *C. elegans*. $PM_{2.5}$ collected from these areas (25.9, 41.7, and 28.9 μg m^{-3} in urban, TRAP, and industrial areas, respectively) caused significant toxic effects on development (body growth, 85.8%–95.9% reduction), reproduction (brood size, 52.5%–85.8% reduction), and neurology (locomotion, 49.7%–84.3% and 72.3%–94.5% declines in head thrashing and body bending frequencies, respectively), along with changes in the expression of genes regulating antioxidant mechanisms. Despite its lowest $PM_{2.5}$ concentration, the industrial site induced stronger reproductive and locomotor toxicity, accompanied by heightened antioxidant gene expression. To further elucidate the role of $PM_{2.5}$ composition in these effects, concentrations of PAHs, PAH-BaPeq, and elements were quantified in the nematode exposure models. Our results showed that $PM_{2.5}$-bound Σ_{16}PAH (0.501–0.658 ng m^{-3}) and PAH-BaPeq (0.0497–0.0698 ng BaPeq m^{-3}) levels did not differ significantly among the three areas; however, toxic metal concentrations—particularly lead and copper—were substantially higher in the industrial samples than in those from the urban and TRAP environments. The reproductive and locomotor toxicity, along with increased superoxide dismutase (SOD)-related reactive oxygen species (ROS) production observed in nematodes exposed to low-dose industrial $PM_{2.5}$, is likely more strongly associated with elevated

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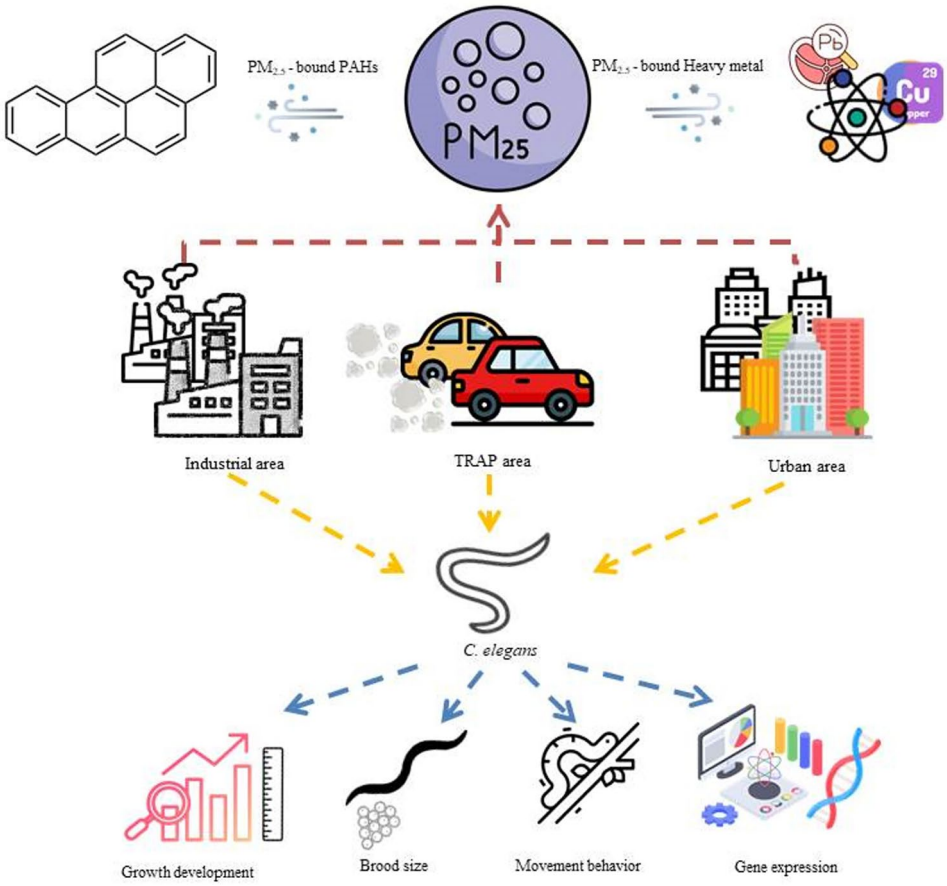
levels of $PM_{2.5}$-bound toxic metals than with exposures from urban or TRAP regions. Compared with $PM_{2.5}$-bound PAHs, $PM_{2.5}$-bound lead or copper might contribute more substantially in triggering the toxic responses of $PM_{2.5}$ in nematodes, particularly on oxidative stress, reproduction, and locomotion, according to the findings of the current study.

Graphical Abstract



Keywords $PM_{2.5}$ · Polycyclic aromatic hydrocarbons (PAHs) · Lead (Pb) · *Caenorhabditis elegans* · Antioxidant gene · Health effects

1 Introduction

Particulate matter (PM), consisting of intricate blend of adsorbed metals and organic pollutants which poses one of the greatest environmental health issues. Particularly smaller size of PM ($PM_{2.5}$-aerodynamic diameter less than 2.5 micron), owning to its smaller size can travel deep into the respiratory or bronchial tracts, reach the lung alveoli, enter the bloodstream, and potentially cause serious health effects (EPA 2024). Various inorganic elements (e.g., metals) and organic chemicals (e.g., semi-volatile organic hydrocarbons like polycyclic aromatic hydrocarbons (PAHs)) adhered to $PM_{2.5}$ particles' surfaces can disrupt normal physiological activity of organs and tissues through oxidative stress (Wang et al. 2021; Hou et al. 2024). After $PM_{2.5}$ exposure, such effects increase the risks related to lung and cardiovascular diseases (Hou et al. 2024), type II diabetes (Liu et al. 2023a), metabolic disorders (Li et al. 2023), reproductive toxicity (Wang et al. 2021), cerebrovascular diseases (Chen et al. 2024), and neurological impairments (Chuang et al. 2020), and are correlated with increased in mortality and morbidity (Alexeeff et al. 2023; EPA 2024). According to our previous reports, $PM_{2.5}$ triggers adverse effects in in vitro (Tseng et al. 2024), in vivo (Chung et al. 2019, 2020; Lu et al. 2023), and epidemiological studies (Chao et al. 2018; Kao et al. 2019; Tseng et al. 2022). Traffic conductors and school-age children exposed to $PM_{2.5}$ in TRAP areas had higher serum levels of tumor necrosis factor-α (TNFα) compared to office policemen and school-age children in low-$PM_{2.5}$-pollution areas (Chao et al. 2018; Tseng et al. 2022). TRAP-derived

$PM_{2.5}$ disrupts reproduction, locomotion, longevity, and specific gene expression in nematode models (Chung et al. 2020; Lu et al. 2023). The human lung epithelial cells (A549 cells) with exposure to $PM_{2.5}$ led to a clear increase in cytotoxicity, a reduction in cell growth, and delayed wound closure or impaired tissue repair potential in our cellular study (Tseng et al. 2024).

Fine particles could be directly released into ambient or specific microenvironments (Chao et al. 2016). In southern Taiwan, $PM_{2.5}$ levels, particularly during the spring and winter, are affected by multiple factors, including the Northeast Monsoon, Asian Dust storms, long-range transport from mainland China and northern parts of Taiwan (Liang et al. 2015; Ting et al. 2023), natural events (Kollanus et al. 2017), and local emissions such as vehicular exhaust (Chung et al. 2020) and the combustion from the industrial activities (Shen et al. 2020). $PM_{2.5}$ particles possess a high surface area-to-mass ratio and remain suspended or resuspended in the atmosphere for prolonged periods, allowing them to accumulate harmful substances and adsorb a variety of toxic chemicals, including PAHs and heavy metals (Kim et al. 2015). In Taiwan, several studies have examined the seasonal variation, source apportionment, emission patterns of $PM_{2.5}$-bound PAHs in industrial, TRAP, and urban environments, to highlight their health impacts such as cancer risks, inflammation, and asthma (Chen et al. 2016b; Zhu et al. 2019; Hsu et al. 2020; Pan et al. 2024). Heavy metals commonly found in $PM_{2.5}$ have also been investigated in different ambient air conditions (Fang et al. 1999, 2017; Lin et al. 2016), and are associated with several toxic effects, including exacerbation of allergic airway inflammations (e.g., asthma) (Tu et al. 2022), damaging lung macrophages (Hou et al. 2024), negatively impacting the pulmonary indices such as forced volume capacity (FVC) in school-age children (Huang et al. 2018), induction of reactive oxidative stress (Tu et al. 2022), and increasing the cancer risk (Lin et al. 2016). $PM_{2.5}$-bound reactive oxygen species (ROS), such as PAHs and certain heavy metals, can induce cytotoxicity, inflammation, DNA damage, oxidative stress, and further can activate inflammatory responses in severe respiratory diseases like chronic obstructive pulmonary disease (COPD) (Hamad et al. 2016; Yang et al. 2016; Chao et al. 2018; Wang and Liu 2023).

An in vivo (non-mammalian) model of *Caenorhabditis elegans (C. elegans)* is cost-effective, time-saving, and easy to culture, operate, and maintain in the laboratory without concerns regarding animal ethics (Lu et al. 2023). With a fully sequenced genome, a short life span, and a transparent body structure, *C. elegans* has turn out to be an ideal in vivo model for studying environmental toxicology (Chung et al. 2019; Chuang et al. 2020; Lu et al. 2023; Yang et al. 2023) and nanotoxicology (Tsai et al. 2021; Huang et al. 2023; Lu et al. 2023). Previous studies, including our previous ones, have observed that the nematodes' exposure to $PM_{2.5}$ can cause delayed development, shortened lifespan, reduced heat tolerance, altered gene expression, oxidative damage, an enhanced antioxidant capacity, and lipid metabolism disorders (Chung et al. 2019; Cáceres Quijano et al. 2022; Lu et al. 2023; Zhang et al. 2023a). This study aimed to clarify the different compositions of PAHs and elements bound to $PM_{2.5}$ found in industrial, TRAP, and urban areas, and to evaluate their detrimental effects on the *C. elegans* models. Furthermore, we investigate the adverse effects on multiple toxicological endpoints after *C. elegans* are exposed to $PM_{2.5}$, including growth rate, reproductive capacity, locomotion behaviors, and oxidative stress responses.

2 Materials and Methods

2.1 $PM_{2.5}$ Sampling in the Selected Environments

The sampling programs of $PM_{2.5}$ and pretreatment procedure of the air samples were conducted as described previously (Chung et al. 2020). To eliminate residual organic pollutants, the glass-fiber $PM_{2.5}$ filters were conditioned at the temperature of 600 °C for 2 h prior to the sampling. The $PM_{2.5}$ filter samples were then placed in an electronic desiccator for 24 h before and after on-site $PM_{2.5}$ sampling. $PM_{2.5}$ concentrations were determined by the gravimetric method, based on the difference in filter weights before and after sampling, measured with a six-digit balance accurate to 0.100 μg. The airborne samplers of SIBATA HV-1000R (Sibata, Japan) were investigated in the range of the flow rates from 800 to 1000 L min^{-1} for 24-h sampling, following the Taiwanese EPA NIEA A205.11C or US EPA Reference Method TO9A, to collect $PM_{2.5}$ from industrial (Xiaogang District, Kaohsiung City), TRAP (Pingtung City, Pingtung County), and urban (Chaozhou Township, Pingtung County) areas. After air sampling, the filters were subsequently transported in sealed, contaminant-free containers to our laboratory located at the National Pingtung University of Science and Technology and kept at low temperature of − 20 °C in a refrigerator to minimize volatilization losses and prevent cross-contamination until chemical analysis. The sampling program was carried out once a month between October 2022 and March 2023.

Airborne $PM_{2.5}$ concentrations were initially measured in μg m^{-3} and subsequently converted into equivalent aqueous concentrations (mg L^{-1}) for use in *C. elegans* in vivo exposure experiments. The $PM_{2.5}$ filters underwent extraction with (DCM) assisted by sonication as part of the pretreatment process. The extract was then cleaned by passing 15 mL dichloromethane through an acid–silica column (Chung et al. 2020). Finally, this extract after the cleanup was concentrated to 1 mL in a pre-cleaned vial, further

reduced to near-dryness using a gentle flow of high-purity nitrogen gas and then redissolved in 1.00 mL of HPLC-grade DMSO prior to assessing toxic effects in *C. elegans* models.

2.2 *Chemical Characterization of $PM_{2.5}$*

2.2.1 Polycyclic Aromatic Hydrocarbons (PAHs) Analysis

Extraction of $PM_{2.5}$-bound PAH samples were processed via microwave extraction with a mixed solution of dichloromethane, acetone, and n-hexane (1:2:2 v/v/v) for 24 h following Kung et al.'s method (Kung et al. 2024) (The detailed information was listed in the supplementary materials). The internal standards of PAHs (acenaphthene-d_{10}, chrysene-d_{12}, naphthalene-d_8, perylene-d_{12}, and phenanthrene-d_{10}) were spiked into the mixed solution prior to extraction. The extracts underwent a series of the multi-column clean-up procedures, including passed through a multi-column silica gel column, and then reconcentrated to 0.500 ml in a vial. Sixteen PAHs were analyzed using a triple quadrupole gas chromatograph/mass detector (Agilent Technologies, CA, USA) with a 30-m DB-17MS column. Helium was the carrier gas (1.2 mL min^{-1}, 280 °C injector), and the oven program was 60.0 °C (0.500 min) → 265 °C at 15.0 °C min^{-1} → 305 °C at 5.00 °C min^{-1} (10.0 min hold). Quality assurance and control (QA/QC) in this study was also listed in the supplemental materials.

The 16 PAH species were subsequently sorted into three groups based on molecular weights: low (2- and 3-ringed PAHs: naphthalene (Nap), acenaphthylene (AcPy), acenaphthene (AcP), fluorene (Flu), phenanthrene (PA), anthracene (Ant)), middle (4-ringed PAHs: fluoranthene (FL), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (CHR)), and high (5- and 6-ringed PAHs: benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno(123-cd)pyrene (IND), dibenz[a,h]anthracene (DBA), and benzo[g,h,i]perylene (BghiP)). The carcinogenic potencies of the 16 PAHs in the investigated airborne samples were determined based on the BaP equivalency levels (BaPeq) from the toxic equivalency factor (TEF) of the PAHs.

2.2.2 Elemental Analysis

$PM_{2.5}$-bound elements [strontium (Sr), chromium (Cr), arsenic (As), rubidium (Rb), manganese (Mn), cadmium (Cd), palladium (Pd), titanium (Ti), barium (Ba), lead (Pb), copper (Cu), platinum (Pt), nickel (Ni), silver (Ag), iron (Fe), ruthenium (Ru), beryllium (Be), tin (Sn), potassium (K), antimony (Sb), magnesium (Mg), vanadium (V), zinc (Zn), sodium (Na), calcium (Ca), mercury (Hg), selenium (Se), rhodium (Rh), lithium (Li), molybdenum (Mo), thallium (Tl), aluminium (Al), and cobalt (Co)] were quantified using the standard NIEA W105 method established by the Taiwanese Ministry of Environment. $PM_{2.5}$ filters were treated with concentrated nitric and hydrochloric acids (1:3 volume-to-volume ratio) in a microwave digestion process. The solvent of metals was analyzed using by a high-resolution inductively coupled plasma mass spectrometry (ICP-MS, ULTIMA 2000, Jobin Yvon Horiba, NJ, USA). A standard solution of metal elements was used for an automatic wavelength search to select the most suitable wavelength for elemental analysis. At least five concentrations of the elemental standards, within ranges based on the analytical concentrations from the samples, were generated to test the calibration curves, ensuring the absolute errors are within 10%. Recovery rates of the selected elements in this study were checked and confirmed every 10 samples using additional spiked standards to ensure that the recovery rates were within 80%–120%. Blank sample tests showed the analytical levels to be below MDLs and confirmed the purity of the samples.

2.3 Maintenance and Toxic Tests of C. elegans

The assays for survival, growth, reproduction, and locomotion under contaminant exposure were performed according to our previous study with minor changes (Chung et al. 2019, 2020; Liu et al. 2023a, b). The detailed description was performed in the supplemental materials.

2.3.1 Maintenance, Age Synchronization, and Exposure of the nematodes

The nematodes (wild-type N2 *C. elegans*) were maintained on nematode growth medium (NGM) agar plates seeded with OP50 *E. coli* at 22 °C. Age-synchronized L1 larvae were obtained by bleaching gravid adults with NaOCl/KOH, leaving only eggs. After development, L3/young L4 larvae were washed off plates with K-medium (2.36 g L^{-1} KCl and 3 g L^{-1} NaCl) and centrifuged (4 min at 2500 × g). The synchronized worms were diluted in K-medium for experiments. For age synchronization, gravid worms were bleached to obtain synchronized larvae of L1, which were grown up to the larvae of L3/L4. Worms (~ 200 per well) were placed in 12-well plates with 1-mL K-medium containing $PM_{2.5}$ at designated concentrations and exposed for 24 h at 22 °C without food. Toxicity endpoints included survival, reproduction, and locomotion. Each treatment was tested in triplicate and repeated three times.

2.3.2 Nematodes' Assay of Growth, Reproduction, and Locomotion

Nematodes exposed to $PM_{2.5}$ for 24 h were moved to OP50-seeded NGM plates at 48-h incubation. Body lengths at

the L4 stage were measured from micrographs (Olympus SZX10) using ImageJ. Twenty worms per concentration were measured in triplicate assays for the growth measurement. For reproductive assay, $PM_{2.5}$ extracts were obtained by DCM sonication, cleaned with an acid–silica column, concentrated under N_2, and redissolved in DMSO. After exposure, 20 synchronized L3/L4 worms were individually moved to OP50-seeded NGM plates. Adults were transferred to fresh plates each 2 days, and progeny were counted at the L4 stage. Total brood size per worm was calculated. For locomotion assay, twenty synchronized L3/L4 worms were exposed to $PM_{2.5}$ for 24 h. Locomotor activity was assessed by counting head thrashes (1 min) and body bends (20 s) on unseeded NGM plates with M9 buffer. Assays were conducted in triplicate for each dose.

2.4 Assays of Quantitative Real-Time PCR (qRT-PCR) in nematodes

Approximately 500–1000 synchronized L3 to early L4 *C. elegans* were placed in 6-cm Petri dishes and exposed to varying doses of $PM_{2.5}$ for 24 h. Following nematode collection, extraction of total RNA in the nematode's sample was tested with TRIzol Reagent purchased from Gibco, Life Technologies (Carlsbad, CA, USA). cDNA synthesis was performed with 1000 ng of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Gibco, Life Technologies), following the manufacturer's instructions. Real-time PCR amplification was carried out using 50 ng of cDNA and SYBR Green PCR Master Mix (Gibco, Life Technologies, Carlsbad, CA, USA) on an Applied Biosystems PRISM 7500 Fast Real-Time PCR System. The amplification program comprised an initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Following amplification, the cycle threshold (Ct) values were collected for subsequent analysis. Relative mRNA expression of antioxidant-related genes was calculated between untreated control and treatment groups using the $2^{-\Delta\Delta Ct}$ method. In *C. elegans*, the target genes included catalase (CTL-1, CTL-2, CTL-3), superoxide dismutases (SOD-1, SOD-2, SOD-3, SOD-4, SOD-5), and ACTIN as the reference gene. The list of primer sequences was shown in Table 1.

2.5 Statistical Analysis

Data analysis was conducted by SAS 9.3 (SAS Institute, Cary, NC, USA). The Shapiro–Wilk test indicated that locomotion, reproduction, and body length measurements were not normally distributed. Therefore, the nonparametric method of Mann–Whitney *U* tests were applied to test differences between control and exposed nematodes, as well as among the three $PM_{2.5}$-exposed sampling sites. Statistical analyses were examined using12.0-version SPSS Statistics software (IBM Corp., Armonk, NY, USA). Measurements are reported as means ± SD, and a p value < 0.05 was statistically significant.

3 Results and Discussion

3.1 *Concentrations of $PM_{2.5}$-Bound Elements and PAHs in Three Different Environments*

The $PM_{2.5}$ levels of the investigated ambient air in urban (n = 6), TRAP (n = 6), and industrial (n = 6) areas were 25.9 ± 8.58, 41.7 ± 24.9, and 28.9 ± 16.3 μg m^{-3}, respectively. The meteorological conditions, including wind speed (m sec^{-1}), relative humidity (%), wind direction (degrees), temperature (°C), and rainfall (mm) were gathered in the current study (Table S1). For Table 1, the $PM_{2.5}$-bound Σ_{16}PAH concentrations in urban, TRAP, and industrial areas were 0.658 ± 0.391 (mean ± standard deviation (SD)), 0.501 ± 0.193, and 0.517 ± 0.216 ng m^{-3}, respectively. $PM_{2.5}$-bound PAHs with LMW, MMW, and HMW were 29.8% (0.196 ng m^{-3}), 11.7% (0.077 ng m^{-3}), and 58.4% (0.385 ng m^{-3}) in urban areas, 30.7% (0.154 ng m^{-3}), 13.2% (0.066 ng m^{-3}), and 56.8%

Table 1 Primer sequences

Gene Code	Forward primer (5' to 3')	Reverse primer (5' to 3')
C. elegans		
SOD-1	TCAGGTCTCCAACGCGATTT	ACCGGGAGTAAGTCCCTTGA
SOD-2	GAGGCGGTCTCCAAAGGAAA	CGCTCTTAATTGCGGTGAGC
SOD-3	CTCCAAGCACACTCTCCCAG	TCCCTTTCGAAACAGCCTCG
SOD-4	GACGCGGTACTTCAGACCAA	CTGGAGGAAGGGATGCTGTC
SOD-5	CCGATAAGGTGGTCAGCCTC	CAAAGACTCCTCGGCCTTGT
CTL-1	GTGTCGTTCATGCCAAGGGAG	TGGATTGCGTCACGAATGAAG
CTL-2	TCCCAGATGGGTACCGTCAT	GGTCCGAAGAGGCAAGTTGA
CTL-3	ATGCCAATGCTTCCCCACAT	GCAGGTGGGGTTCCTGATTT
ACTIN-1	AGAAGAGCACCCAGTCCTCC	GAAGCGTAGAGGGAGAGGAC

(0.285 ng m^{-3}) in TRAP areas, and 29.9% (0.154 ng m^{-3}), 15.2% (0.078 ng m^{-3}), and 55.0% (0.284 ng m^{-3}) in industrial areas, respectively, of the total PAHs. In terms of the total Bap_{eq} levels for the 16 PAH species, $PM_{2.5}$-bound PAH in urban areas (0.0698 ± 0.0589 ng Bapeq m^{-3}) had a higher magnitude compared to those in TRAP (0.0497 ± 0.0264 ng Bapeq m^{-3}) and industrial (0.0518 ± 0.0172 ng Bapeq m^{-3}) areas, but their differences were insignificant (Table S2). This result may be due to the higher level of $PM_{2.5}$-bound Bap in urban areas (0.0380 ng Bap_{eq} m^{-3}) compared with those in TRAP (0.0277 ng Bap_{eq} m^{-3}) and industrial (0.0251 ng Bap_{eq} m^{-3}) areas in southern Taiwan. When considering $PM_{2.5}$-bound Bap_{eq} levels, $PM_{2.5}$-bound HMW-PAHs accounted for 96.6%–97.8% of Σ_{16}PAH in these three areas (Table 2).

Concentrations of $PM_{2.5}$-bound elements for 33 species, including essential (Mg, K, Ca, Na, Al, Mn, Zn, Co, Ni, Se, Mo, Ba, and Li), toxic (Cr, As, Cd, Cu, Hg, and Pb), trace (Ti, Rb, Sr, Sn, Sb, and Be), and transition (V, Ru, Rh, Pd, Pt, Tl, and Ag) elements, collected from urban, TRAP, and industrial areas are in Fig. 1. Levels of $PM_{2.5}$-bound elements are presented as 283 ± 68.5, 382 ± 186, and 231 ± 99.4 ng m^{-3} in urban, TRAP, and industrial areas. Levels of $PM_{2.5}$-bound toxic elements in industrial areas (8.49 ± 2.77 ng m^{-3}) was significantly higher than those in urban (3.91 ± 1.22 ng m^{-3}, $p < 0.001$) and TRAP (4.24 ± 1.80 ng m^{-3}, $p < 0.001$) areas. The predominant toxic elements found in $PM_{2.5}$ in urban, TRAP, and industrial areas of southern Taiwan were lead (Pb) (32%–54%), chromium (Cr) (17%–38%), and copper (Cu) (16%–31%). $PM_{2.5}$-bound lead concentrations in urban (1.39 ± 0.301 ng m^{-3}) and TRAP (1.30 ± 0.702 ng m^{-3}) areas had lower magnitudes than that in industrial (4.61 ± 1.62 ng m^{-3}, $p < 0.001$) areas (Table S3).

3.2 $PM_{2.5}$-Induced Reproductive Effects in *C. elegans*

Fine particulate has the potential effects to induce oxidative stress, provoke inflammation, and interfere with the mitochondrial function, as well as disrupt endocrine signaling, leading to reduced fertility (Nääv et al. 2020; Zheng et al. 2024). Based on the present result, $PM_{2.5}$-induced reproductive toxicity was found in nematodes. Figure 2 shows the brood size after a 24-h exposure to $PM_{2.5}$ from industrial, urban, and TRAP regions at various concentrations. In urban $PM_{2.5}$ exposure, compared with the untreated control (153 ± 25.6 progenies), the brood size significantly decreased by 79.4%, 80.8%, and 64.5% at 1.00, 10.0, and 100 mg L^{-1}, respectively (Fig. 2A and D). For TRAP $PM_{2.5}$, egg production declined by 85.8%, 64.0%, and 52.5% at 1.00, 10.0, and 100 mg L^{-1}, respectively, compared with the untreated control (156 ± 43.6 progenies) (Fig. 2B and D). Furthermore, industrial $PM_{2.5}$ exposure reduced the brood size by 83.8%, 73.4%, and 71.0% at 0.100, 1.00, 10.0, and 100 mg L^{-1}, respectively, compared to the control group (146 ± 35.4 progenies) (Fig. 2C and D). These findings in the present study recommend that the exposed $PM_{2.5}$ concentrations from 1.00 to 100 mg L^{-1} induces reproductive toxicity in *C. elegans* across urban, TRAP, and industrial areas. According to the analysis of the proportionate decrease in brood size, it can be inferred that $PM_{2.5}$ from industrial areas causes greater harm than that from TRAP and urban areas (Fig. 2). The toxic and endocrine-disrupting effects of $PM_{2.5}$ have been attributed to its content of particulate PAHs, phthalate esters, and various elements (Zhou et al. 2022). Among the heavy metals most recognized to cause disruption to endocrine system are Pb, Hg, Cd, As, Ni, Mn, Zn, and Cu, (Liu et al. 2023b). The present study demonstrated that higher $PM_{2.5}$ concentrations were associated with more severe reproductive toxicity in nematodes, particularly following exposure to $PM_{2.5}$ from industrial zones,

Table 2 $PM_{2.5}$-bound PAH concentrations in urban, TRAP, and industrial areas from southern Taiwan

PAHs	Urban	TRAP	Industrial
Concentrations (ng m^{-3})			
LMW	0.196 ± 0.027	0.154 ± 0.012	0.154 ± 0.033
MMW	0.077 ± 0.051	0.066 ± 0.027	0.078 ± 0.044
HMW	0.385 ± 0.313	0.285 ± 0.152	0.284 ± 0.139
Total	0.658 ± 0.391	0.501 ± 0.193	0.517 ± 0.216
BaP_{eq} levels (ng BaP_{eq} m^{-3})			
LMW	$2.23\times10^{-5}\pm3.35\times10^{-5}$	$1.66\times10^{-4}\pm1.36\times10^{-5}$	$1.73\times10^{-4}\pm3.88\times10^{-5}$
MMW	$1.32\times10^{-3}\pm9.02\times10^{-4}$	$1.07\times10^{-3}\pm4.49\times10^{-4}$	$1.49\times10^{-3}\pm9.76\times10^{-4}$
HMW	$6.83\times10^{-2}\pm5.80\times10^{-2}$	$4.85\times10^{-2}\pm2.59\times10^{-2}$	$5.02\times10^{-2}\pm1.62\times10^{-2}$
Total	$6.98\times10^{-2}\pm5.89\times10^{-2}$	$4.97\times10^{-2}\pm2.64\times10^{-2}$	$5.18\times10^{-2}\pm1.72\times10^{-2}$

Note: PM, particulate matter; TRAP, traffic-related air pollution; LMW, low molecular weight; MMW, medium molecular weight; HMW, high molecular weight

Elements (ng m^{-3})	Urban	TRAP	Industrial
Essentials elements	277 ± 66.7	375 ± 183	220 ± 96.1
Toxic elements	3.91 ± 1.22	4.24 ± 1.80	8.49 ± 2.77
Trace elements	2.53 ± 0.496	2.89 ± 1.20	2.60 ± 0.500
Transition elements [1]	0.204 ± 0.101	0.299 ± 0.103	0.100 ± 0.0100
Total	283 ± 68.5	382 ± 186	231 ± 99.4

[1] Only Vanadium concentrations are higher than MDLs.

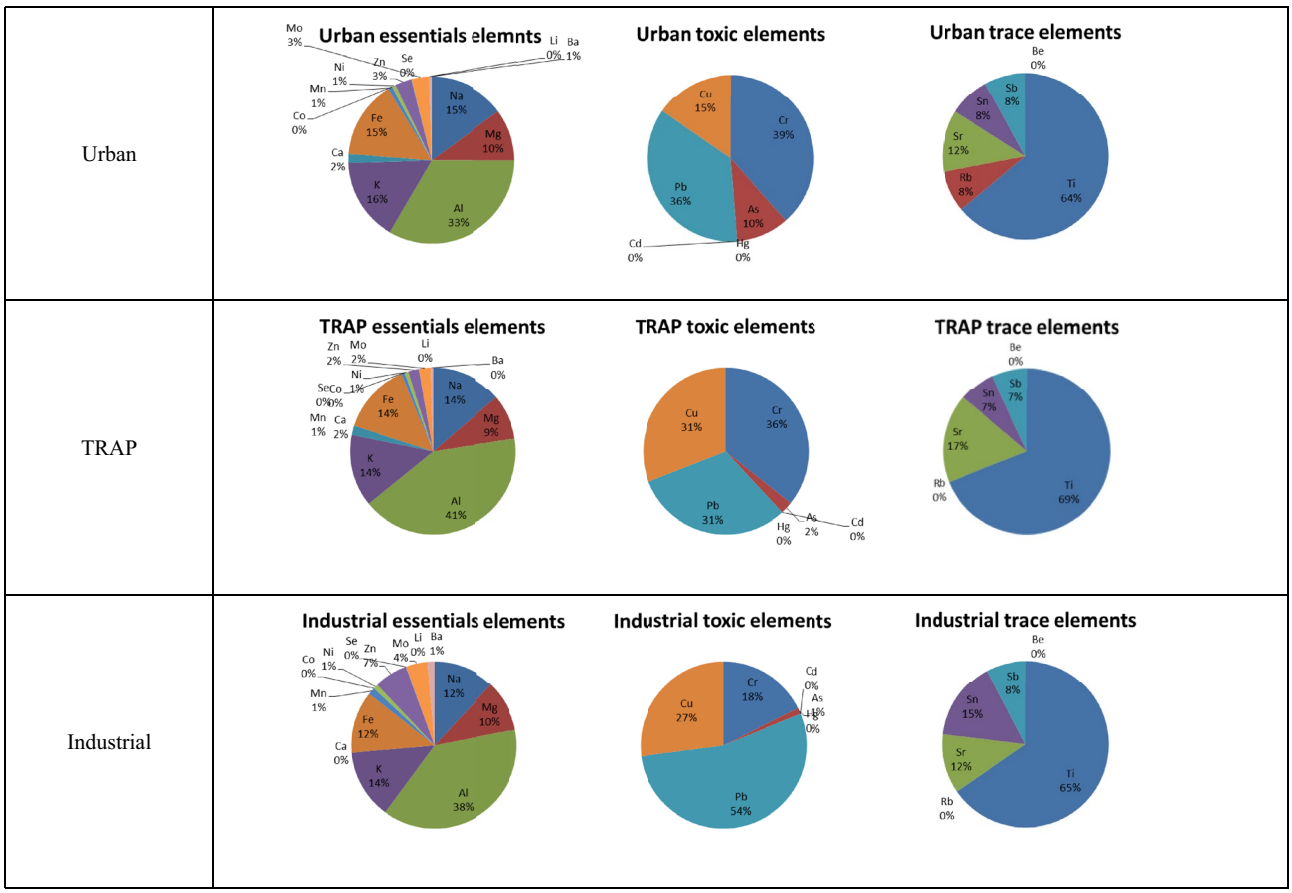
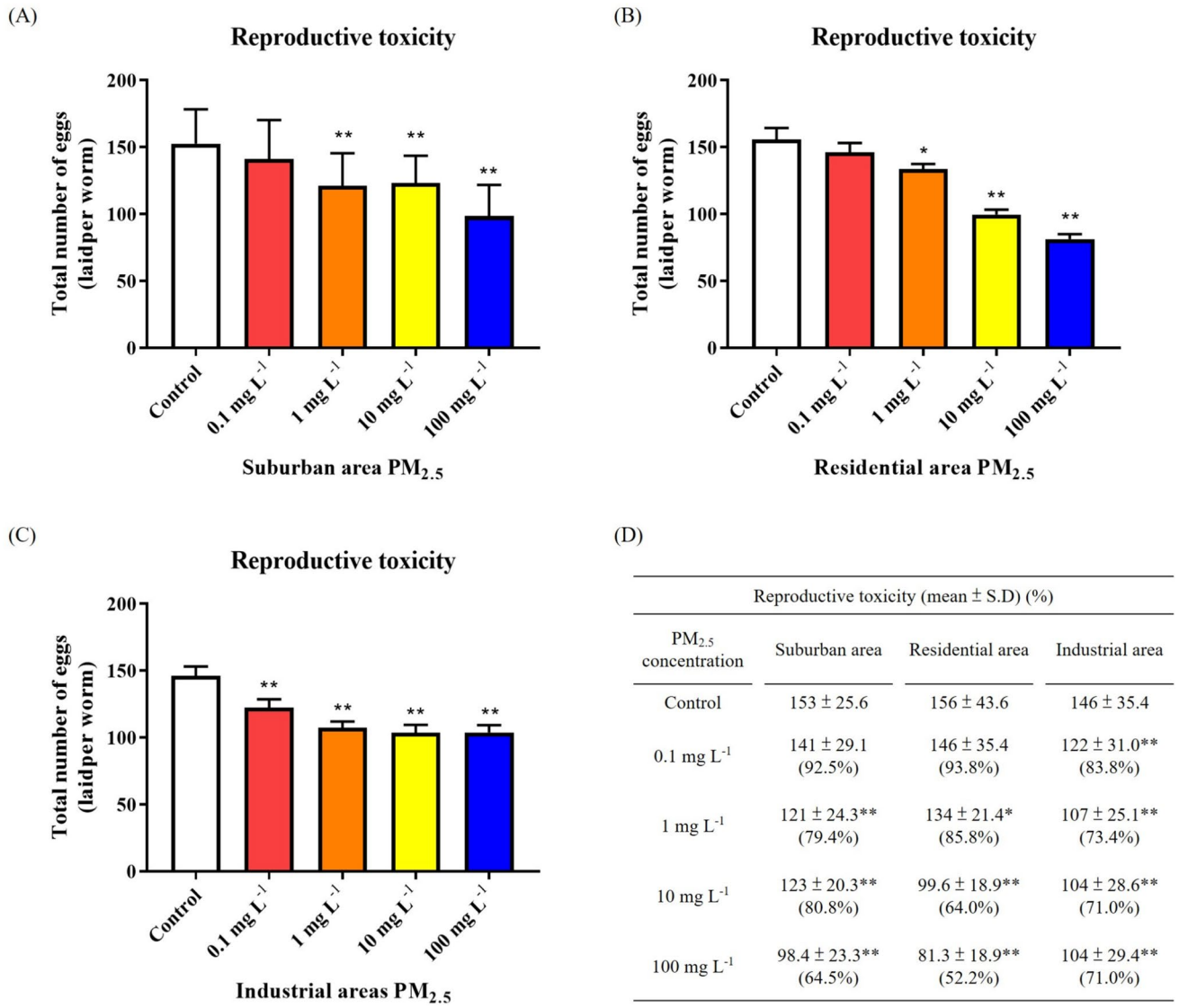


Fig. 1 Levels of $PM_{2.5}$-bound elements including essential, toxic, and crustal elements in urban, TRAP, and industrial areas. Note: PM, particulate matter; TRAP, traffic-related air pollution

which also exhibited higher levels of Pb and Cu compared to urban and TRAP zones. The findings from this animal study might be applied to epidemiological studies. A clinical study has shown that pregnant women with elevated blood Pb levels (≥ 10.0 μg dL^{-1}) had shorter gestation periods and elevated risks of preterm delivery and small-for-gestational-age outcomes (Jelliffe-Pawlowski et al. 2006). Serum Cu levels in women with miscarriage or infertility were 30% and 35% lower, respectively, compared with levels in pregnant women. (Skalnaya et al. 2019), suggesting that adequate Cu concentration may support positive outcomes related to pregnancy and reduce spontaneous abortion risk (Thaker et al. 2019). However, elevated serum Cu concentrations are associated with embryo implantation failure (Matsubayashi et al. 2017) and pathological pregnancies (Alebic-Juretic and Frkovic 2005). In this report, the lower levels of $PM_{2.5}$-bound Pb found in urban and TRAP areas, as compared to industrial areas, indicated that $PM_{2.5}$-bound lead may be a key factor contributing to increased reproductive toxicity (Figs. 1 and 2).

3.3 *$PM_{2.5}$-Induced Growth Effects in C. elegans*

Clinically, it has been found that $PM_{2.5}$ exposure can affect children's growth trajectory (Ma et al. 2024). Additionally, $PM_{2.5}$ exposure disrupts key signaling pathways

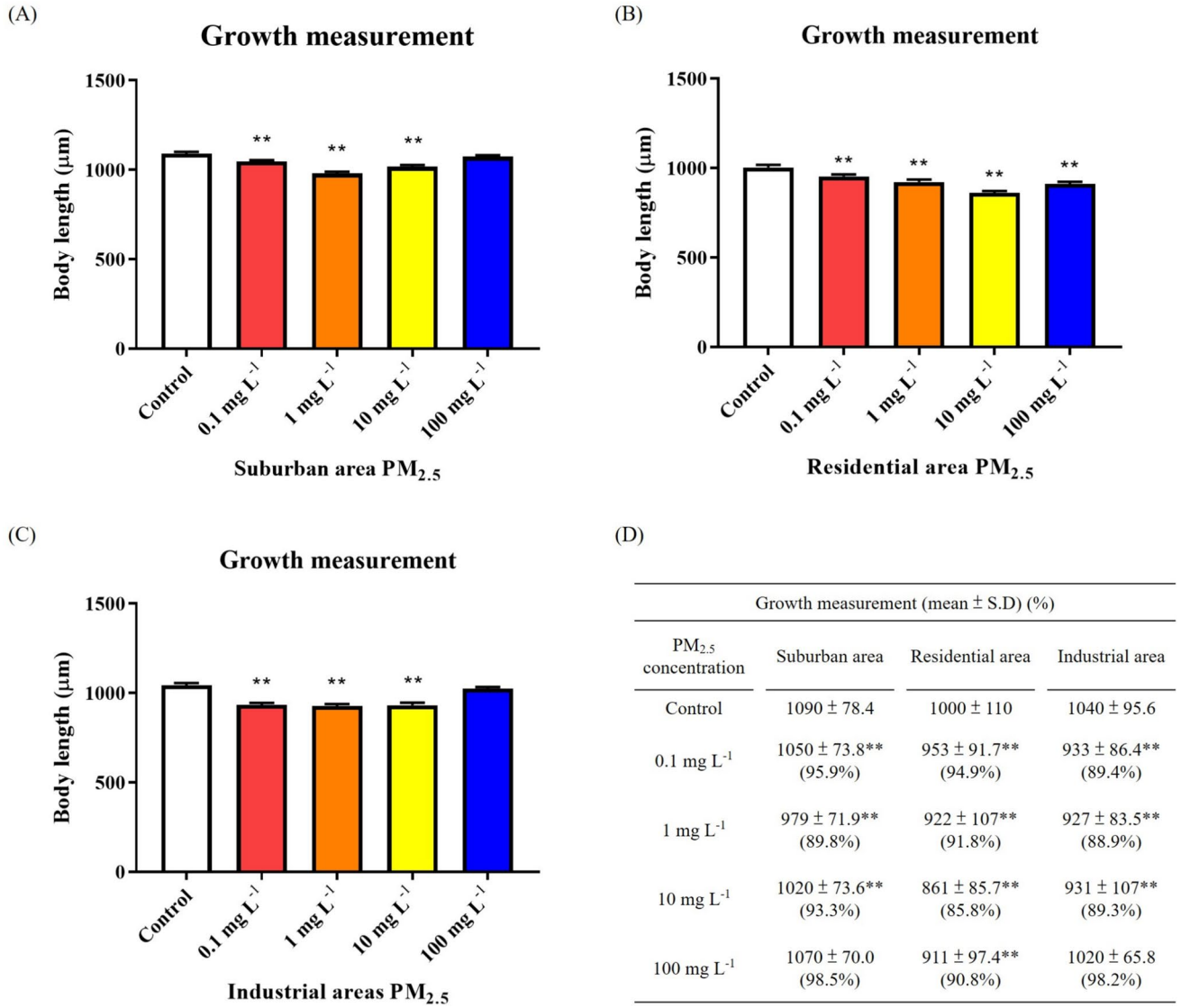


(D)

Reproductive toxicity (mean ± S.D) (%)			
$PM_{2.5}$ concentration	Suburban area	Residential area	Industrial area
Control	153 ± 25.6	156 ± 43.6	146 ± 35.4
0.1 mg L^{-1}	141 ± 29.1 (92.5%)	146 ± 35.4 (93.8%)	122 ± 31.0** (83.8%)
1 mg L^{-1}	121 ± 24.3** (79.4%)	134 ± 21.4* (85.8%)	107 ± 25.1** (73.4%)
10 mg L^{-1}	123 ± 20.3** (80.8%)	99.6 ± 18.9** (64.0%)	104 ± 28.6** (71.0%)
100 mg L^{-1}	98.4 ± 23.3** (64.5%)	81.3 ± 18.9** (52.2%)	104 ± 29.4** (71.0%)

Fig. 2 The reproductive toxicity effects of $PM_{2.5}$ from **A** urban, **B** TRAP, and **C** industrial areas on *Caenorhabditis elegans* after a 24-h exposure. Note: PM, particulate matter; TRAP, traffic-related air pollution. *$p < 0.05$, **$p < 0.01$ versus the control group

involved in growth, such as insulin-like growth factor signaling (IGF), which plays a critical role in *C. elegans* development (Zhang et al. 2023b). The body length serves as a crucial indicator of growth and development in *C. elegans*. This study evaluated the impact of $PM_{2.5}$ from urban, TRAP, and industrial areas by exposing nematodes to various $PM_{2.5}$ concentrations for 24 h. After exposure, the worms were moved to fresh NGM plates free of $PM_{2.5}$ and had the incubation for 2 days (48 h) until they developed to the young-L4 stage. As shown in Fig. 3A–D, exposure to urban, TRAP, and industrial $PM_{2.5}$ significantly impaired *C. elegans* growth. Urban $PM_{2.5}$ (0.100–10.0 mg L^{-1}) reduced the body length from 1090 ± 78.4 µm to 1050 ± 73.8, 979 ± 71.9, and 1020 ± 73.6 µm. TRAP $PM_{2.5}$ (0.1–100 mg L^{-1}) shortened the body length from 1000 ± 110 µm to 953 ± 91.7, 922 ± 107, 861 ± 85.7, and 911 ± 97.4 µm. Industrial $PM_{2.5}$ (0.1–10 mg L^{-1}) decreased the body length from 1040 ± 95.6 µm to 933 ± 86.4, 927 ± 83.5, and 931 ± 107 µm. All reductions were significant compared to the controls. These findings suggest that the worms with acute exposure to $PM_{2.5}$ between 0.100 and 10.0 mg L^{-1} in urban, TRAP, and industrial areas negatively impacts the growth and development of the nematodes. Moreover, the analysis of the decreased body-length proportions indicated that the detrimental effects of $PM_{2.5}$ in TRAP and industrial areas outweigh those in urban areas.



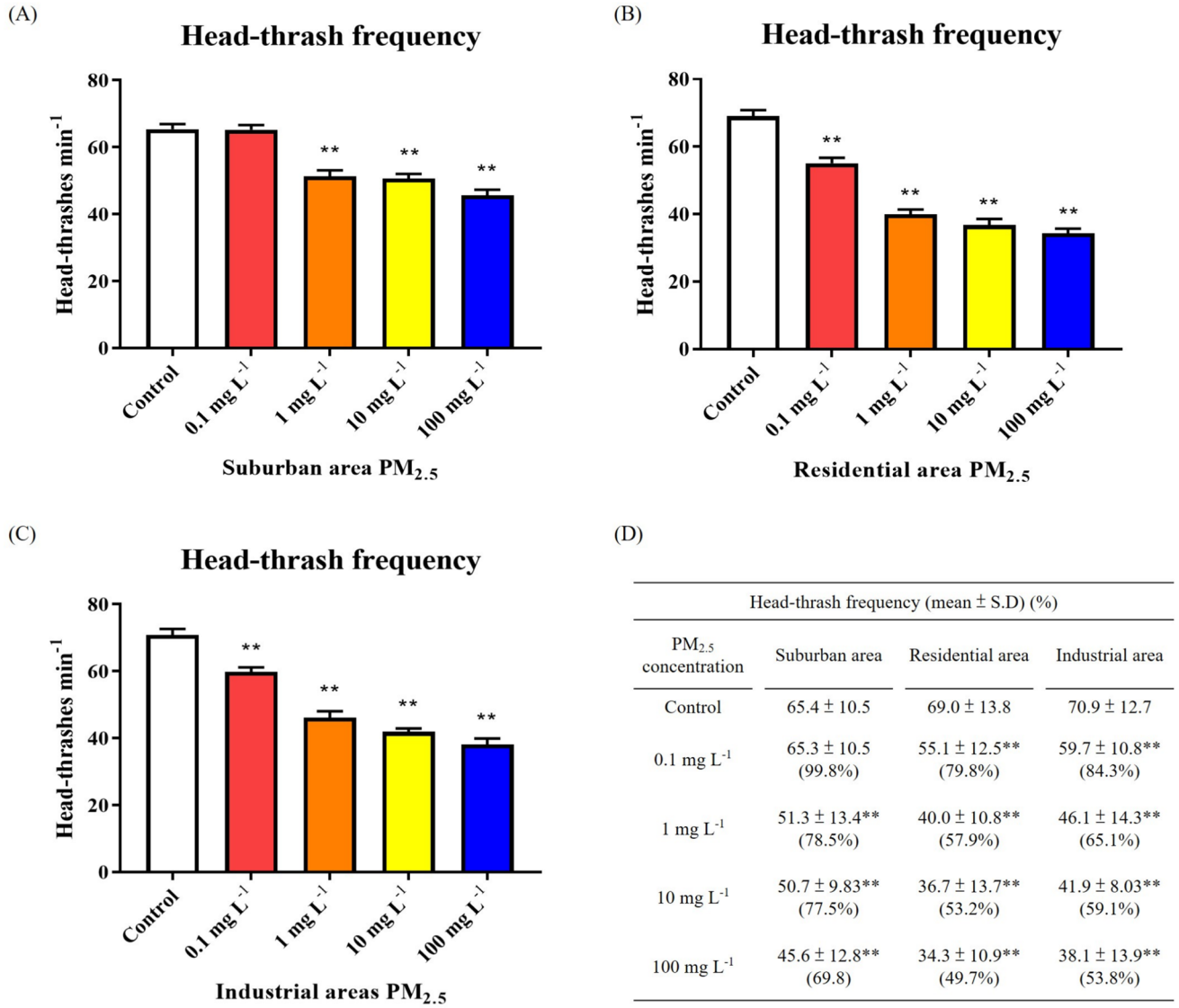
(D)

Growth measurement (mean ± S.D) (%)			
$PM_{2.5}$ concentration	Suburban area	Residential area	Industrial area
Control	1090 ± 78.4	1000 ± 110	1040 ± 95.6
0.1 mg L^{-1}	1050 ± 73.8** (95.9%)	953 ± 91.7** (94.9%)	933 ± 86.4** (89.4%)
1 mg L^{-1}	979 ± 71.9** (89.8%)	922 ± 107** (91.8%)	927 ± 83.5** (88.9%)
10 mg L^{-1}	1020 ± 73.6** (93.3%)	861 ± 85.7** (85.8%)	931 ± 107** (89.3%)
100 mg L^{-1}	1070 ± 70.0 (98.5%)	911 ± 97.4** (90.8%)	1020 ± 65.8 (98.2%)

Fig. 3 Effects of $PM_{2.5}$ from **A** urban, **B** TRAP, and **C** industrial areas on body length of *Caenorhabditis elegans* after a 24-h exposure. Note: PM, particulate matter; TRAP, traffic-related air pollution. $*p < 0.05$, $**p < 0.01$ versus the control group

3.4 *$PM_{2.5}$-Induced Neurobehavior Effects in C. elegans*

The humans or animals with exposure to $PM_{2.5}$ has been related with neurological impairments that influence neurodevelopment, neuroplasticity, and behavior (Lee et al. 2023). It is well-established that exposure to PAHs and excessive elements can result in abnormal nervous system development and neurobehavioral disorders in animals, with detrimental effects also observed in humans. These effects include reduced learning abilities, cognitive decline, and neural tube defects (Chen et al. 2016a; Xu et al. 2024). Therefore, it is crucial to understand the neurotoxic effects of $PM_{2.5}$ from different sources and its relationship with specific pollutants. In the current study, the neurotoxicity induced by $PM_{2.5}$ from urban, TRAP, and industrial areas was assessed through the frequencies of body bending and head thrashing in our worms' models (Figs. 4 and 5). The head thrashing frequency significantly decreased upon exposure to urban $PM_{2.5}$ levels from 1.00 to 100 mg L^{-1} compared to the untreated control (65.4 ± 10.5 thrashes min^{-1}). Specifically, exposure to 1, 10, and 100 mg L^{-1} $PM_{2.5}$ reduced head thrashing by 78.5%, 77.5%, and 69.8%, respectively (Fig. 4A and D). Similarly, TRAP $PM_{2.5}$ exposure (0.100 to 100 mg L^{-1}) notably and significantly lowered the head-thrashing frequencies (69.0 ± 13.8 thrashes min^{-1}) in the control. The reductions were 79.8% at 0.1 mg L^{-1}, 57.9% at 1 mg L^{-1}, 53.2% at 10 mg L^{-1}, and 49.7% at 100 mg L^{-1} (Fig. 4B and D). Additionally, industrial $PM_{2.5}$ exposure (0.100 to 100 mg L^{-1}) also significantly shortened the

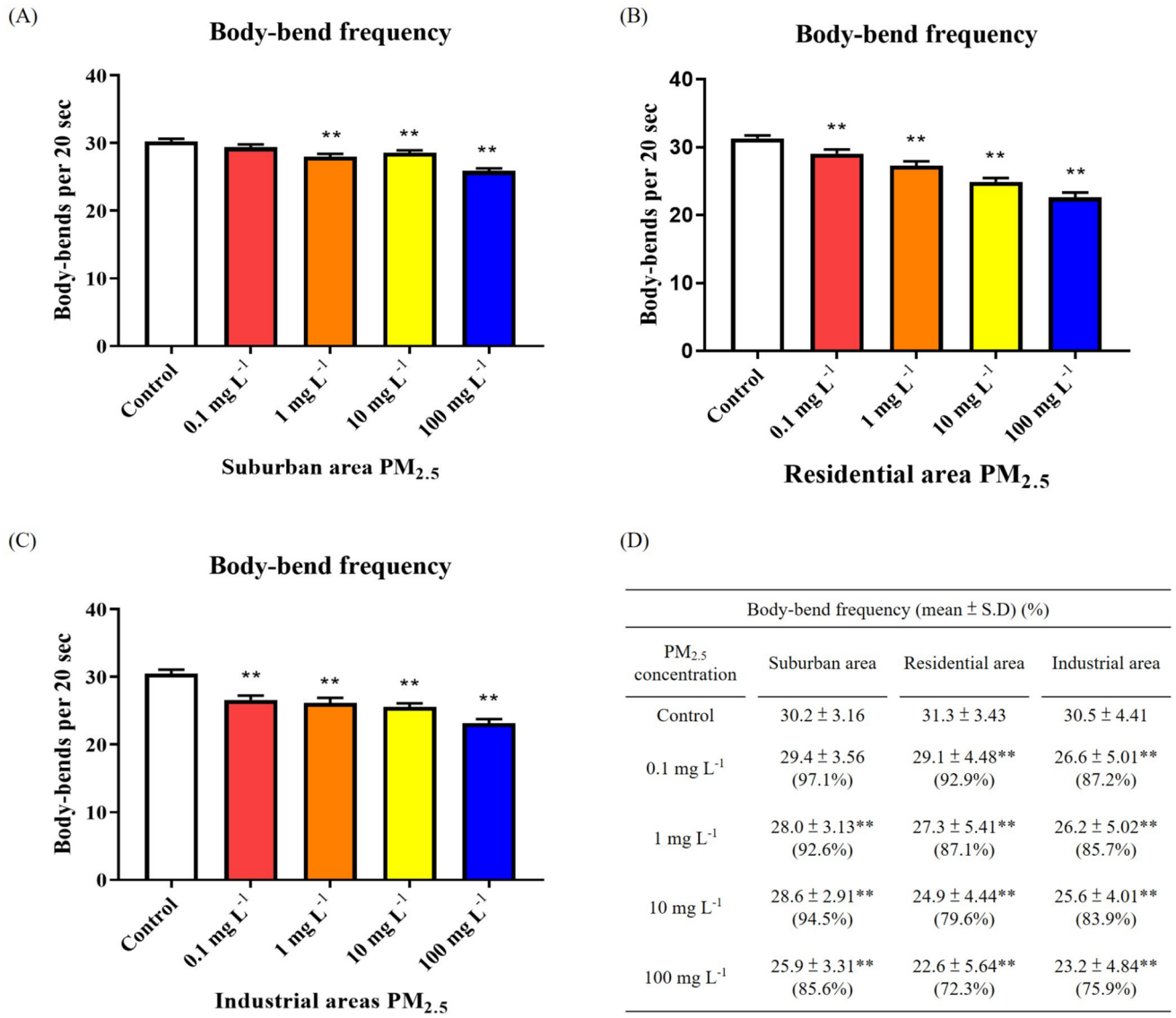


Head-thrash frequency (mean ± S.D) (%)			
$PM_{2.5}$ concentration	Suburban area	Residential area	Industrial area
Control	65.4 ± 10.5	69.0 ± 13.8	70.9 ± 12.7
0.1 mg L^{-1}	65.3 ± 10.5 (99.8%)	55.1 ± 12.5** (79.8%)	59.7 ± 10.8** (84.3%)
1 mg L^{-1}	51.3 ± 13.4** (78.5%)	40.0 ± 10.8** (57.9%)	46.1 ± 14.3** (65.1%)
10 mg L^{-1}	50.7 ± 9.83** (77.5%)	36.7 ± 13.7** (53.2%)	41.9 ± 8.03** (59.1%)
100 mg L^{-1}	45.6 ± 12.8** (69.8)	34.3 ± 10.9** (49.7%)	38.1 ± 13.9** (53.8%)

Fig. 4 Effects of $PM_{2.5}$ from **A** urban, **B** TRAP, and **C** industrial areas on head thrashes of *Caenorhabditis elegans* after a 24-h exposure. Note: PM, particulate matter; TRAP, traffic-related air pollution. ** $p < 0.01$ versus the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus the glucose group

head-thrashing frequencies compared to the untreated group (70.9 ± 12.7 thrashes min^{-1}). The reductions were 84.3% at 0.1 mg L^{-1}, 65.1% at 1 mg L^{-1}, 59.1% at 10 mg L^{-1}, and 53.8% at 100 mg L^{-1} (Fig. 4C and D).

Furthermore, it was observed that the exposed urban $PM_{2.5}$ levels ranged from 1.00 to 100 mg L^{-1} markedly and significantly decreased the *C. elegans*' body-bending frequency compared to the control group (30.2 ± 3.16 body-bends per 20 s). At 1.00 mg L^{-1}, the frequency decreased by 92.6%, at 10 mg L^{-1} by 94.5%, and at 100 mg L^{-1} by 85.6% (Fig. 5A and D). Similarly, exposure to TRAP $PM_{2.5}$ levels of 0.100, 1.00, 10.0, and 100 mg L^{-1} resulted in significant decreases in the body bending frequency of 92.9%, 87.1%, 79.6%, and 72.3%, respectively, compared to the control group (31.3 ± 3.43 body-bends per 20 s) (Fig. 5B and D). Furthermore, the exposed concentrations between 0.100 and 100 mg L^{-1} of industrial $PM_{2.5}$ led to a significant decrease in the body bending frequency of the nematodes compared to the controls (30.5 ± 4.41 body-bends per 20 s). At 0.100 mg L^{-1}, the decrease was 87.2%, at 1 mg L^{-1} 85.7%, at 10 mg L^{-1} 83.9%, and at 100 mg L^{-1} 75.9% (Fig. 5C and D). From the aforementioned results, it is evident that the exposed $PM_{2.5}$ within the range of 1.00 to 100 mg L^{-1} in urban, TRAP, and industrial areas may potentially induce brain/neural damage in *C. elegans*, resulting in a delay in the nematode's locomotor behavior. Furthermore, the findings of a reduced body bending frequency and head thrashing frequency suggest that $PM_{2.5}$ from TRAP and industrial areas exerts greater detrimental effects compared to that from urban areas.



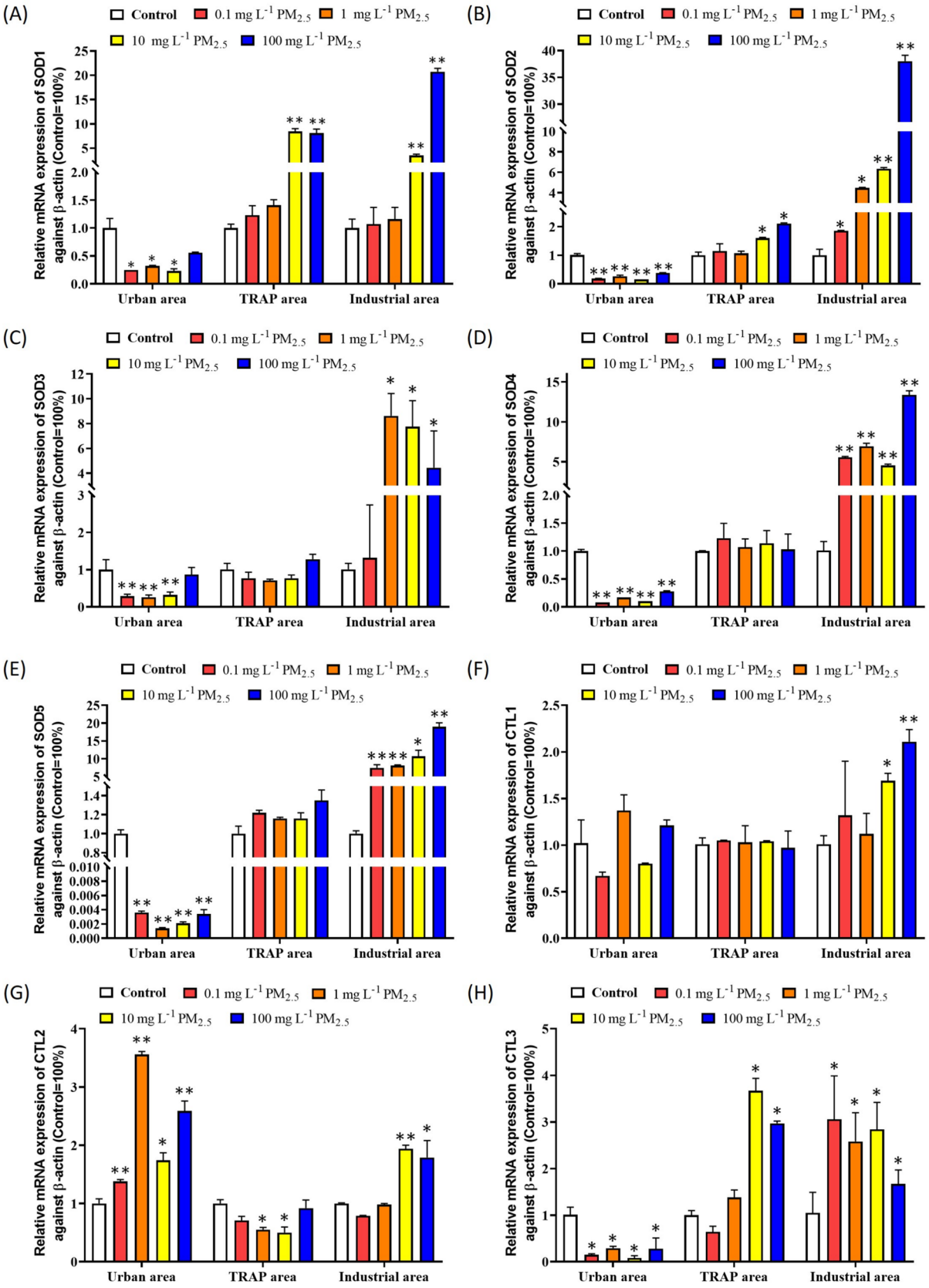
Body-bend frequency (mean ± S.D) (%)			
$PM_{2.5}$ concentration	Suburban area	Residential area	Industrial area
Control	30.2 ± 3.16	31.3 ± 3.43	30.5 ± 4.41
0.1 mg L^{-1}	29.4 ± 3.56 (97.1%)	29.1 ± 4.48** (92.9%)	26.6 ± 5.01** (87.2%)
1 mg L^{-1}	28.0 ± 3.13** (92.6%)	27.3 ± 5.41** (87.1%)	26.2 ± 5.02** (85.7%)
10 mg L^{-1}	28.6 ± 2.91** (94.5%)	24.9 ± 4.44** (79.6%)	25.6 ± 4.01** (83.9%)
100 mg L^{-1}	25.9 ± 3.31** (85.6%)	22.6 ± 5.64** (72.3%)	23.2 ± 4.84** (75.9%)

Fig. 5 Effects of $PM_{2.5}$ from **A** urban, **B** TRAP, and **C** industrial areas on body bends of *Caenorhabditis elegans* after a 24-h exposure. Note: PM, particulate matter; TRAP, traffic-related air pollution. $^{**}p < 0.01$ versus the control group

Exposure to Pb during development may alter the neurodevelopmental trajectory, causing lasting neuropathological consequences and enhancing susceptibility to additional stressors, as demonstrated in both in vivo and epidemiological studies. Clinical studies demonstrate that elevated blood and bone Pb levels impair intellectual functioning, verbal and nonverbal memories, sensory-motor reaction times, and fine motor skills, while also contributing to progressive cognitive decline, hyperactivity and attention deficits (Mason et al. 2014; Ahmad and Liu 2020). Copper required for normal brain physiological activity. Imbalance Cu homeostasis contributes to the onset of neurodegenerative diseases, such as movement disorders, multiple sclerosis, Wilson's, Parkinson's, Menkes', and Alzheimer's disease, amyotrophic lateral sclerosis, tremors, and muscle weakness (Zhu et al. 2024; Wang et al. 2025). The elevated Pb and Cu in industrial $PM_{2.5}$ likely amplified these effects in C. elegans, reflecting potential human neurological risks.

3.5 *$PM_{2.5}$-Induced Several Gene-Expression Effects in C. elegans*

To access the possible toxicity and repair mechanisms of $PM_{2.5}$ collected from TRAP, urban and industrial zones in terms of their impact on the expression of antioxidant genes, *C. elegans* individuals were exposed to $PM_{2.5}$ for 24 h during their adult stage. After exposure, the antioxidant-genes expression of SOD-1, SOD-2, SOD-3, SOD-4,


(A)
Control
0.1 mg L-1 PM2.5
1 mg L-1 PM2.5
10 mg L-1 PM2.5
100 mg L-1 PM2.5
Relative mRNA expression of SOD1 against β-actin (Control=100%)
Urban area
TRAP area
Industrial area
(B)
Relative mRNA expression of SOD2 against β-actin (Control=100%)
(C)
Relative mRNA expression of SOD3 against β-actin (Control=100%)
(D)
Relative mRNA expression of SOD4 against β-actin (Control=100%)
(E)
Relative mRNA expression of SOD5 against β-actin (Control=100%)
(F)
Relative mRNA expression of CTL1 against β-actin (Control=100%)
(G)
Relative mRNA expression of CTL2 against β-actin (Control=100%)
(H)
Relative mRNA expression of CTL3 against β-actin (Control=100%)

◂**Fig. 6** Effects of $PM_{2.5}$ from urban, TRAP, and industrial areas on *Caenorhabditis elegans* **A** SOD1, **B** SOD2, **C** SOD3, **D** SOD4, **E** SOD5, **F** CTL1, **G** CTL2, and **H** CTL3 mRNA expression after a 24-h exposure. Note: PM, particulate matter; TRAP, traffic-related air pollution; CTL, catalase; SOD, superoxide dismutase. $^{*}p < 0.05$, $^{**}p < 0.01$ versus the control group

SOD-5, CTL-1, CTL-2, and CTL-3 were analyzed using real-time PCR. As can be seen in Fig. 6, the exposed $PM_{2.5}$ in the urban areas at 0.100 mg/L significantly lowered the mRNA expression of the genes SOD-1 (0.25-fold), SOD-2 (0.18-fold), SOD-3 (0.29-fold), SOD-4 (0.08-fold), SOD-5 (0.0035-fold), and CTL-3 (0.14-fold) compared to the control group. Exposure to urban $PM_{2.5}$ of 1.00 mg L^{-1} had the significant reduction of the mRNA expression of the SOD1 (0.31-fold that of the control group), SOD2 (0.25-fold), SOD3 (0.26-fold), SOD4 (0.17-fold), SOD5 (0.0014-fold), and CTL-3 (0.28-fold) genes, while increasing the mRNA expression of the CTL-2 gene to 3.56-fold that of the control group (Fig. 6). The exposed $PM_{2.5}$ of 10.0 mg L^{-1} in the urban environment significantly inhibited the mRNA expression of the SOD1 (0.23-fold that of the control group), SOD2 (0.15-fold), SOD3 (0.32-fold), SOD4 (0.10-fold), SOD5 (0.0021-fold), and CTL-3 (0.08-fold) genes, while increasing the mRNA expression of the CTL-2 gene to 1.74-fold that of the control group (Fig. 6). Furthermore, the exposed urban $PM_{2.5}$ level of 100 mg L^{-1} markedly and significantly attenuated the mRNA expression of SOD-2 (0.38-fold that of the control group), SOD-4 (0.27-fold), SOD-5 (0.0034-fold), and CTL-3 (0.28-fold), but increased the mRNA expression of the CTL-2 gene to 2.59-fold that of the control group.

Figure 6 was shown that *C. elegans* with acute exposure to $PM_{2.5}$ in industrial areas had the opposite effect on the antioxidant-gene expression in *C. elegans* compared to that in urban areas. Our results revealed that the exposed $PM_{2.5}$ at 0.100 mg L^{-1} in the industrial areas markedly inhibited the mRNA expression of the SOD-2 (1.86-fold that of the control group), SOD-4 (5.55-fold), SOD5 (7.46-fold), and CTL-3 (3.06-fold) genes (Fig. 6). Exposure to industrial $PM_{2.5}$ of 1.00 mg L^{-1} significantly and notably increased the mRNA expression of the SOD-2 (4.46-fold compared to the control group), SOD3 (8.63-fold), SOD-4 (6.95-fold), SOD-5 (8.12-fold), and CTL-3 (2.58-fold) genes (Fig. 6). The exposed industrial $PM_{2.5}$ level at 10.0 mg L^{-1} had the significant increase in the mRNA expression of the SOD-1 (3.49-fold that of the control group), SOD-2 (6.35-fold), SOD-3 (7.76-fold), SOD-4 (4.54-fold), SOD-5 (10.9-fold), CTL-1 (1.68-fold), CTL-2 (1.94-fold), and CTL-3 (2.84-fold) genes (Fig. 6). Additionally, the exposed industrial $PM_{2.5}$ concentration of 100 mg L^{-1} obviously and significantly increased the mRNA expression of the SOD-1 (20.75-fold that of the control group), SOD-2 (38.03-fold), SOD-3 (4.45-fold), SOD-4 (13.38-fold), SOD-5 (19.00-fold), CTL-1 (2.11-fold), CTL-2 (1.79-fold), and CTL-3 (1.67-fold) genes (Fig. 6).

There were no significant differences on the antioxidant genes by the mRNA expression, including SOD-1, SOD-2, SOD-3, SOD-4, SOD-5, CTL-1, CTL-2, and CTL-3, compared to the control group after nematodes were acutely exposed to TRAP $PM_{2.5}$ at 0.100 mg L^{-1}. Exposure to TRAP $PM_{2.5}$ at 1.00 mg L^{-1}significantly and markedly inhibited the mRNA expression of CTL-2 to 0.55-fold that of the control group. The exposed TRAP $PM_{2.5}$ at the level of 10.0 mg L^{-1} significantly increased the mRNA expression of SOD-1 (8.43-fold that of the control), SOD-2 (1.60-fold), and CTL-3 (3.67-fold), while reducing CTL-2 expression to 0.50-fold that of the control group. Similarly, the nematodes' exposure to TRAP-$PM_{2.5}$ level of 100 mg L^{-1} markedly and significantly increased the mRNA expression as SOD-1 (8.08-fold that of the control), SOD-2 (2.11-fold), and CTL-3 (2.97-fold) (Fig. 6).

The findings of this study indicate that $PM_{2.5}$ exposure in urban areas may reduce the expression of antioxidant genes in *C. elegans*, resulting in oxidative stress that negatively affects its reproduction, growth, and locomotion. In contrast, $PM_{2.5}$ exposure in TRAP and industrial areas may increase antioxidant gene expression thereby inhibit ROS formation and offer a protective effect for cells (Kang et al. 2005; Hayes and McMahon 2009). However, $PM_{2.5}$ exposure can disrupt the balance between the NRF2-regulated antioxidant system and ROS production in the cellular environment (Chu et al. 2019), leading to increased ROS generation. This imbalance causes oxidative stress in *C. elegans*, with negative impacts on reproduction, growth, and locomotion. Additionally, overexpression of antioxidant genes induced by $PM_{2.5}$ may excessively suppress endogenously produced ROS to disrupt redox homeostasis and then to further adversely affect *C. elegans* in terms of reproduction, growth, and locomotion.

The differing antioxidant gene expression results observed under $PM_{2.5}$ exposure across urban, TRAP, and industrial areas may be influenced by variations in the $PM_{2.5}$ particles specific to each region. Specifically, $PM_{2.5}$ exposure in urban areas generally downregulated antioxidant genes (e.g., SOD-2 to 0.15-fold at 10.0 mg L^{-1}), potentially leading to increased oxidative stress and impaired cellular defenses. In contrast, industrial $PM_{2.5}$ significantly upregulated these genes (e.g., SOD-2 to 38.03-fold at 100 mg L^{-1}), which may represent an adaptive response to higher levels of toxic elements such as lead (4.61 ± 1.62 ng m^{-3} vs. urban 1.39 ± 0.30 ng m^{-3}). We speculate that differences in the composition of heavy metals or PAHs within $PM_{2.5}$ may account for these responsible, for instance, the higher lead and copper concentrations in industrial samples may trigger stronger compensatory mechanisms against metal-induced oxidative stress (Wu et al. 2016), thereby

potentially affecting several aspects of *C. elegans* biology. Their impacts may include changes in the population size, lifespan, reproductive output, peroxisome morphology, vulval development, and the metabolism of both exogenous chemicals (e.g., xenobiotics) and endogenous substances (e.g., signaling chemicals) (Eom et al. 2015; Schaar et al. 2015; Tullet et al. 2017).

Exposure to heavy metals in biological systems can lead to oxidative stress, resulting in DNA damage, lipid peroxidation, and other adverse effects (Wu et al. 2016). Specifically, the toxicity of elements such as lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) is well-documented. For the occupational exposure, chronic Pb exposure could elevate oxidative stress markers, reduce glutathione levels, and alter antioxidant enzyme activity in workers, while subacute exposure modifies glutathione-related enzyme activity (Kasperczyk et al. 2012, 2014; Dobrakowski et al. 2016). Similarly, excessive Cu might act as a potent oxidant, increasing ROS production, which impairs mitochondrial integrity and creates a feedback loop of heightened ROS generation (Vo et al. 2024). Cadmium, in contrast, could indirectly generate ROS by displacing iron and copper in biological systems (Videla et al. 2003; Liu et al. 2008). In this study, the cadmium contents in urban and industrial areas were lower than MDLs, while lead levels were notably higher in the industrial zone compared with the urban zone, suggesting that lead (Pb) has the possible contribution to the overall higher expression of antioxidant genes in the industrial zone. The current findings show that the exposed to $PM_{2.5}$ of 100 mg L^{-1} in industrial areas leads to statistically significantly increased expression of antioxidant genes (SOD1, SOD2, SOD3, SOD4, SOD5) compared with the untreated control. This confirms that exposure to high concentrations of $PM_{2.5}$ induces higher oxidative stress, ultimately leading to DNA damage. Previous studies have found that *Caenorhabditis elegans* shares homologs with many human genes (60%–80%), including those involved in basic physiological and redox reactions, such as various signaling pathways (Kaletta and Hengartner 2006). Therefore, this study suggests that *C. elegans* may offer a unique perspective or a more accessible and reliable method than other animal models for assessing the toxicity of $PM_{2.5}$.

According to our previous reports, $PM_{2.5}$ collected from rural areas significantly disrupted the head thrashing frequencies and the brood size numbers (Chung et al. 2019), and $PM_{2.5}$ from TRAP areas induced significant reductions in frequencies of locomotion, such as body bending and head thrashing, reproduction, such as brood size numbers, and longevity, such as lifespan, as well as enhanced the responses of oxidative stress (Chung et al. 2020; Lu et al. 2023). We focused on $PM_{2.5}$-bound PAHs and elements in areas of high $PM_{2.5}$ levels to test the toxic effects after the *C. elegans* were prolongedly exposed to $PM_{2.5}$ in this study. According to the results of the present study, exposure to $PM_{2.5}$-bound PAHs and elements in three areas of high $PM_{2.5}$ levels clearly has toxic effects on the nematodes, including toxicity to their reproduction, locomotion, and development and the generation of oxidative stress. The levels of $PM_{2.5}$-bound PAHs and PAH-Bap_{eq} did not show differences in the three areas of high $PM_{2.5}$ levels, but the levels of $PM_{2.5}$-bound toxic elements significantly differed, especially for lead in the industrial areas. When compared with the toxic effects of $PM_{2.5}$-bound PAHs in the nematodes' models, $PM_{2.5}$-bound toxic elements presented significantly more harmful effects, particularly for reproductive and neurological toxicity (Fig. 2) and SOD-related ROS production (SOD1–5) (Fig. 6) induced by lead and copper contaminating the fine particulate from industrial areas. According to the Yang's report (Yang et al. 2023), acute exposure of *C. elegans* to $PM_{2.5}$ resulted in elevated ROS production, intestinal autofluorescence, germ cell apoptosis, and delayed development. In Yang et al.'s (2023) study (Yang et al. 2023), these adverse effects (except for ROS production) were highly correlated with PAHs, Pb, Cu, and As in $PM_{2.5}$, and a high correlation coefficient was found between the nematodes' exposure to Cd and their induced ROS production. However, both $PM_{2.5}$-bound toxic elements and PAHs may interact additively or synergistically to disrupt redox balance, and future studies isolating these components could confirm their combined effects on redox balance and related toxicities.

This study was limited as the followings: (1) Model selection: The present study utilized *C. elegans* as an initial animal model for screening purposes. However, the toxic effects of $PM_{2.5}$ require further confirmation in more complex systems, such as mammalian models. (2) Exposure method differences: The method of $PM_{2.5}$ exposure used in this study differed from real-world exposure pathways, which may impact the applicability of the findings to real-world scenarios. (3) Target points: The tests of head thrashing, body bending, and several ROS genes evaluated in this study were specific for the examination of *C. elegans* models; these results may be difficult to replicate in mammalian models.

4 Conclusions

The findings of the current study are shown that $PM_{2.5}$ collected from urban (25.9 ± 8.58 μg m^{-3}), TRAP (41.7 ± 24.9 μg m^{-3}), and industrial (28.9 ± 16.3 μg m^{-3}) areas had toxic impact on the development, reproductive capacity and locomotor activity (neurological function) of *C. elegans* and induced oxidative stress. Exposure to $PM_{2.5}$ at 0.100–100 mg L^{-1} for 24 h significantly impaired nematode reproduction (52.5%–85.8% brood size reduction), growth (85.8%–95.9% body length reduction), and locomotion

behaviors (49.7%–84.3% and 72.3%–94.5% declines in head thrashing and body bending frequencies, respectively) across all areas compared to controls. Additionally, $PM_{2.5}$ induced oxidative stress, with industrial exposure upregulating antioxidant gene expression (SOD-1 by 3.49–20.75-fold, SOD-2 by 1.86–38.03-fold, SOD-3 by 4.45–8.63-fold, SOD-4 by 4.54–13.38-fold, SOD-5 by 7.46–19.00-fold, CTL-1 by 1.68–2.11-fold, CTL-2 by 1.79–1.94-fold, and CTL-3 by 1.67–3.06-fold), whereas urban exposure led to downregulation (SOD-1 by 0.23–0.31-fold, SOD-2 by 0.15–0.38-fold, SOD-3 by 0.26–0.32-fold, SOD-4 by 0.08–0.27-fold, SOD-5 by 0.0014–0.0035-fold, and CTL-3 by 0.08–0.28-fold). Levels of $PM_{2.5}$-bound PAHs and elements were also determined to examine their relationship with the outcomes of toxic effects in *C. elegans* models. Considering contribution of $PM_{2.5}$-bound PAHs or elements to $PM_{2.5}$ toxicity in nematode models, we found that the levels of $PM_{2.5}$-bound PAHs (Σ16PAH: 0.501–0.658 ng m^{-3}) and PAH-Bap_{eq} (0.0497–0.0698 ng BaPeq m^{-3}) did not present consistent results with regard to their toxic effects on the *C. elegans* models in these three areas. In contrast, the levels of $PM_{2.5}$-associated toxic elements (3.91 ± 1.22 ng m^{-3} in urban, 4.24 ± 1.80 ng m^{-3} in TRAP, and 8.49 ± 2.77 ng m^{-3} in industrial areas), particularly Pb and Cu were highly linked with the toxic outcomes of $PM_{2.5}$ observed in the nematode models. The higher levels of $PM_{2.5}$-bound lead (4.61 ± 1.62 ng m^{-3} vs. 1.30–1.39 ng m^{-3} elsewhere) and copper (2.29 ± 0.90 ng m^{-3} vs. 0.598–1.30 ng m^{-3} elsewhere) found in industrial areas, in comparison with those in urban and TRAP areas, may seriously harm the reproductive function (brood size) and neurological behavior (body bending and head thrashing) and induce more serious ROS responses, which have been correlated with the SODs in nematode models. These findings suggest that $PM_{2.5}$-bound Pb and Cu might have a more significant contribution to toxicity in industrial environments compared to urban and TRAP environments. To mitigate these public health risks from emission of heavy metals in the industrial zones, we recommend to implementing stricter regulations on industrial emissions to further reduce heavy metal concentrations in ambient air, conducting continuous air quality monitoring and epidemiological studies (e.g., measuring blood levels of Pb and Cu in local residents), educating communities near industrial areas about the risks associated with $PM_{2.5}$ exposure while promoting protective behaviors (e.g., wearing masks or limiting outdoor activities), and adopting advanced air filtration systems to reduce the dispersion of industrial pollutants.

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Declarations

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