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Assessing developmental and transcriptional effects of PM2.5 on zebrafish embryos

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ABSTRACTS

Investigating fine particulate matter (PM2.5) toxicity is crucial for health risk assessment and pollution control. This study explores the developmental toxicity of two PM2.5 sources: standard reference material 2786 (NIST, USA) and PM2.5 from Chakri Naruebodindra Medical Institute (CNMI, Thailand) located in the Bangkok Metropolitan area. Zebrafish embryos exposed to these samples exhibited embryonic mortality, with 50% lethal concentration (LC_{50}) values of 1476 μg/mL for standard PM2.5 and 512 μg/mL for CNMI PM2.5. Morphological analysis revealed malformations, including pericardial and yolk sac edema, and blood clotting in both groups. Gene expression analysis highlighted source-specific effects. Standard PM2.5 downregulated *sod1* and *cat* while upregulating *gstp2*. Inflammatory genes *tnf-α* and *il-1b* were upregulated, and *nfkbi-αa* was downregulated. Apoptosis-related genes *bax*, *bcl-2*, and *casp3a* were downregulated. CNMI PM2.5 consistently downregulated all examined genes. These findings underscore PM2.5 source variability's significance in biological system impact assessment, providing insights into pollutant-gene expression interactions. The study emphasizes the need for source-specific risk assessment and interventions to address PM2.5 exposure's health impacts effectively.

1. Introduction

Air pollution, particularly in the form of fine particulate matter, commonly referred to as PM2.5, is a pressing concern with far-reaching implications for both human health and the environment. PM2.5, defined as particles with a diameter of 2.5 micrometers or smaller, is a complex mixture of microscopic solids and liquid droplets suspended in the air. These tiny particles originate from various sources, including combustion processes from vehicles, industrial facilities, and natural sources like dust and wildfires [9]. While PM2.5 is nearly invisible to the naked eye, its health effects are far from inconspicuous. Exposure to PM2.5 has been linked to a myriad of adverse health outcomes, ranging from respiratory and cardiovascular issues to premature mortality [13,22,4]. Consequently, understanding the sources and health impacts of PM2.5 is paramount for comprehending its environmental health concerns, highlighting the need for robust research, and informing public health and policy initiatives.

In our pursuit of comprehending the multifaceted challenges posed by PM2.5, it is essential to acknowledge the intricate landscape of PM2.5 source variability. The composition and impact of PM2.5 can diverge significantly based on its source, emphasizing the critical need to discern between these origins. Vehicular exhaust, industrial processes, wildfires, and natural dust each contribute to PM2.5 with distinct chemical compositions and associated health risks [1,18,24]. Moreover, regional variations further complicate the PM2.5 equation, with urban areas, rural regions, and industrial hubs experiencing unique mixes of PM2.5 constituents [16,23,7]. The influence of source on PM2.5 toxicity cannot be underestimated; specific sources, such as diesel engines, are notorious for emitting PM2.5 laden with more toxic compounds, including polycyclic aromatic hydrocarbons and heavy metals [15,17]. Understanding these source-dependent effects is paramount for developing targeted strategies to reduce health risks and environmental impacts while guiding region-specific research and policy interventions.

Zebrafish (*Danio rerio*) has emerged as a highly advantageous model

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organism in the realm of environmental health research, particularly for toxicity testing [2,8]. Their transparency, small size, and genetic similarity to humans make them exceptionally suitable for studying the impacts of PM2.5 exposure. The rapid growth of zebrafish embryos enables high-throughput experiments and facilitates assessments at individual and population levels [3]. Beyond toxicity testing, their relevance in environmental health research extends to exploring the intricate relationship between PM2.5 and various health outcomes, from developmental abnormalities to neurotoxicity. Utilizing the genetic manipulability of zebrafish is instrumental in revealing the underlying molecular mechanisms [10,26,5]. This enhances their role as an invaluable resource for understanding the impacts of pollutants on both human health and the environment. It also contributes to developing evidence-based policies and strategies in public health.

In recent years, the Bangkok Metropolitan area has faced significant challenges due to its dense population and high concentration of industrial factories [20]. The combination of industrial emissions and vehicular exhaust emissions contributes to the elevated levels of PM2.5 in the air, creating a pressing environmental and public health concern for residents of the Bangkok Metropolitan area. In the present study, our objective was to assess the impact of PM2.5 particles obtained from the Bangkok Metropolitan area, specifically from Chakri Naruebodindra Medical Institute (CNMI), on the developmental processes of zebrafish embryos. This investigation involved a comparative analysis with a standard PM2.5 reference material collected from the National Institute of Standards and Technology (NIST), USA. We conducted a zebrafish embryo acute toxicity test to assess the impact of these particulate samples on various parameters, including survival, hatching, heart rate, and embryo morphology. Furthermore, we explored the transcriptional effects of both particulate samples on biological processes related to toxicological outcomes. This involved an examination of genes associated with oxidative stress, inflammation, and apoptosis.

2. Materials and methods

2.1. PM2.5 collection and preparation

The present study used two types of PM2.5 samples. The standard PM2.5 reference material® 2786 was purchased from NIST (Gaithersburg, USA). Additionally, PM2.5 samples from Chakri Naruebodindra Medical Institute (CNMI), Bangkok Metropolitan, Thailand, were collected every two weeks between October 2021 and April 2022 using a dust collector (Envotech®, Thailand). Subsequently, each collected bag was cut into small pieces and subjected to a five-time cycle of sonication in ultrapure water using a Cole-Parmer ultrasonic cleaner (Cole-Parmer, USA). The extracted solution was then filtered through Whatman® No.2 filter paper with an 8 μm pore size. The filtrate was partially filled in a 50 mL tube (1/3 of the total volume) and frozen at −20°C for a minimum of 12 h. Subsequently, the frozen tubes were subjected to freeze-drying using a high-capacity freeze dryer (Supermodulyo-230, Thermo Scientific, United States) for 24 h. Upon completion of the cycle, each resulting particulate sample was transferred to a container and stored in a dry cabinet.

2.2. Zebrafish maintenance and embryo collecting

Adult wild-type zebrafish were raised at the National Nanotechnology Center (NANOTEC, Thailand) and housed in a recirculating system (AAB-074, Yakos65, Taiwan) under standard conditions. Zebrafish embryos were obtained from natural mating pairs and kept in egg water (60 mg/L of sea salt). Only normal fertilized eggs were selected for the experiments using a stereomicroscope (SZX7, Olympus, Tokyo, Japan). All experimental procedures in the present study were approved by the NSTDA Institutional Animal Care and Use Committee (No. 003–2564).

2.3. Zebrafish embryo acute toxicity test of PM2.5 samples

Acute toxicity test of PM2.5 samples was performed on zebrafish embryos according to OECD test guideline 236. Healthy embryos at 4 hour post-fertilization (hpf) were transferred to a 12-well plate with twenty embryos per well. Stock solutions of PM2.5 samples were prepared by dissolving in DMSO and sonicated for 30 minutes. Various concentrations (200, 400, 600, 800, and 1000 μg/mL) of PM2.5 samples were prepared, and each test solution was vortexed for 30 seconds before adding to the respective well. The chosen PM2.5 doses were based on established research, including a previous study by [21], which utilized similar concentrations to produce observable effects within a reasonable timeframe. The plate was then incubated at 28.5 °C. DMSO with 0.48% v/v was used as a vehicle control, a dose below the threshold known to induce notable morphological or molecular-level abnormalities in zebrafish embryos [12]. Test solutions were renewed every 24 h concurrently with the removal of the dead embryos, and the number of dead embryos was recorded. The morphology of zebrafish larvae at 96 hpf were documented using a stereomicroscope (SZX16, Olympus, Japan) equipped with a DP73 camera (Olympus, Tokyo, Japan). In addition, heartbeats of zebrafish larvae at 96 hpf were recorded via one-minute videos. The data on survival rate, hatching rate, and heart rate were obtained from three independent experiments. Experiments in which the control group exhibited a mortality rate exceeding 10% were excluded from the analysis.

2.4. Quantitative reverse transcription-PCR analysis

After exposure to 1000 μg/mL of standard PM2.5 or 600 μg/mL of CNMI PM2.5, total RNA was extracted from whole zebrafish embryos using the RNeasy Plus Mini Kit (Qiagen, Germany). The purity and concentration of the RNA were analyzed using a Nanodrop 2000c (Thermo Scientific, USA). Equal amounts of total RNA from each sample were then reverse transcribed into complementary DNA (cDNA) using the iScript™ Reverse Transcription Supermix (Bio-Rad, California, USA). The genes associated with oxidative stress, inflammation, and apoptosis were selected, and their corresponding primers are listed in Table 1. The qPCR reactions were carried out using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using Bio-Rad SsoAdvanced™ Universal SYBR Green Supermix. Gene expression levels were normalized to the glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh*) as an internal control. Data analysis was conducted using CFX Manager Software (Bio-Rad).

2.5. Statistical analysis

Statistical analyses were conducted with SPSS Statistics version 28.0.0.0, and Regression Probit analysis was performed using IBM

Table 1
List of commercial reference IDs used for qRT-PCR analysis.

Primer name	Gene name	Bio-rad assay ID	T_a (°C)
cat	catalase	qDreCED0018362	60°C
sod1	superoxide dismutase 1	qDreCID0011220	60°C
gstp2	glutathione S-transferase pi 2	qDreCID0006153	60°C
tnf-α	tumor necrosis factor alpha	qDreCED0010044	60°C
nfkbiaa	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha a	qDreCED0012341	60°C
Il-1β	interleukin 1, beta	qDreCID0003801	60°C
bax	BCL2 associated X, apoptosis regulator a	qDreCID0002305	60°C
Bcl-2	BCL2 apoptosis regulator a	qDreCED0010463	60°C
casp3a	caspase 3, apoptosis- related cysteine peptidase a	qDreCED0019244	60°C
gapdh	glyceraldehyde-3-phosphate dehydrogenase	qDreCED0021000	60°C

software (Chicago, USA). The statistical significance between experimental groups was assessed through a one-way analysis of variance (ANOVA) and subsequently compared using Tukey's test to identify any significant differences between the groups. Significance was defined as $p < 0.05$.

3. Results

3.1. Effects of PM2.5 on the survival and hatching of zebrafish embryos

In the acute toxicity test, zebrafish embryos were exposed to the standard PM2.5 and the CNMI PM2.5 for 96 h. The results revealed that both samples induced embryonic mortality. Notably, only the highest concentration (1000 μg/mL) of standard PM2.5 significantly reduced the survival rate of zebrafish embryos (61.7±11.5%) compared to the control group (98.3±2.9%), as shown in Fig. 1A. On the other hand, at concentrations of 800 and 1000 μg/mL of CNMI PM2.5, no embryos survived, and the embryonic survival rate was significantly lower after treatment with 600 μg/mL of CNMI PM2.5 compared to the control group (96.7±4.1%), as shown in Fig. 1B. Meanwhile, at 96 hpf, the estimated 50% lethal concentration (LC_{50}) values of standard PM2.5 and CNMI PM2.5 to zebrafish embryos were 1476 and 512 μg/mL, respectively.

Additionally, the hatching rates of treated zebrafish embryos were also determined. At 72 hpf, the zebrafish in the control group exhibited a 100% hatching rate. Notably, except for 800 and 1000 μg/mL of CNMI PM2.5, which resulted in 100% mortality, all concentrations tested of both particulate samples did not significantly change the hatching rates of zebrafish embryos (Fig. 1C and D).

3.2. Effects of PM2.5 on morphological development of zebrafish larvae

Representative images of zebrafish larvae treated with standard PM2.5 and CNMI PM2.5 at 96 hpf are presented in Fig. 2, respectively. The assessment of embryonic morphology revealed that both particulate samples caused malformations in zebrafish embryos, including pericardial edema, yolk sac edema, and blood clotting. Malformed embryos were particularly prevalent in the treatment groups exposed to 800 and 1000 of standard PM2.5 and 600 μg/mL of CNMI PM2.5. Quantitative analysis showed a significant increase in malformed embryos in these higher concentration groups compared to the control groups (Fig. 3).

3.3. Effects of PM2.5 on heart rate of zebrafish embryos

After treatment, both particulate samples did not significantly alter the heart rate of zebrafish larvae at 96 hpf (Fig. 4). The heart rates of standard PM2.5- and CNMI PM2.5-treated zebrafish embryos ranged from 129±2–137±3 beats/min and 137±11–142±11 beats/min, respectively, while the control group exhibited a heart rate of 133±2 beats/min.

3.4. Transcriptional effects of standard PM2.5 on oxidative stress, inflammatory, and apoptosis-related genes in zebrafish embryos

Using qRT-PCR, we examined the expression levels of oxidative stress, inflammatory, and apoptosis-related genes in zebrafish embryos exposed to standard PM2.5. Superoxide dismutase 1 (*sod1*), catalase (*cat*), and glutathione S-transferase pi 2 (*gstp2*) were chosen for the study of oxidative stress-related genes. The results revealed that standard PM2.5 significantly downregulated the expression of *sod1* and *cat*, while the expression level of *gstp2* was significantly upregulated compared to the control group (Fig. 5).

For inflammatory-related genes, we selected tumor necrosis factor *α* (*tnf-α*), interleukin 1 beta (*il-1β*), and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha *a* (*nfkbi-αa*) for the study. After exposure to standard PM2.5, the expression levels of *tnf-α* and *il-1b* were significantly upregulated in treated zebrafish embryos compared to the control group (Fig. 5). In contrast, the expression of *nfkbi-αa* was significantly downregulated compared to the control. Moreover, the expression of apoptosis-related genes, including BCL2-associated X apoptosis regulator *a* (*bax*), BCL2 apoptosis regulator *a*

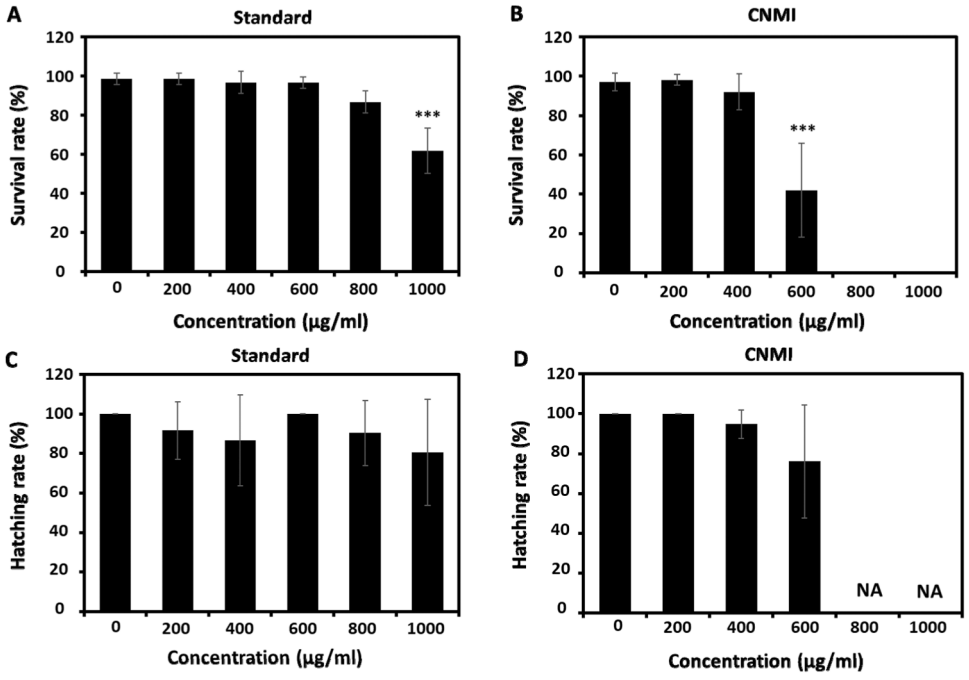

Fig. 1. Survival and hatching rates of zebrafish embryos exposed to different concentrations of particulate samples at 96 hpf (A, B) and 72 hpf (C, D). (A) Survival rate of zebrafish embryos exposed to standard PM2.5. (B) Survival rate of zebrafish embryos exposed to CNMI PM2.5. (C) Hatching rate of zebrafish embryos exposed to standard PM2.5. (D) Hatching rate of zebrafish embryos exposed to CNMI dust. The data are presented as mean ± SD from three independent experiments, each utilizing 20 embryos per experimental condition. *** $p < 0.001$ compared to the control.

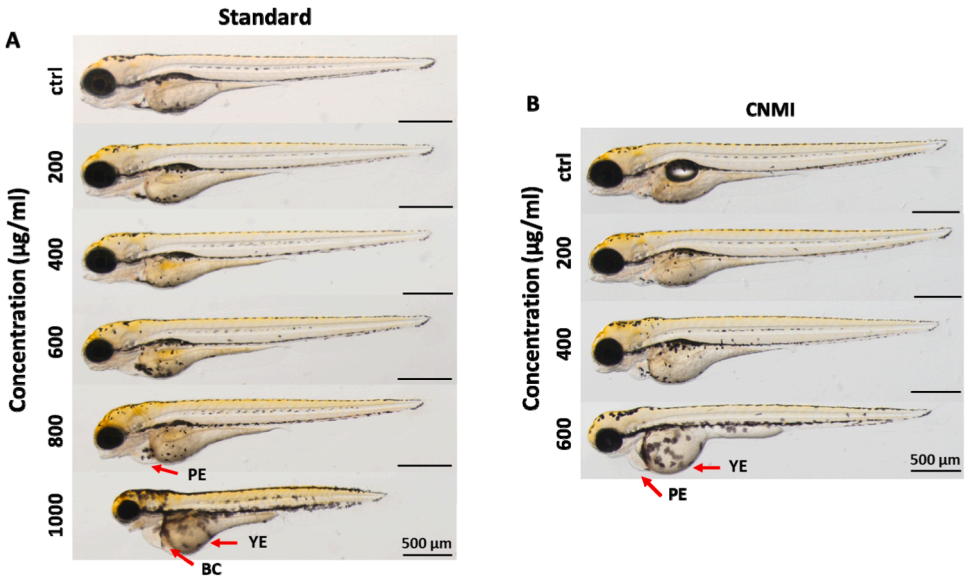

Fig. 2. Representative images showing the morphology of zebrafish larvae at 96 hpf exposed to different concentrations of (A) standard PM2.5 and (B) CNMI PM2.5. PE = Pericardial edema, YE = Yolk sac edema, BC = Blood clotting.

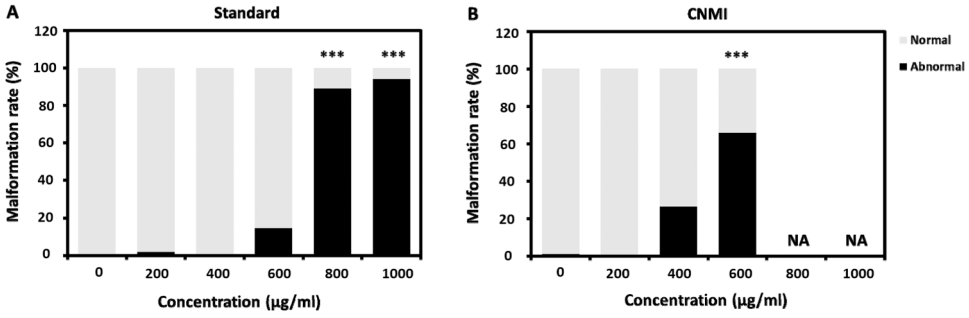

Fig. 3. Malformation rates in zebrafish embryos exposed to different concentrations of (A) standard PM2.5 and (B) CNMI PM2.5 at 96 hpf. The data are presented as means from three independent experiments. *** $p < 0.001$ compared to the control.

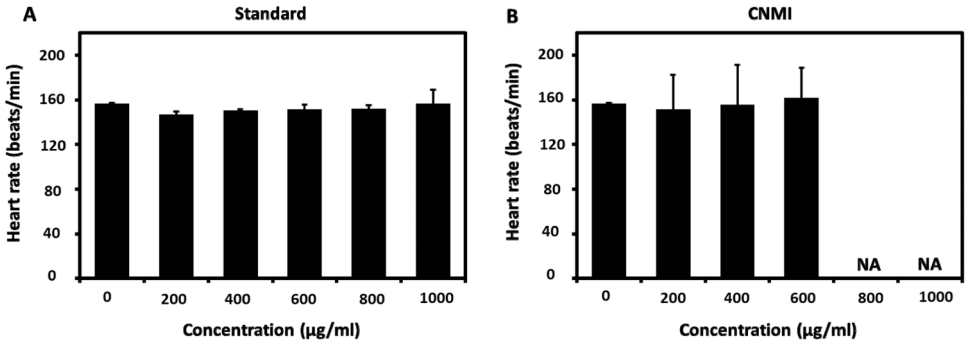

Fig. 4. Heart rates of zebrafish embryos exposed to different concentrations of (A) standard PM2.5 and (B) CNMI PM2.5 at 96 hpf. The data are presented as mean ± SD from three independent experiments, each utilizing five embryos per experimental condition. *** $p < 0.001$ compared to the control.

(*bcl-2*), and caspase 3 apoptosis-related cysteine peptidase *a* (*casp3a*), also exhibited downregulation, as shown in Fig. 5.

3.5. Transcriptional effects of CNMI PM2.5 on oxidative stress, inflammatory, and apoptosis-related genes in zebrafish embryos

By qRT-PCR analysis, we observed alterations in the gene expression levels of *sod1*, *cat*, and *gstp2* caused by CNMI PM2.5 exposure (Fig. 6). Notably, the expression levels of all oxidative stress-related genes were significantly downregulated compared to the control group. Similarly, CNMI PM2.5 reduced the expression of inflammatory-related genes *tnf-α* and *nfkbi-αa*, though *il-1b* expression remained unchanged (Fig. 6). Furthermore, all apoptosis-related genes in the treated groups showed significant downregulation compared to the control group (Fig. 6).

4. Discussion

The present study aimed to assess the developmental and

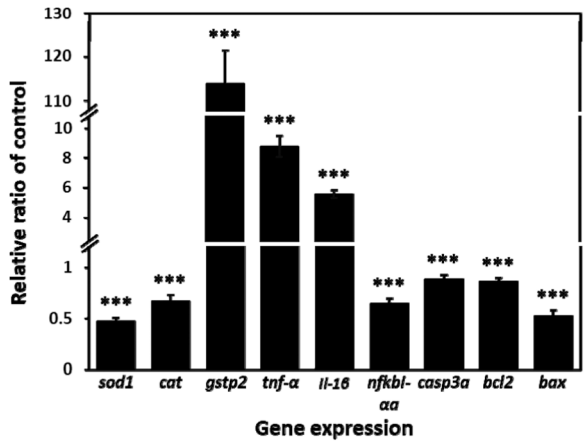

Fig. 5. Transcriptional effects of standard PM2.5 (1000 μg/mL) on the expression of genes related to oxidative stress (*sod1*, *cat*, and *gstp2*), inflammation (*nf-α*, *il-1b*, and *nfkbi-αa*), and apoptosis (*casp3a*, *bcl-2*, and *bax*). The data are shown as the mean ± S.D from three independent experiments. ***p < 0.001 compared to the control.

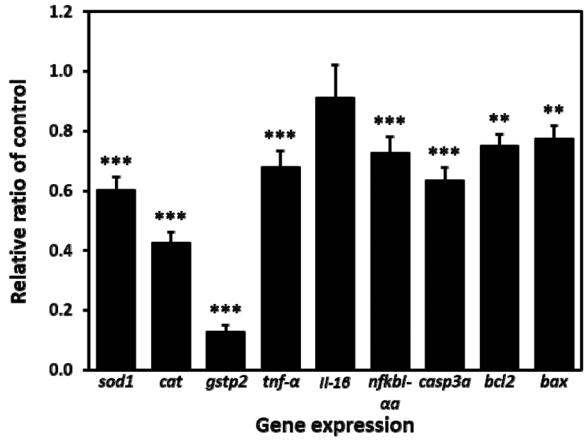

Fig. 6. Transcriptional effects of CNMI PM2.5 (600 μg/mL) on the expression of genes related to oxidative stress (*sod1*, *cat*, and *gstp2*), inflammation (*nf-α*, *il-1b*, and *nfkbi-αa*), and apoptosis (*casp3a*, *bcl-2*, and *bax*). The data are shown as the mean ± S.D from three independent experiments. **p < 0.01 and ***p < 0.001 compared to the control.

transcriptional effects of PM 2.5 collected from central Bangkok, Thailand (CNMI PM2.5), compared to standard PM2.5 (NIST Material 2786 from the USA) on zebrafish embryos. Our findings revealed that both particulate samples led to zebrafish embryo mortality but did not affect the hatching process. Additionally, both samples induced embryonic malformations, including pericardial edema, yolk sac edema, and blood clotting. Furthermore, both standard and CNMI PM2.5 significantly altered the expression levels of genes related to oxidative stress, inflammation, and apoptosis in treated groups compared to the control groups.Top of Form

We investigated the developmental toxicity of both particulate samples using the zebrafish embryo acute toxicity test, following OECD test guideline 236. Exposure to standard and CNMI PM2.5 samples resulted in embryonic mortality with estimated LC_{50} values of 1476 and 512 μg/mL, respectively. This finding indicates that the local particulate, CNMI PM2.5, is more toxic to zebrafish embryos than standard PM2.5. Interestingly, both samples had no impact on the hatching rate of zebrafish embryos. The differential response of zebrafish embryos to PM2.5, where they experience increased mortality, but hatching rates remain unaffected, suggests that the embryos are more sensitive to the toxic effects of PM2.5 during post-hatching stages. Furthermore, the protective role of the chorion, which features pores with a diameter of 0.77 μm [6], may account for the observed PM2.5-induced mortality in zebrafish embryos without interfering with the hatching rates. The chorion acts as a barrier that prevents direct PM2.5 exposure during the early developmental stages. While hatching rates remain unaffected, some embryos may experience mortality or sublethal effects after hatching when they are no longer protected by the chorion. This differential response underscores the importance of considering developmental stages and protective barriers in understanding the impact of pollutants on embryonic development.

In our assessment of zebrafish embryos exposed to both PM2.5, we observed morphological malformations, including pericardial edema, yolk sac edema and blood clotting. These embryonic abnormalities have also been documented in previous studies involving PM2.5 collected from China [11,25,27]. Our findings underscore the consistency of these embryonic abnormalities in response to PM2.5 exposure. The observed morphological abnormalities in zebrafish embryos following exposure to standard and CNMI PM2.5 could result from the presence of harmful substances or pollutants within the particulate samples. Alternatively, these embryonic malformations may be induced by PM2.5 affecting biological processes involved in toxicological outcomes, including oxidative stress, inflammation, and apoptosis.

Gene expression plays a pivotal role in toxicology as it provides insights into how environmental factors, such as exposure to PM2.5, influence biological responses. Examining changes in gene expression allows us to understand the molecular mechanisms behind the observed toxic effects, offering valuable information for assessing the impact of pollutants on living organisms [14,19].

Through qRT-PCR analysis, the results revealed that standard PM2.5 downregulated two oxidative stress-related genes, *sod1* and *cat*, but upregulated *gstp2*. The upregulation of *gstp2* while the downregulation of *sod1* and *cat* occurred in response to standard PM2.5 exposure, indicating a differential response among these oxidative stress-related genes. Cells can adapt to various environmental stressors by modulating gene expression. In this instance, the upregulation of *gstp2* signifies that the cell recognizes the need to enhance the expression of this gene, which may play a critical role in countering the effects of PM2.5 exposure. Conversely, the downregulation of *sod1* and *cat* may imply that the cell regards other defense mechanisms as less necessary or that they are being suppressed due to standard PM2.5 exposure.

In contrast, CNMI PM2.5 downregulated all three oxidative stress-related genes. The uniform downregulation of all three oxidative stress-related genes in response to CNMI PM2.5 exposure suggests a highly coordinated and consistent cellular response. This consistency in gene expression changes may be indicative of a specific signaling pathway or regulatory mechanism that is consistently triggered by CNMI PM2.5 particles. Additionally, the downregulation of these genes may collectively reduce the cell's capacity to combat oxidative stress effectively. This implies that CNMI PM2.5 exposure leads to a decrease in the cell's ability to activate these critical defense mechanisms, potentially increasing susceptibility to the harmful effects of oxidative stress.

Furthermore, gene expression analysis of inflammatory-related genes (*tnf-α*, *il-1b*, and *nfkbi-αa*) revealed upregulation of *tnf-α* and *il-1b* and downregulation of *nfkbi-αa* in response to standard PM2.5 exposure. *Tnf-α* and *il-1b* are key pro-inflammatory cytokines, while *nfkbi-αa* is an inhibitor of *nf-κb* (nuclear factor-kappa b), which plays a pivotal role in regulating immune and inflammatory responses. These findings regarding the differential regulation of inflammatory-related genes in response to standard PM2.5 exposure hold significant implications for our understanding of the cellular response to environmental pollutants. The upregulation of *tnf-α* and *il-1b*, both potent pro-inflammatory cytokines, indicates that PM2.5 exposure triggers an inflammatory response in cells. This response may lead to increased levels of these cytokines, contributing to inflammation within the affected tissues. In contrast, the downregulation of *nfkbi-αa*, an inhibitor of *nf-κb*,

suggests that the cellular machinery responsible for dampening inflammation may be compromised. *Nf-κb* is a central player in regulating immune and inflammatory responses, and its inhibition can result in the uncontrolled activation of pro-inflammatory pathways.

On the other hand, CNMI PM2.5 exposure resulted in the downregulation of *tnf-α* and *nfkbi-αa*, with no significant effect on *il-1b*. The downregulation of *tnf-α* and *nfkbi-αa* implies a possible attenuation of inflammatory pathways in response to CNMI PM2.5, suggesting a different modulation of immune and inflammatory responses compared to standard PM2.5 exposure. The lack of an effect on *il-1b*, however, raises questions about the specificity of the response, as *il-1b* is another crucial pro-inflammatory cytokine. These findings necessitate further research to delve into the intricate molecular mechanisms underlying CNMI PM2.5-induced changes in gene expression and to clarify the potential implications for the health of exposed populations.

In addition, the analysis of apoptosis-related genes (*bax*, *bcl-2*, and *casp3a*) revealed that both standard and CNMI PM2.5 significantly downregulated all three genes. These findings unveil a notable impact on the cellular mechanisms that regulate programmed cell death, known as apoptosis. *Bax* and *bcl-2* are key regulators of apoptosis, with *bax* promoting cell death and *bcl-2* inhibiting it. The simultaneous downregulation of these opposing factors implies a potential disruption in the delicate balance between pro-apoptotic and anti-apoptotic signals within the exposed cells. *Casp3a*, a caspase enzyme, is central to the execution of apoptosis. Its downregulation further suggests an interference with the apoptotic process. These results indicate a significant alteration in the cellular response to PM2.5, possibly affecting cell survival and the overall health implications for individuals exposed to these particulate matters.

The significant downregulation of apoptosis-related genes (*bax*, *bcl-2*, and *casp3a*) following PM2.5 exposure suggests a potential mechanism contributing to decreased survival rates and increased malformations. The downregulation of *bax* and *bcl-2* implies a disruption in the balance between cell survival and cell death pathways, which may hinder the normal development of embryos and contribute to increased mortality. Additionally, the downregulation of *casp3a*, a critical enzyme in apoptosis execution, could further impede the embryo's ability to undergo programmed cell death, potentially leading to malformations. These changes in gene expression patterns may, in part, explain the increased occurrence of pericardial edema, yolk sac edema, and blood clotting in zebrafish embryos exposed to PM2.5. Furthermore, the interplay between the downregulation of oxidative stress and inflammation-related genes and the gene expression changes related to survival and malformations underscores the intricate web of responses initiated by PM2.5 exposure. These findings support the hypothesis that alterations at the genetic level are contributing to the observed phenotypic outcomes and highlight the need for further exploration of the molecular mechanisms underlying PM2.5-induced developmental effects.

The present study underscores the critical importance of source-dependent effects in environmental studies. Notably, the research revealed differential outcomes in response to standard and CNMI PM2.5 exposure, highlighting that the origin of particulate matter significantly influences its impact on biological systems. The fact that CNMI PM2.5 was more toxic to zebrafish embryos than standard PM2.5 exemplifies how the geographical source of pollutants can lead to different levels of toxicity, emphasizing the need to consider regional variations in environmental assessments. Furthermore, the divergent gene expression responses to the two PM2.5 sources illustrate that source-specific chemical compositions can result in distinct cellular reactions.

These findings underscore that generalizing the effects of environmental pollutants, such as PM2.5, needs to account for their origin to be accurate and sufficient for developing effective mitigation strategies. Therefore, source-dependent effects should be a central consideration in environmental research, policy, and regulation to accurately evaluate and address the diverse health and ecological impacts of pollutants and to tailor interventions to the specific sources and characteristics of environmental contaminants. However, this study primarily examined acute effects and a more comprehensive understanding of the long-term consequences of PM2.5 exposure is needed. Future research should delve into the specific chemical components and characteristics of PM2.5 from various sources, as these can vary significantly. Understanding the molecular mechanisms underlying source-specific effects is critical for refining risk assessments and informing targeted strategies for mitigating the health impacts of environmental pollutants. Moreover, studying the impacts of PM2.5 on different developmental stages and addressing potential intergenerational effects could provide a more holistic view of the health consequences of particulate matter exposure.

5. Conclusions

Our findings underscore the critical importance of considering the source of environmental pollutants, particularly PM2.5 when assessing their impact on biological systems. It reveals that PM2.5 from different sources can exert varying toxicity, with source-specific gene expression patterns affecting oxidative stress, inflammation, and apoptosis. Moreover, these source-dependent effects are linked to the observed survival rates and malformations in zebrafish embryos. These findings have far-reaching implications for environmental health, emphasizing the need for tailored risk assessments and interventions that account for regional variations in pollutant sources. They illuminate the intricate molecular mechanisms underpinning the effects of PM2.5 on living organisms, offering valuable insights for public health protection and policy development in the ongoing global battle against air pollution's adverse health consequences.

CRediT authorship contribution statement

Phisit Khemawoot: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Wittaya Pimtong:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Pornpun Vivithanaporn:** Supervision, Funding acquisition. **Siwapech Sillapaprayoon:** Visualization, Software, Investigation. **Pinnakarn Techapichetvanich:** Writing – original draft, Visualization, Software, Investigation, Formal analysis.

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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