

Aerosol and Air Quality Research

# Cytotoxicity and Exposure Assessment of PM<sub>2.5</sub> in a Residential Home during COVID-19 Lockdown

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# ABSTRACT

As individuals become more confined to their homes, especially during the COVID-19 lockdown and the post-pandemic era, human activities will continue to generate more indoor particles. However, the toxicity effects of indoor particles remain unknown during residents' occupancy time. Eighteen 24 hours of indoor and outdoor PM<sub>2.5</sub> samples were collected using 37 mm polyvinyl chloride (PVC) filter within a residential terrace house in Serdang, Selangor, during the 2021 Malaysia lockdown between February and March 2021. PM<sub>2.5</sub> samples were then extracted using methanol. MTT assay determined the cytotoxic activity of extracted indoor and outdoor PM<sub>2.5</sub> treated at different concentrations (25–200  $\mu$ g mL<sup>-1</sup>) on human lung cells (MRC-5) at a 24-hour incubation period. The 24-h mass concentration of outdoor PM<sub>2.5</sub> ( $41.4 \pm 1.99 \,\mu g \,m^{-3}$ ) was significantly three times higher than indoor PM<sub>2.5</sub> ( $11.8 \pm 0.60 \,\mu g \,m^{-3}$ ) (p < 0.05). However, exposure to indoor  $PM_{2.5}$  at higher concentrations (100 and 200  $\mu$ g mL<sup>-1</sup>) on lung cells (MRC-5) significantly reduces cell viability compared to outdoor PM<sub>2.5</sub>, suggesting that exposure to indoor PM<sub>2.5</sub> causes toxicity to the lung cells compared to outdoor PM2.5. In parallel, indoor real-time PM2.5 measurements were recorded in the kitchen during cooking and non-cooking days. We found cooking days generated higher indoor PM<sub>2.5</sub> concentrations (maximum PM<sub>2.5</sub> = 75.0  $\mu$ g m<sup>-3</sup>), suggesting that cooking activity might contribute to the toxicity of indoor PM<sub>2.5</sub>. Due to the limited yield of indoor and outdoor PM<sub>2.5</sub>, further optimization on the extraction of PM<sub>2.5</sub> should be carried out to evaluate further the mechanism of cytotoxicity of indoor PM<sub>2.5</sub> on the lung cells.

Keywords: Cooking, Lockdown, Cytotoxicity, Cancer, Indoor air quality

# **1 INTRODUCTION**

People spent a significant time indoors throughout the COVID-19 lockdown and subsequent post-pandemic period due to the altered dynamics of COVID-19's emergence, particularly regarding adopting remote work practices. This shift in behaviour has shown an impact on their level of exposure and the subsequent effects experienced (Roh *et al.*, 2021). Internal sources such as indoor combustion, including cooking, smoking, and particle re-suspension, lead to even worse air quality than outdoor, with an indoor/outdoor (I/O) ratio above one (Martins and Carrilho da Graça, 2018). Extensive cooking activities during the lockdown period have increased the emission of cooking oil (Du and Wang, 2020). It is also known that the cooking process releases significant amounts of particulate matter (PM), hence contributing to the emission of cooking effluents (Zhao *et al.*, 2019).

Fine particulate matter ( $PM_{2.5}$ ) is a significant air pollutant that harms human health, particularly cardiorespiratory health.  $PM_{2.5}$  mainly comprises several compounds, including organic fractions, trace elements, crustal elements, heavy metals, and carbonaceous species. Approximately 30–37%



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of outdoor PM<sub>2.5</sub> in urban Southeast Asia cities were released from traffic motor vehicles (Karagulian *et al.*, 2015). Significant contributions are from exhaust fume particles of diesel-powered exhaust, brake and tire wear, and resuspension of the settled particle on the road surface (Tunno *et al.*, 2016; Hatzopoulou *et al.*, 2013). Another 27–34% of emissions are from industry and power generation (Karagulian *et al.*, 2015). In addition, limited studies have conducted to collect indoor and outdoor PM<sub>2.5</sub> oxidative potential during COVID-19 lockdown (Altuwayjiri *et al.*, 2021).

Epidemiological studies demonstrate significant associations between individuals' exposure to particulate matter (PM) and adverse health effects. Indoor particle composition and toxicity can be quite complex, with both resembles and differences from outdoor PM (Morawska *et al.*, 2013) depending on the source and environment. The presence of particulate bound-heavy metals and polycyclic aromatic hydrocarbons (PAHs) can lead DNA damage, cell death and genotoxicity (Dou *et al.*, 2018; Figliuzzi *et al.*, 2020). When the airborne particles travel down to the lung, they will cause injury along the tract due to free radical peroxidation-producing oxidate stressor (OS), which is the underlying factor of those injuries (Greenwell *et al.*, 2002). This will lead to inflammation associated with lung damage and cardiovascular changes (Aztatzi-Aguilar *et al.*, 2018). Secondary systemic effects that modify the translocation of particulate matter (PM) or PM-induced mediators from the alveoli into circulation have been hypothesized to cause direct toxic cardiovascular effects once the OS and inflammation mechanisms are activated (Hamanaka and Mutlu, 2018). Chronic exposure to PM<sub>2.5</sub> promotes the proliferation of lung cancer cells through the activation of epidermal growth factor receptor (EGFR) signalling (Wang *et al.*, 2023).

Even with extensive research on PM<sub>2.5</sub> exposure, there is still a knowledge gap concerning the cytotoxicity of PM<sub>2.5</sub> exposure toward lung cells in specific settings, notably in suburban regions with diverse pollution sources. PM<sub>2.5</sub> concentrations in suburban Malaysia vary significantly due to various sources, including commercial and industrial development, motor vehicles, and transboundary haze (Ab. Rahman *et al.*, 2022). The suburban area of Serdang, Selangor, Malaysia, represents a unique setting with a combination of residential, educational, business, and industrial zones and transportation infrastructure. This study aims to investigate the cytotoxic effects of PM<sub>2.5</sub> in indoor and outdoor environments of a single terrace house in Serdang, shedding light on the toxicity analysis of PM<sub>2.5</sub> in a typical suburban residential setting. This study considered data from a limited sampling site due to the Malaysia Movement Control Order (MCO) lockdown and observed a unique opportunity to assess the average integrated daily residential exposure given the occupancy time is 24 hours. To progress toward this broader objective, we assessed PM<sub>2.5</sub> concentrations during cooking events, contributing to the occupant's total daily integrated personal exposure in a lockdown home.

# 2 METHODS

#### **2.1 Study Location**

To collect the indoor and outdoor PM<sub>2.5</sub> samples, field monitoring was performed in a residential terrace house located in a township of the suburban area of Serdang of Selangor state, Malaysia (Fig. 1). Serdang is located in the Petaling District of Selangor and approximately 15 km south from Kuala Lumpur City Centre. It covers an area of 10.62 km<sup>2</sup> with a population density of about 9,929/km<sup>2</sup>. The site includes residential, higher educational institutions, business and industrial zones, and railway transit stations. This area is connected to other significant parts of Greater Kuala Lumpur via major roads and the North-South Expressway PLUS, which becomes an important source of traffic-related air pollution. Putrajaya is the nearest air quality monitoring station, 9 km southwest of the study location.

The single-story residential house has a built-up area of 816 square meters and is equipped with three bedrooms, a toilet, a kitchen, and a living room over fifteen years old. This residence relies solely on natural ventilation via open windows and is fully occupied by three non-smoking occupants. In addition, the house's LPG-fuelled kitchen does not have a range hood and only has an exhaust vent. All samplings were carried out between February and March 2021, which coincided with 2021 Malaysia's third wave of the COVID-19 pandemic and movement control order (MCO) lockdown (Zamri *et al.*, 2021). The mean  $\pm$  SD of indoor and outdoor temperature during 24 hours





Fig. 1. Location of the study area city of Serdang.

of sampling was  $28.4^{\circ}C \pm 0.3$  and  $28.6^{\circ}C \pm 1.0$ , respectively. The recorded mean  $\pm$  SD of indoor and outdoor humidity readings during the sampling was  $62.2\% \pm 4.2$  and  $73.9\% \pm 6.0$ , respectively.

#### 2.2 PM<sub>2.5</sub> Mass Concentrations and Real-Time Monitoring

A portable low-volume personal air sampler, Escort ELF (Zefon International, FL, USA), fitted with a nylon cyclone, was used with a flow rate of  $1.7 \text{ L} \text{min}^{-1}$ . Indoor and outdoor PM<sub>2.5</sub> samples were collected for 24 hours on a 5.0  $\mu$ m PVC 37 mm filter (Zefon International, FL, USA). Before sampling, the filter was pre-baked at 40°C in a laboratory incubator oven (Memmert INB200, Schwabach, Germany) for two hours. The gravimetric analysis determined the PM<sub>2.5</sub> mass concentration using an analytical weighing balance (A&D Weighing GX-400, Japan) in a controlled temperature and relative humidity weighing room. Filter weights were analysed in triplicate after conditioning for 24 hours in desiccators before and after each sampling session.

Meanwhile, PM<sub>2.5</sub> concentrations during cooking and non-cooking days were measured using AM520 side Pak (TSI Instruments, Minnesota, USA). During sampling, the environmental condition, including wind speed, temperature, humidity, and pressure, was measured using a weather meter, Kestrel 5500 (Kestrel instruments<sup>®</sup>, Pennsylvania, USA). Ambient PM<sub>2.5</sub> levels were obtained from the nearest government monitoring station, Putrajaya.



# **2.3 Reagents and Chemicals**

Methanol was purchased from Fischer Scientific (Seoul, Korea); Roswell Park Memorial Institute medium (RPMI-1640), sodium bicarbonate (NaHCO3), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Aldrich® (Merck, St. Louis, USA); fetal bovine serum (FBS) was purchased from Tico Europe (Amstelveen, Netherland); dimethyl sulfoxide (DMSO) was purchased from EMSURE® (Massachusetts, USA); 95% ethanol was purchased from Alchemy Supplies (Exabytes, Selangor, Malaysia); 0.5% Trypsin-EDTA (10×) was purchased from Gibco® (Thermo Fischer Scientific, Loughborough, USA); penicillin-streptomycin solution (100×) was purchased from BBI Life Science (Shanghai, China); trypan blue solution (0.4%) was purchased from ScienCell<sup>™</sup> Research Laboratories (Carlsbad, USA); and phosphate-buffered saline (PBS) tablet was purchased from WWR® Life Science AMRESCO (Ohio, USA).

#### 2.4 PM<sub>2.5</sub> Extraction

 $PM_{2.5}$  filter samples were extracted using an ultrasonication method (Roper *et al.*, 2019) with a slight modification. The samples and blank PVC filters were placed in a 50 mL falcon tube containing 5 mL methanol. The filters were sonicated for 10 minutes in a water-bath sonicator at room temperature (27°C). The filters were then rinsed with 1 mL methanol to remove any residual particles before discarding them. The extraction procedure was repeated three times for each of the filters. The extracts were then dried in the oven at 40°C for 24 hours. The concentrated  $PM_{2.5}$  extract was stored at  $-20^{\circ}$ C for further MTT assay use.

#### 2.5 Cell Line

Human lung fibroblast cells (MRC-5) were purchased from American Type Culture Collection (ATCC®) (ATCC accession number MRC-5 ((CCL-171<sup>M</sup>), Manassas, USA). MRC-5 was cultured in RPMI-1640 and supplemented with 10% FBS and 1% of penicillin-streptomycin solution (10×). Cells were grown in a T75 tissue culture flask and incubated at 37°C with 5% CO<sub>2</sub>.

#### 2.6 MTT Assay

Cytotoxicity of the extracted indoor and outdoor PM<sub>2.5</sub> samples on MRC-5 cells was determined using MTT assay as previously described (Gouvea *et al.*, 2012). MRC-5 cells ( $2 \times 10^4$  cells well<sup>-1</sup>) were seeded in 96-well plates for 24 hours and then treated with different concentrations (25, 50, 100, and 200 µg mL<sup>-1</sup>) of extracted indoor and outdoor PM<sub>2.5</sub> samples. The untreated cells were included as the control group. Following 24 hours of incubation at 37°C, 20 µL of MTT solutions (5 mg mL<sup>-1</sup>) was added to each well and incubated for three hours at dark conditions. After removal of the supernatant containing MTT solution, dark blue formazan crystals formed and were dissolved in 100 µL DMSO. Absorbance (Abs) was read at a wavelength of 570 nm and a reference wavelength of 620 nm using a microplate reader (Tecan, Infinite® F50, Männedorf, Switzerland). The percentage of cell viability for each concentration was calculated using Eq. (1). The experiment was performed in three independent experiments (triplicate for each concentration).

Percentage of cell viability (Abs\_sample/Abs\_control)  $\times$  100%

(1)

# 2.7 Statistical Analysis

Statistical analysis is performed using Statistical Package for Social Science (SPSS) 25.0 software (IBM, New York, NY) and Microsoft Excel 365 (Microsoft Inc., USA). Results were presented as mean  $\pm$  SD. Independent T-test and One-Way Analysis of Variance (ANOVA) were used to analyse the results. Dunnett's multiple comparisons test was used to determine the significant differences from the control. A *p*-value of less than 0.05 (*p* < 0.05) was considered significant.

# 2.8 Quality Assurance and Quality Control

During air sampling, the portable low-volume personal air sampler was located away from any obstacles to prevent restricted airflow. A secured electrical supply was obtained to operate the sampler, and the sampler was ensured to be working properly. Also, the sampler flow rate was



calibrated and adjusted to  $\pm$  5% before and after each sampling session using a flow metre calibrator model 4148 (TSI Instruments, Minnesota, USA) to ensure the flow rate remained constant during the sampling. Moreover, field blanks with approximately 5–10% of the total samples were collected with the sampling pump turned off to evaluate any potential contamination.

All filters were visually inspected thoroughly before being equilibrated in the conditioning environment (20°C) inside a desiccator for 24 hours before and after sampling. At the end of sampling, the filter was removed carefully by only touching the outer edge. In the process of weighing the filter paper and chemical substances, the weighing scale was ensured to be calibrated. Before weighing, the filter was passed through the mini-ioniser fan model 6213 (NRD Staticmaster®, New York, USA). The weighing scale was swiftly brushed with an anti-static brush to eliminate any static charge affecting the balance reading.

For quality assurance and control during the MTT assay, a control group containing untreated cells was prepared for each of the tested concentrations of all samples. It is a must since, to obtain a cell viability percentage, the absorbance of treated cells with indoor and outdoor  $PM_{2.5}$  samples must be deducted from the absorbance of untreated cells. Besides, control is important to determine whether the cell viability of cells treated with  $PM_{2.5}$  samples at a certain concentration was significant compared to untreated cells using one-way ANOVA statistical analysis.

The mean and standard deviation of cell viability were calculated to represent each of the tested indoor and outdoor PM<sub>2.5</sub> concentrations. Moreover, the complete MTT assay protocol for indoor and outdoor PM<sub>2.5</sub> samples was run in an independent experiment (triplicate for each experiment) using different passage numbers on different days. This biological replicate concept was practiced ensuring that the cell viability results obtained were parallel and the probability of errors was reduced. The mean and standard error of the mean of three independent experiments were calculated to represent the obtained MTT assay result.

# **3 RESULTS AND DISCUSSION**

# **3.1** Outdoor PM<sub>2.5</sub> Concentrations is Significantly Higher Compared to Indoor PM<sub>2.5</sub>

Fig. 2 shows the average of PM<sub>2.5</sub> mass concentrations collected from 24-hours accumulated indoor and outdoor samples. Levels of PM2.5 recorded from the Putrajaya monitoring station were also shown in Fig. 2 during the period coincided with our sampling campaign. The average PM<sub>2.5</sub> mass concentration measured outdoors was significantly three times higher than indoors (indoor:  $11.8 \pm 0.60 \ \mu g \ m^{-3}$  and outdoor:  $41.4 \pm 1.99 \ \mu g \ m^{-3}$ ) (p < 0.05) and almost two times higher than measured at Putrajaya Monitoring Station (23.8  $\pm$  6.5  $\mu$ g m<sup>-3</sup>). This result may be due to the relaxation of mobility for business and occupational purposes, contributing to increased traffic on a nearby highway near the study location. The mean 24-hour PM<sub>2.5</sub> mass concentration measured slightly exceeded the New Malaysia Ambient Air Quality Standard (NMAAQS) for daily  $PM_{2.5}$  exposures with a mean limit of 35  $\mu$ g m<sup>-3</sup>.  $PM_{2.5}$  levels were highly dependent on meteorological conditions, with lower levels recorded during the northeast monsoon (Othman et al., 2022), which coincided with our study period. The ambient PM<sub>2.5</sub> in the suburban area was reported to have a lower concentration than in urban areas, with an almost 20% reduction (Strosnider et al., 2017). This is due to the fact that, while pollutants can be carried downwind from urban sources and contribute to pollution levels in neighbouring areas, the sources of these air pollutants are more concentrated in urban areas with higher levels of urbanisation and industrial development. Thus, the increased wind speed and precipitation may contribute to the relatively low ambient PM<sub>2.5</sub> concentrations.

The average indoor/outdoor (I/O) ratio obtained in this study was  $0.33 \pm 0.17$  with a correlation coefficient (*r*) value of 0.73, indicating that outdoor sources significantly influence the indoor PM<sub>2.5</sub> level (Han *et al.*, 2016). The correlation between indoor and outdoor pollutant levels was affected by penetration rates of ambient PM<sub>2.5</sub> indicated by Air exchange rate (AER) reading, indoor sources of emission, and indoor decay rates (Tofful *et al.*, 2020; Meier *et al.*, 2015). Both natural and mechanical ventilation controlled the kitchen's airflow in our study location. The windows were opened during daylight hours, and the installed exhaust fan was switched on.



**Fig. 2.** Summary of PM<sub>2.5</sub> concentrations ( $\mu$ g m<sup>-3</sup>) obtained from average values collected from indoor and outdoor samples and Putrajaya Monitoring Station, Malaysia. Box plots denote minimum values, 1<sup>st</sup> quartile, median, 3<sup>rd</sup> quartile and maximum values.

There were no apparent cracks or openings in the walls or ceiling, which explains why the indoor PM<sub>2.5</sub> level was lower than the outdoor level. Malaysia has a hot and humid climate, so most residential houses are built to be airtight to keep temperature, humidity, and energy consumption within ideal limits (Reynolds, 2019). However, with limited natural ventilation and human-related heat-released activities indoors, negative air pressure worsened indoor air quality (Ibiyeye *et al.*, 2015). As a result, mechanical ventilation systems heavily rely on providing comfort and better IAQ for occupants (Kubota *et al.*, 2009).

#### 3.2 Higher Indoor PM<sub>2.5</sub> during Cooking Days in a Lockdown House

Further analysis was conducted to compare real-time PM2.5 concentrations on cooking and non-cooking days in a study location of a lockdown house. Our finding found that cooking days generated higher PM<sub>2.5</sub> concentrations with maximum PM<sub>2.5</sub> recorded at 75.0  $\mu$ g m<sup>-3</sup> (average = 34.0  $\mu$ g m<sup>-3</sup> ± 17) compared to non-cooking days (average = 23.9  $\mu$ g m<sup>-3</sup> ± 8) (Fig. 3). During cooking days, occupants prepared food at least once a day for breakfast, primarily by frying, followed by broiling on an LP gas stove, microwave, or air fryer. The highest real-time PM<sub>2.5</sub> peak was recorded between 10:00 and 11:00 a.m. on each cooking day when occupants were busy preparing breakfast and lunch. The lowest PM<sub>2.5</sub> peak was observed during dinner, indicating that the occupants rarely ate dinner or ordered delivery food. Compared to other households, occupants' cooking activities were lower than usual. The average modern adult spends more than 20% of their daytime in the kitchen, including cooking, eating, and cleaning up after meals, especially during this COVID-19 pandemic, when citizens are not allowed to dine in. Increased frequency of cooking activities during the MCO resulted in a significant increase in PM<sub>2.5</sub> concentrations measured indoors, with the highest average concentration exceeding the NMAAQS 2020 standard. Staying indoors was predicted to pose a 25% increased risk of lung cancer (Ezani et al., 2021). The findings from various studies consistently indicate substantial reductions in the ambient PM2.5 concentrations during COVID-19 lockdown (Yao et al., 2022). However, it is necessary to acknowledge the increased of indoor PM<sub>2.5</sub> due to consequence of intensified domestic activities such as solid-fuel burning and smoking during stay-home restriction. When these solid fuels burn inefficiently, particles like PM<sub>2.5</sub> are released into the house, exposing occupants to indoor air pollution.

Wan *et al.* (2011) reported that during the cooking episode, the average concentrations of  $PM_{2.5}$  were approximately 20–40 times the kitchen background level. In contrast, they were about ten times the living room background level. They accounted for at least 60% and 73% of





Fig. 3. Levels of  $PM_{2.5}$  concentrations ( $\mu g m^{-3}$ ) measured during cooking and non-cooking days.

the kitchen and living room surface area concentration, respectively. Depending on the cooking procedures, cooking generated PM<sub>2.5</sub> emission rates ranging from 2.14 mg min<sup>-1</sup> to 22.84 mg min<sup>-1</sup> (Kang *et al.*, 2019). Surprisingly, broiling was the most polluting cooking technique, followed by frying, including deep and stir-frying. Broiling foods resulted in average PM<sub>2.5</sub> concentrations three times that of frying (Jung and Su, 2020). The average indoor temperature and relative humidity were recorded at  $27.9^{\circ}$ C ± 0.5 and  $65.8\% \pm 1.8$  respectively during cooking days. While during non-cooking days, indoor temperature and relative humidity were recorded at  $28.3^{\circ}$ C ± 0.4 and  $63.2\% \pm 4.2$  respectively. Air temperature during cooking days is slightly higher compared to the cooking days, however this may influence by high outdoor temperature  $28.6^{\circ}$ C ± 1.0. During cooking, natural ventilation aids to regulate relative humidity in an effective way (Chen *et al.*, 2023).

#### 3.3 Reduction in the Cells Viability of MRC-5 Cells Treated with Indoor PM<sub>2.5</sub>

The cytotoxic effect of indoor and outdoor PM2.5 on MRC-5 cells was assessed using the MTT assay. This assay relies on the conversion of soluble yellow tetrazolium (MTT) into insoluble purple formazan crystals (MTT formazan) by the reduction in viable cells due to the cell's metabolic activity (Stoddart, 2011). Normal human lung cells, MRC-5 cells, were used since they are commonly utilized in toxic agents' research and have been widely used in the medical treatment industry (Gouvea et al., 2012). Fig. 4 shows the percentage of MRC-5 cell viability after treatment with various concentrations (25–200  $\mu g$  mL<sup>-1</sup>) of collected indoor and outdoor PM<sub>2.5</sub> samples. Our finding demonstrates that the cell viability of MRC-5 cells treated with the highest concentration of outdoor PM<sub>2.5</sub> samples (100–200  $\mu$ g mL<sup>-1</sup>) is significantly higher compared to the untreated cells (p < 0.05). In contrast, exposure to indoor PM<sub>2.5</sub> on MRC-5 cells induces a reduction in cell viability at higher concentrations (100–200  $\mu$ g mL<sup>-1</sup>) than untreated cells (p < 0.05) (Figs. 4(A) and 4(B), suggesting that exposure to indoor PM<sub>2.5</sub> cause reduction in the cell viability of lung cells as compared to outdoor PM2.5. The use of alveolar epithelial cells from the human lung adenocarcinoma cell line A549 has also been applied, apart from MRC-5, for in vitro studies into PM mechanisms. Cell death (30-40%) and reactive oxidative stress (ROS) production were observed in 24-hour exposure during the winter season in Beijing and Shanxi (Lai et al., 2021).

Our current finding agrees with Chen *et al.* (2020) and Tong *et al.* (2019). The increased concentration of exposure to  $PM_{2.5}$  resulted in decreased viability of exposed cells, as indicated by increased lactate dehydrogenase (LDH) release (Chen *et al.*, 2020; Tong *et al.*, 2019). At a treatment concentration of 100 µg mL<sup>-1</sup> of total  $PM_{2.5}$ , the cell viability percentage dropped to 65.5% compared to control cells (Song *et al.*, 2019). Furthermore, at a 1000 µg mL<sup>-1</sup> of urban particulate matter (NIST-1648a) concentration (using standard reference material), the cell viability was reduced to



**Fig. 4.** Reduction in percentage of cell viability of MRC-5 cells treated with highest concentration of indoor PM<sub>2.5</sub> as compared to the (A) untreated cells and (B) outdoor PM<sub>2.5</sub> for 24-hours incubation using MTT assay. Data were presented as mean  $\pm$  SEM of three independent experiments. Oneway ANOVA and independent t-test were used to analyse the results presented in A and B, respectively. Dunnet test was used to analyse the significant differences among the concentration as compared to the untreated cells (control). The significant mean difference of *p*-value less than 0.05 and 0.01 is shown as \* and \*\* respectively.

40% (Das *et al.*, 2021). Interleukin 6 level (IL-6), the pro-inflammatory cytokine pathway, and 8-isoprostane level, a marker of OS, were elevated in exposed cells when treated with indoor and outdoor  $PM_{2.5}$  samples. Cell viability rose when cells were exposed to water-soluble fraction, but it decreased dramatically when exposed to total  $PM_{2.5}$  and organic soluble fraction (Song *et al.*, 2019).

Since cooking was identified as the source of indoor PM in this study, it may contribute to the toxicity of PM<sub>2.5</sub>. Indoor PM<sub>2.5</sub> were proved to be more toxic than indoor as it caused severer DNA damage in mice (Wierzbicka *et al.*, 2022). It has been found that increased relative amounts of metals, PAHs, and endotoxins may influence the indoor toxicity compared to the outdoor PM<sub>2.5</sub>. Additionally, the COVID-19 partial and full lockdown reduced ambient PM<sub>2.5</sub> toxicity by approximately 25% in the Milan metropolitan area due to reduced vehicle traffic (Altuwayjiri *et al.*, 2021).



Due to the restricted yield of indoor and outdoor of our PM<sub>2.5</sub> samples, further study on PM<sub>2.5</sub> extraction should be conducted to examine further the mechanism of cytotoxicity of indoor PM<sub>2.5</sub> on lung cells. The study's limitation was the low number of samples collected due to the lockdown period. It is possible that the lack of cytotoxicity was due to the low PM<sub>2.5</sub> mass concentration collected in this study. As a result, it is recommended that the effects of indoor and outdoor PM<sub>2.5</sub> on human health to be further investigated. Additionally, since the composition of PM<sub>2.5</sub> toxicities varies depending on the sources of emission, chemical analysis is required to determine the composition of extracted PM<sub>2.5</sub>. Chemical analysis methods such as thermal optical carbon analysis for carbonaceous species, ion chromatography for water-soluble inorganic ions, and inductively coupled plasma atomic emission spectroscopy for elemental fractions can be included in the future study.

# **4 CONCLUSIONS**

A significantly three-fold higher concentration of outdoor  $PM_{2.5}$  was recorded in the present study compared to indoor  $PM_{2.5}$  during the COVID-19 lockdown period in the study location. A strong correlation of indoor/outdoor ratio obtained proved that outdoor  $PM_{2.5}$  does affect indoor pollutant levels as a whole (r = 0.73). Furthermore, our finding found that cooking days generated higher  $PM_{2.5}$  concentrations than non-cooking days. In future studies, it is worth to characterize indoor exposure profiles among home dwellers and occupational exposure among home-cook business vendors living in the post-COVID world.

MTT assay analysis revealed that exposure to the collected indoor PM<sub>2.5</sub> on MRC-5 cells reduces cell viability at higher concentrations (100–200  $\mu$ g mL<sup>-1</sup>) compared to outdoor PM<sub>2.5</sub>. Further works on reactive oxidative stress (ROS) assessment related to PM<sub>2.5</sub> and other combustion-related indoor emissions are needed to examine acute and long-term health effects. This study identified cooking as the source of indoor PM, so it is possible that cooking contributes to the toxicity of PM<sub>2.5</sub>. Due to the limited yield of indoor and outdoor PM<sub>2.5</sub> samples, however, further optimization on PM<sub>2.5</sub> extraction is required to examine the mechanism of cytotoxicity of indoor PM<sub>2.5</sub> on lung cells.

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