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# The cytotoxicity and genotoxicity of $PM_{2.5}$ during a snowfall event in different functional areas of a megacity

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**Abstract**

Atmospheric fine particulate matter ($PM_{2.5}$) can harm human health, but the chemical composition and toxicity of $PM_{2.5}$ pollution might vary with weather conditions. In order to investigate the impacts of snowfall weather on aerosol characteristics and toxicity by changing particle sources and components, the daily $PM_{2.5}$ samples were collected before, during, and after a snowfall event in urban, industrial, suburban, and rural areas of Nanjing city in eastern China, for both

chemical composition analysis and cytotoxicity tests. After 24 h exposure to these $PM_{2.5}$, the cell activity, oxidative stress indicators and inflammatory factor expression levels of human lung epithelial cells A549 were measured by ELISA, and DNA damage was determined by comet assay. Although the concentrations of $PM_{2.5}$ in the air were reduced during snowfall, they posed stronger cytotoxicity, genetic toxicity and the ability to inflammatory responses to A549 cells. Related to the elevated mass concentrations of some components accumulated in $PM_{2.5}$ during snowfall, As, Co, Cr, Sr, V, water-soluble $Na^+$ and $Ca^{2+}$ showed positive correlations with toxicity indicators. Therefore, snowfall will clean air by deposition, but also make the $PM_{2.5}$ components remaining in air mostly anthropogenic by covering ground soil/dust, thus increase the particle's mass-based cytotoxicity and their health risks still cannot be ignored, such as the heavy metals and water-soluble ions from automobile exhaust and coal combustion.

**Keywords**: Atmospheric fine particles; Weather events; Cell toxicity; Health risks; Chemical composition; Pollution sources

## 1. Introduction

With the rapid development of global industrialization and urbanization, air pollution has been serious environmental issue in many regions, especially the risks of atmospheric fine particulate matters ($PM_{2.5}$; Chowdhury et al., 2018; Heft-Neal et al., 2018). Owing to the characteristics of long-term retention and long-range transport in the atmosphere, $PM_{2.5}$ has important impacts on environmental quality, atmospheric

visibility, and climate change (Sofiev et al., 2018). Evidence showed that $PM_{2.5}$ can enter the body through ingestion, skin contact and inhalation exposure, causing a variety of respiratory and cardiovascular diseases (Makkonen et al., 2010).

The $PM_{2.5}$ mixture has complex compositions from diverse sources, thus can induce different health effects (Liu et al., 2016). Some studies found that Pb, Cr, Cd, $NO_3^-$, $SO_4^{2-}$, and $Fe^{2+}$ can cause the formation of reactive oxygen species and oxidative stress, and enhance oxidative DNA damage induced by the generated free radicals (Rezaei et al., 2014; Xing et al., 2016). In addition to the influences of various pollution sources on $PM_{2.5}$, varied meteorological conditions also control the processes of dilution, diffusion, deposition, transportation and transformation of particles, thereby affecting the concentration and composition of $PM_{2.5}$ in the air (Ghio et al., 2012; Liao et al., 2017). Snowfall is such a special but overlooked weather event. Snowflake has a large specific surface area, high porosity and slow settling speed, thus can trap pollutants. Snowfall can effectively remove gaseous and particulate pollutants from the air, and the larger the particles, the more obvious (Liu et al., 2019; Nazarenko et al., 2017). Snow covering the ground can also block the soil/dust suspension to air, which reduces the natural sources of atmospheric particulates and makes them mostly anthropogenic. Therefore, snowfall will clean air by decreasing the concentration of $PM_{2.5}$, but how about the toxicity of those $PM_{2.5}$ still rest in air owing to their entirely anthropogenic components? These answers related to human activity and public health could be much helpful for guiding the oriented control of air pollution emission. However, no study has investigated this

point.

Since the harm of $PM_{2.5}$ to human health depends on both air concentration and particle toxicity, in order to accurately evaluate the integrated health risks of $PM_{2.5}$ under different weather conditions, the composition and toxicity of $PM_{2.5}$ in snowfall events were studied in this paper. The scientific hypothesis is that snowfall will cover most of the ground and roads, which can reduce the contribution of natural sources to air $PM_{2.5}$ and thus change particle toxicity. To achieve it, $PM_{2.5}$ samples from four different functional areas of a megacity during different periods of a snowfall event were collected. Both the cytotoxicity and genotoxicity differences of these $PM_{2.5}$ on A549 cells were compared, and combined with chemical analysis to further analyze their correlations with source-related particle components.

## 2. Material and methods

### 2.1 Study areas and $PM_{2.5}$ sampling

The air particle samples were collected from an industrial, an urban, a suburban and a rural site in Nanjing city, eastern China, during January 23-29, 2018 covering a snowfall event period. The urban sampling site is located in an institute in downtown, and the industrial site is in a campus near large petrochemical and metallurgical factories. The suburban site is located in the campus area of the southern suburbs, and the rural site is surrounded by orchards and farmlands. According to local meteorological conditions and air quality monitoring data, the snowflake quickly covered the ground after snowfall. Therefore, the sampling period was divided into

three intervals, Jan. 23-24 was before snowfall (BS), 25-27 was during snowfall (DS), and 28-29 was after snowfall (AS). Totally 28 $PM_{2.5}$ samples were collected for 23h daily by a high-volume sampler (1000 L $min^{-1}$) using quartz fiber filters (QMA, 203 mm × 254 mm, Whatman, UK) which were prebaked at 400 °C for 4 h to remove organic substances before sampling. The filters were equilibrated under a constant temperature and humidity condition before and after sampling and weighted by a high-precision electronic balance. After weighing, $PM_{2.5}$ filters were cut into subsamples by ceramic scissors and stored in the refrigerator for following chemical analyses and toxicity tests.

## 2.2 Chemical analysis of $PM_{2.5}$ samples

Subsamples of $PM_{2.5}$ filters were taken for analyses of heavy metals, water-soluble ions and organic carbon contents. For water-soluble components, filter pieces immersed in ultrapure water were sonicated for 1.5 h in a pre-cooled ultrasonic cleaner, and then the $PM_{2.5}$ suspension was filtered by 0.22 μm filtration membranes. The major anions ($Cl^-$, $NO_3^-$ and $SO_4^{2-}$) were determined by Dionex ICS-1100 (Thermo Fisher Scientific, USA), the major cations ($Na^+$, $K^+$, $NH_4^+$, $Ca^{2+}$, and $Mg^{2+}$) were analyzed by Dionex DX-600 (Thermo Fisher Scientific, USA), and the water-soluble organic carbon (WSOC) was determined by TOC-V-CPH/CPN. For the contents of metal, filter samples were digested by $HNO_3$-$HClO_4$-HF acids, and determined by inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima8000, PerkinElmer) and ICP mass spectrometer (ICP-MS, NexION300X,

PerkinElmer).

**2.3 Cell culture**

Human lung epithelial A549 cells (American Type Culture Collection, ATCC) for cytotoxicity assay were cultured in RMPI-164 (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, USA) and 1% antibiotic penicillin-streptomycin (100 U/mL) at 37℃ in a 5% $CO_2$ atmosphere. When the cell growth density reached more than 80%, the 0.25% trypsin digestion was performed. Cells within 10 generations after passage of primary cells were used for toxicity tests, all of which were collected in the exponential growth period.

**2.4. *In vitro* toxicity assays**

For toxicity tests, each $PM_{2.5}$ filter was cut into small pieces and sonicated within ultrapure water for 3 h in a pre-cooled ultrasonic cleaner, and then the $PM_{2.5}$ suspension was collected into sterile centrifuge tubes after removing the pieces of QMA materials by 2.5 μm pore-size filtering. The culture medium was fixed to a $PM_{2.5}$ concentration of 80 μg $mL^{-1}$ suspension. A549 cells were supplemented with 0.25% trypsin-digested, and $2\times10^4$/ml cell suspension was seeded into the 96-well (100 mL/well) culture plate (Costar, USA) at 37 ℃ in a 5 % $CO_2$ incubator. After 24h incubation, 10 μL $PM_{2.5}$ suspensions were added to the plate and the blank control and parallel wells (n = 3) were set simultaneously. After 24 h culture, 10 mL of CCK-8 reagent (Beijing Solarbio, CN) was added to blank control and parallel wells, and 2 h

136 further culture was continued in dark. Optical density value for cell viability was
137 measured by a microplate reader (Thermo MULTISKAN FC, USA) at a wavelength
138 of 450 nm. Similarly, after 24 h of cell exposure, the oxidative stress index and
139 inflammatory factor expression level were measured by enzyme-linked
140 immunosorbent assay (ELISA) kits (Jiangsu Enzymatic Biotechnology, CN).
141
142 **2.5 Comet assay**
143 Furthermore, the comet assay (Ji et al., 2019; Karbaschi and Cooke, 2014) was
144 used to measure DNA damage under alkaline conditions (pH > 13) by the following
145 protocol. The cells in 24-well culture plate exposed to 80 μg $mL^{-1}$ $PM_{2.5}$ for 24 h were
146 buried in 0.7 % low melting agarose and then placed on a fully frosted microscope
147 slide coated with a normal melting agarose layer. The treated slides were immersed in
148 lysis buffer (100 mM EDTA disodium salt, 1 % TRITON X-100, 10 mM Tris–HCl,
149 2.5M NaCl and 10 % DMSO), under dark conditions at pH 10 and 4 ℃ overnight.
150 Then slides were placed in alkaline running buffer (300 mM NaOH, 1mM $Na_2EDTA$,
151 pH > 13) for 40 min and ran in the same buffer for 20 min. After the slides were
152 sequentially transferred to neutralization buffer (0.4 M Tris-HCl, pH 7.5, 20 min);
153 cold $UPH_2O$ (20 min); propidium iodide (2.5 μg $mL^{-1}$, 20 min in the dark). Finally,
154 they were washed in cold $UPH_2O$ for 20 min and observed with a fluorescence
155 microscope. CSAP comet analysis software was used to analyze selected cells in each
156 sample. The percentage of tail DNA was selected as a parameter for assessing DNA
157 damage.

158

159 **2.6 Statistical analysis**

160 The data were compiled using Excel 2016 statistics, plotted by Origin (2017),
161 and analyzed by IBM SPSS statistics 25 for correlation and variance analysis. Pearson
162 product-moment correlation coefficient was adopted for the relations between
163 cytotoxicity and components, and T-test was utilized for the differences between the
164 experimental and control group. The variance was statistically significant when the
165 statistical test level was $P < 0.05$, and extremely significant when $P < 0.01$. The
166 one-way analysis of variance (ANOVA) was used to compare the means of three types
167 of $PM_{2.5}$ samples (BS, DS, and AS) from the same site, and Fisher's Least Significant
168 Difference test (LSD) conducted for multiple comparisons.

169

170 **3. Results**

171 **3.1 $PM_{2.5}$ concentrations in different urban functional areas during a snowfall**
172 **event**

173 During the three sampling periods, the average $PM_{2.5}$ concentrations of the four
174 sampling sites were 67.1, 52.4 and 141.4 μg $m^{-3}$ in industrial areas, 54.8, 45.2 and
175 134.6 μg $m^{-3}$ in urban areas, and 90.1, 47.3 and 122.1 μg $m^{-3}$ in suburban areas, and
176 88.8, 54.7 and 117.4 μg $m^{-3}$ in rural areas, respectively. $PM_{2.5}$ levels still exceed the
177 ambient air quality standards of China, USA and WHO. $PM_{2.5}$ concentration variation
178 among the snowfall periods for each site was shown in Fig. 1, indicating a valley
179 shape with the lowest $PM_{2.5}$ level during snowfall owing to the cleaning effects of

snowfall.

### 3.2 Components of $PM_{2.5}$ from different urban functional areas during a snowfall event

Fig. 2 showed the differences in water-soluble ion contents. The $NO_3^-$ and $NH_4^+$ were the major inorganic ions, which form in the atmosphere from their gaseous precursors ($NO_x$ and $NH_3$). The content of nitrate was the highest, reflecting the typical conditions in winter. The low temperature made ammonium nitrate (the form of nitrate in atmospheric PM) stay in the condensed phase, limiting its volatilization (in the form of nitric acid and ammonia). The concentrations of measured cations and $SO_4^{2-}$ during snowfall in most areas were higher than those before snowfall. Fig. 3 showed the variations of WSOC concentrations in $PM_{2.5}$. Except in the industrial area, the WSOC accumulated in fine particles during snowfall was higher than that before snowfall.

As showed in Fig. 4, the metal contents varied with the concentration of $PM_{2.5}$ in air. The mass concentrations of As, Co, Cr, Ni, and Sr in $PM_{2.5}$ samples during snowfall from all sites were highest, among the event period. The changes of these elements which were strongly influenced by human factors indicated that the pollution sources of aerosol have changed. The variation of element concentration in different regions reflected the complexity of the particle sources. In snowing weather, $PM_{2.5}$ contains higher concentrations of most metals, despite the fact that the snowfall decreased the $PM_{2.5}$ concentrations in air. It might cause higher toxicity of the $PM_{2.5}$.

202

203 **3.3 Cell viability exposed to $PM_{2.5}$ from different urban functional areas during a**

204 **snowfall event**

205 As showed in Fig. 5, after 24 h of exposure to $PM_{2.5}$ suspension, the cell viability

206 decreased. In addition, DS samples were more potent in reducing cell viability than

207 BS samples. Cell viability exposed to AS samples from industrial areas was

208 significantly higher than BS and DS samples. The differences of cell viability between

209 the three periods were generally not significant, except that in the industrial area.

210

211 **3.4 Cellular oxidative stress and inflammation induced by $PM_{2.5}$ from different**

212 **urban functional areas during a snowfall event**

213 The levels of ROS production in cell culture supernatants induced by $PM_{2.5}$ from

214 all areas during snowfall were higher than those before snowfall (Fig. 6). It

215 demonstrated that air particles during snowfall had a stronger ability to induce A549

216 cells producing ROS. When the snowfall was over, the ability of $PM_{2.5}$ inducing cell

217 ROS production in urban and industrial areas still increased, while that in suburban

218 and rural areas decreased. The production levels of IL-6 and TNF-α showed different

219 trends (Fig.7). The DS samples from urban and industrial areas posed the strongest

220 effects on inducing TNF-α among the event periods, while the highest ability of DS

221 samples to induce IL-6 were from suburban and rural areas.

222

223 **3.5 DNA damage by $PM_{2.5}$ from different urban functional areas during a**

**snowfall event**

The $PM_{2.5}$ resulted in pronounced tailing of A549 cells by comet assay (Fig. 8), implying the genotoxicity of these aerosol samples through DNA damage. Compared with the control cells, longer tails and smaller comet heads in the exposed cells showed that $PM_{2.5}$ had the potential of DNA damage. They were shown in Fig. 9 that quantitative data from comet assays were analyzed, which could better compare the genotoxicity induced by $PM_{2.5}$. The percentage of DNA in the tail in the exposed cells was higher than 10%. Moreover, among the overall periods, samples during snowfall had the highest genotoxic effects.

### 3.6 Correlations between toxicity and components of $PM_{2.5}$ from different urban functional areas during a snowfall event

The correlations between different chemical components (metals, water-soluble ions and WSOC) and cytotoxicity (production of ROS, TNF-α and IL-6) of $PM_{2.5}$ were analyzed (Table 1 and 2). The WSOC of samples from urban area in snowing events showed a high positive correlation with toxicity, though not significant. Among water-soluble ions, $Cl^-$, $Na^+$, $Ca^{2+}$, and $Mg^{2+}$ showed significant strong correlations with inflammatory mediators. The $Mg^{2+}$ in the rural site also significantly correlated with oxidative stress responses. For airborne trace metals, the IL-6 was strongly correlated with As, Co, Cr, Sr, and V in the industrial area, V in the urban site was related to TNF-α, and Sr in the suburban site showed a relationship with the ROS.

## 4. Discussions

In this study, the toxic effects of $PM_{2.5}$ samples from various urban functional areas of a megacity during different periods of a snowfall event were compared. It was assumed that, after the ground was covered with snow, the natural sources of dust and soil re-suspension contributing to $PM_{2.5}$ became less, therefore the particle compositions were mainly from anthropogenic sources and posed stronger toxicity. By the cytotoxicity and genotoxicity tests of human lung cells exposed to these $PM_{2.5}$, the samples during snowfall confirmed some results. Generally, precipitation weather conditions including snowfall and rainfall have a clearing effect on $PM_{2.5}$ (Zhang et al., 2015; Luo et al., 2017). During the whole snowfall event, the $PM_{2.5}$ concentration curve was valley-shaped. In the winter pollution season of Nanjing (Huang et al., 2017), when it began to snow, there was less $PM_{2.5}$ in air, and after the snowfall was over, the meteorological conditions became favorable for the accumulation of $PM_{2.5}$, therefore, the $PM_{2.5}$ concentration in air rose rapidly. Finally, the $PM_{2.5}$ samples during snowfall caused more cell death and DNA damage.

Air pollution PM can stimulate the expression of inflammatory cytokines and induce inflammatory responses. IL-6 and TNF-α are recognized as markers of inflammation, and are the main cytokines that can cause systemic and local inflammation (Yang et al., 2016). Considering the compositional effects, the water-soluble ions in $PM_{2.5}$ might play the roles in toxic effects, which can accelerate the induction of free radicals and then cause inflammatory damage (Chen et al., 2018). WSOC of the urban area was correlated with IL-6 and TNF-α. There is consistent

evidence indicating the responsibility of humic-like substances (the main fraction of WSOC), which produce pro-inflammatory secretion leading to cell death subsequent to aerosol exposure (Bae et al., 2017; Ma et al., 2019). It could also be seen from Table 1 that $Cl^-$, $Na^+$, $Ca^{2+}$, and $Mg^{2+}$ showed relationships with inflammatory damage (Kolesar et al., 2018). There are several possible explanations. To prevent freezing after a heavy snowfall, the municipal administrators usually sprinkle some salt to melt the snow. Industrial salt contains high contents of Na and Cl (Laffray et al., 2018), and also Ca (Vasic et al., 2012). Due to the weather, industrial emissions of dust and motor vehicle exhaust diffuse slowly in the air, which may be the reason for the increase in particulate concentrations of heavy metals during snowfall. Water-soluble components (including WSOC and secondary ions) were related to the production of proinflammatory cytokines. These compounds have the ability to produce cellular immune response because they can produce excessive free radicals with redox activity (Milnerowicz et al., 2015; Wang et al., 2017). Sulfate and nitrate had a low correlation with inflammatory factors and ROS in this study, although some epidemiological researches shown that these inorganic ions were significantly related to mortality (Rappazzo et al., 2015; Chung et al., 2015). However, further studies are necessary to completely understand how different water-soluble chemicals affect cellular pathways, leading to a variety of inflammatory effects. Affected by the weather, industrial dust and vehicle exhaust spread slowly in the air, which may be the reason for the increase of heavy metal concentration accumulated in $PM_{2.5}$ samples during snowfall, that then increase the cell damage. These above factors may be the reasons for lower cell

viability and greater DNA damage of $PM_{2.5}$ during snowfall. Transition metals in PM2.5 may directly produce reactive oxygen species or activate inflammatory cells that can produce ROS, thus changing the permeability of epithelial cells, activating many signal pathways and enhancing inflammatory response (He et al., 2017; Wang et al., 2017). ROS can initiate cellular stress, lead to the release of transcription factors and inflammatory mediators, and activate the kinase cascade. When the level of intracellular oxidative stress is too high, mitochondria may release apoptotsis factors, which eventually lead to cell damage. Previous studies have shown that $PM_{2.5}$-induced inflammation produces reactive oxygen species in the inflammatory process, which can lead to DNA damage, including various oxidized DNA base modifications, single-strand and double-strand breaks (SSB and DSB) and secondary genotoxicity (Schins and Knaapen, 2007). Moreover, airborne As, Co, Cr, Sr and V are mainly emitted from coal combustion, steelmaking and other industrial production (Agarwal et al., 2017; Ledoux et al., 2017), and these trace metals are significant contributor to health effects (Zeng et al., 2016; Huang et al., 2018; Neisi et al., 2016), such as stimulating ROS generation and then causing inflammation (Zhang et al., 2016) and DNA damage (Senlin et al., 2008; Shang et al., 2014). As and V are not only the indicators of coal emissions, but also important metals related to cytokine induction/inhibition response (Gioda et al., 2011). The significant positive correlation between As, V and IL-6 in current study also proved this point. Cr, mainly from the combustion process and metalliferous smelting, can cause inflammation in A549 cells. It seems possible that the strong correlation between Cr and IL-6 was due to smelting

and steel industries. Only $Ca^{2+}$ and $Mg^{2+}$ were related to the TNF-α release in this study, that implies that the effect of $PM_{2.5}$ on IL-6 might be much significant than that on TNF-α. Generally, the cytotoxicity and genotoxicity of $PM_{2.5}$ varied with seasons, locations, and meteorological conditions (Ghanbarian et al., 2019), owing to the changed sources and through the varied components (Valavanidis et al., 2008). Further study focusing on the toxicity of specific components in $PM_{2.5}$ under different weather conditions is thereby suggested.

## 5. Conclusions

This study attempted to compare both the cytotoxicity and genotoxicity of $PM_{2.5}$ from different urban functional areas during various intervals of a snowfall weather event by relating to pollutant source change and chemical components. Results might help to understand the mechanisms of air particle risks to human health. Compositions of $PM_{2.5}$ and their toxic effects showed spatial and temporal differences influenced by sources and meteorological conditions. Although some specific weather conditions such as snowfall may have a clearing effect on air $PM_{2.5}$ pollution, it may also make the remaining particle sources crucial which are mostly anthropogenic and show higher toxicity. Therefore, while assessing the harm of $PM_{2.5}$ pollution to human health, besides considering the particle concentration in the air, the particulate chemical composition changes induced by weather should also be taken into account.

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## Captions of Figures and Tables

**Fig. 1** Distribution of $PM_{2.5}$ concentrations ($\mu g\ m^{-3}$) in different urban functional areas of Nanjing city during a snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall.

**Fig. 2** Water-soluble ion contents ($mg\ kg^{-1}$) in $PM_{2.5}$ from different areas during a snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall

**Fig. 3** Water-soluble organic carbon contents ($mg\ kg^{-1}$) in $PM_{2.5}$ from different areas during a snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall

**Fig. 4** Heavy metal contents ($mg\ kg^{-1}$) in $PM_{2.5}$ from different functional areas during a snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall

**Fig. 5** Cell viability of A549 exposed to $PM_{2.5}$ from different areas during a snowfall event; a and b are significant groups classified by LSD multiple comparisons within the same area; BS: before snowfall; DS: during snowfall; AS: after snowfall; LSD: Fisher's least significant difference test. (*$p<0.05$, **$p<0.01$ implied the difference between the treatment and control group was significant, n=3)

**Fig. 6** The ROS levels in cell A549 induced by $PM_{2.5}$ from different areas during a snowfall event; a and b are significant groups classified by LSD multiple comparisons

within the same area; BS: before snowfall; DS: during snowfall; AS: after snowfall; LSD: Fisher's least significant difference test. (*p<0.05, **p<0.01 implied the difference between the treatment group and control group was significant, n=3)

**Fig. 7** The IL-6 and TNF-α levels in cell A549 induced by $PM_{2.5}$ from different areas during a snowfall event; a and b are significant groups classified by LSD multiple comparisons within the same area; BS: before snowfall; DS: during snowfall; AS: after snowfall; LSD: Fisher's least significant difference test. (*p<0.05, **p<0.01 implied the difference between the treatment group and control group was significant, n=3)

**Fig. 8** Comet assay of A549 cells exposed to $PM_{2.5}$ for 24 h. **a** $PM_{2.5}$-treated A549 cells in the concentration of 80 μg $mL^{-1}$. **b** Control A549 cells

**Fig. 9** DNA damaged of A549 cells exposed to $PM_{2.5}$ by Comet assay; a and b are significant groups classified by LSD multiple comparisons within the same area; BS: before snowfall; DS: during snowfall; AS: after snowfall; LSD: Fisher's least significant difference test. *Indicates statistically significant differences from the control, $p < 0.05$ (t-test)

**Table 1** Pearson correlation coefficients between cellular responses and water-soluble contents of $PM_{2.5}$

In bold: The content of water-soluble component is related to each indicator level; * and**: the correlation level is statistically significant ($P<0.05$, $P<0.01$)

**Table 2** Pearson correlation coefficients between cellular responses and heavy metal contents in $PM_{2.5.}$

In bold: The content of trace metal component is related to each indicator level; * and**: the correlation level is statistically significant ($P<0.05$, $P<0.01$)

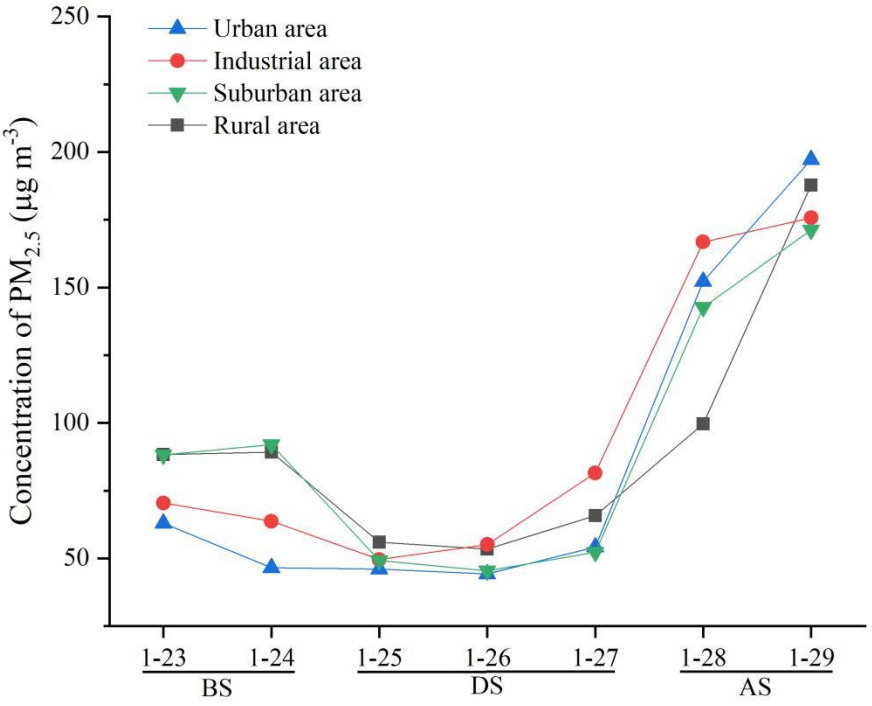
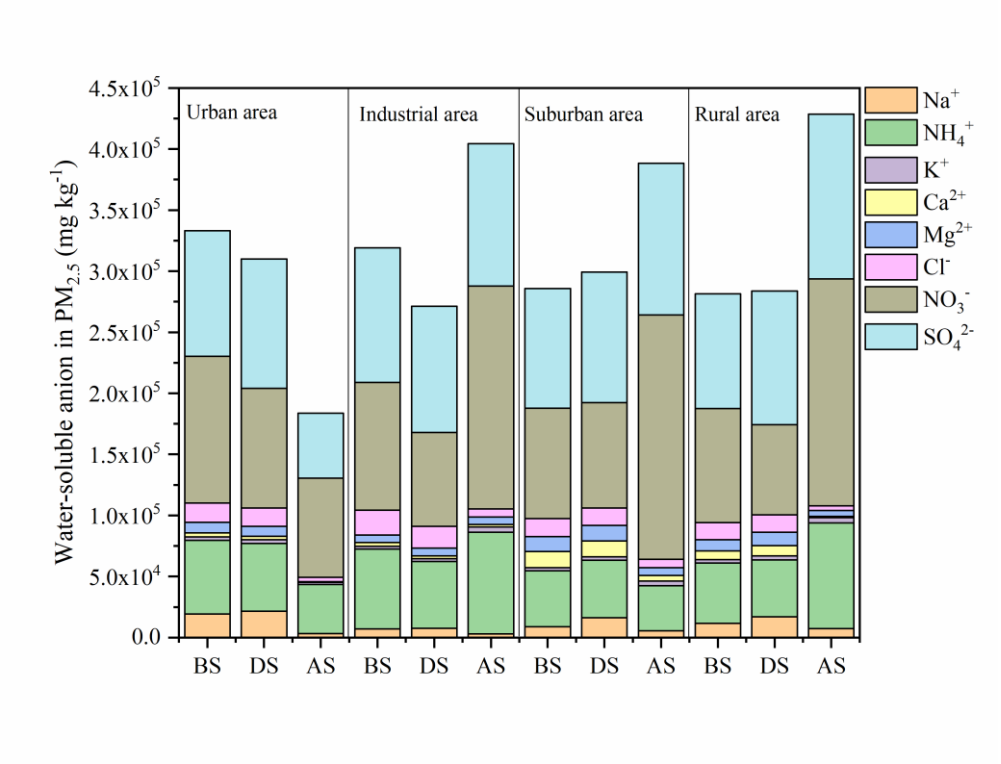



**Fig. 1** Distribution of $PM_{2.5}$ concentrations (μg $m^{-3}$) in different urban functional areas of Nanjing city during a snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall

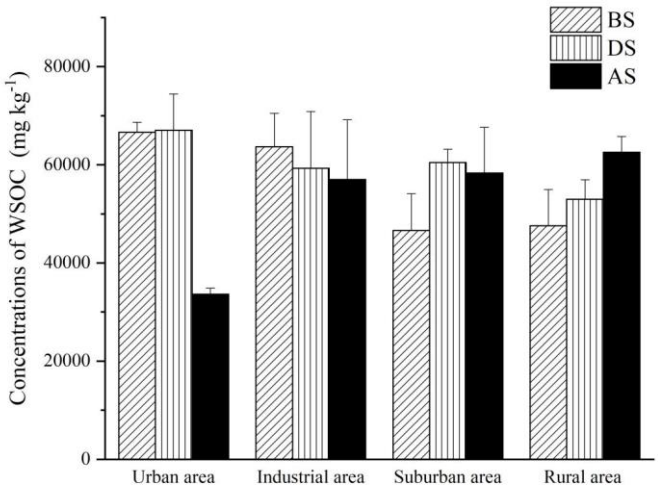



546

547 **Fig. 2** Water-soluble ion contents (mg $kg^{-1}$) in $PM_{2.5}$ from different areas during a

548 snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall

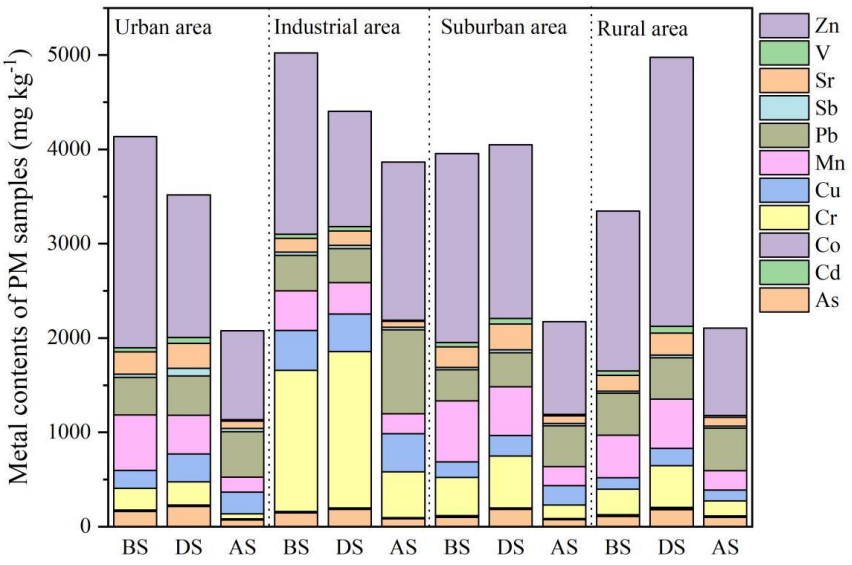
549




550

551 **Fig. 3** Water-soluble organic carbon contents (mg $kg^{-1}$) in $PM_{2.5}$ from different areas

552 during a snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall




553

554 **Fig. 4** Heavy metal contents (mg $kg^{-1}$) in $PM_{2.5}$ from different functional areas during

555 a snowfall event. BS: before snowfall; DS: during snowfall; AS: after snowfall

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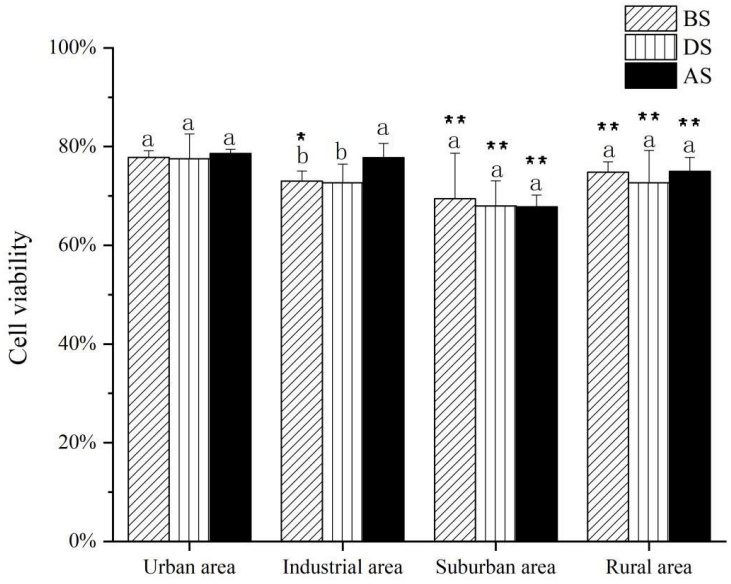


557

558 **Fig. 5** Cell viability of A549 exposed to $PM_{2.5}$ from different areas during a snowfall

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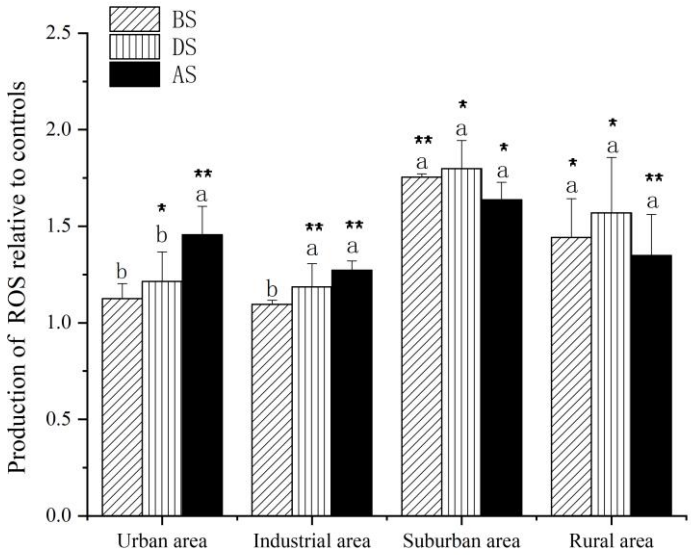


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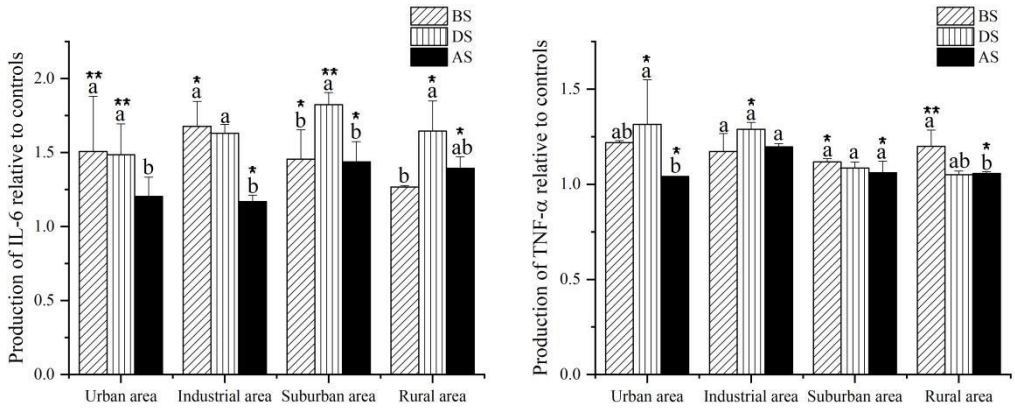


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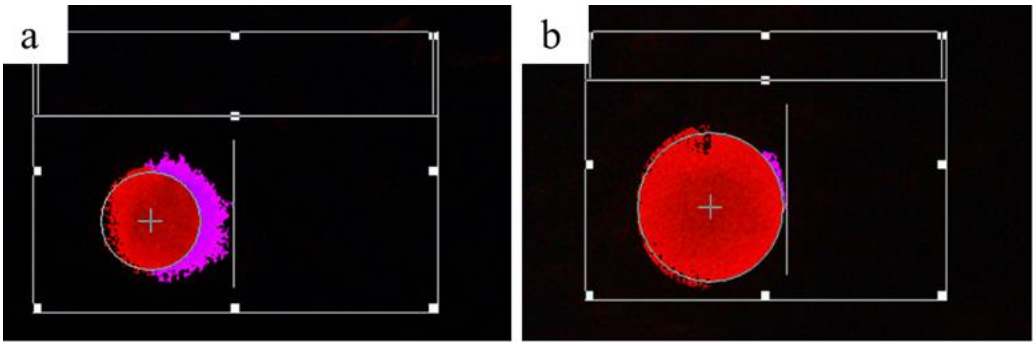
577 n=3)

578

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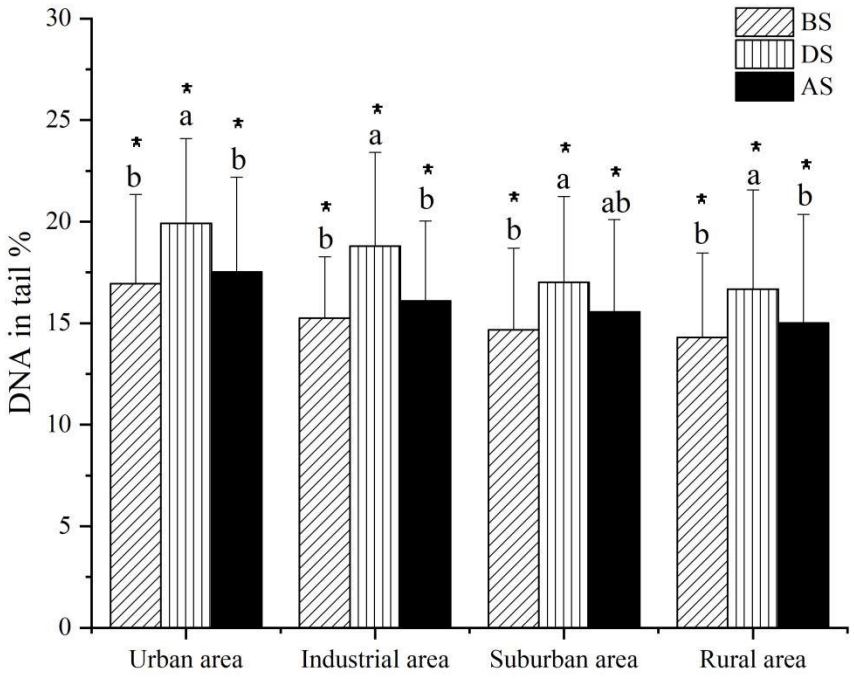


582

583 **Fig. 9** DNA damaged of A549 cells exposed to $PM_{2.5}$ by Comet assay; a and b are

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586 significant difference test. *Indicates statistically significant differences from the

587 control, $p < 0.05$ (t-test)

588 **Table 1** Pearson correlation coefficients between cellular responses and water-soluble

589 contents of $PM_{2.5}$.

| | Urban area | | | Industrial area | | | Suburban area | | | Rural area | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ROS | TNF-α | IL-6 | ROS | TNF-α | IL-6 | ROS | TNF-α | IL-6 | ROS | TNF-α | IL-6 |
| WSOC | -0.721 | 0.603 | 0.727 | -0.278 | 0.006 | 0.307 | -0.218 | 0.063 | 0.047 | -0.092 | -0.686 | -0.340 |
| $Cl^-$ | -0.528 | 0.240 | 0.242 | -0.651 | 0.138 | **0.968**** | 0.132 | 0.318 | 0.248 | 0.180 | 0.174 | 0.160 |
| $NO_3^-$ | -0.856* | 0.523 | 0.239 | 0.368 | -0.340 | -0.820* | -0.637 | -0.096 | -0.616 | -0.462 | -0.248 | -0.414 |
| $SO_4^{2-}$ | -0.465 | 0.287 | 0.590 | 0.448 | -0.247 | -0.121 | -0.517 | 0.074 | -0.496 | -0.264 | -0.764* | 0.098 |
| $Na^+$ | -0.757 | 0.596 | 0.435 | -0.630 | 0.397 | **0.887**** | 0.544 | 0.200 | **0.863*** | 0.719 | -0.240 | 0.489 |
| $NH_4^+$ | -0.459 | 0.270 | 0.614 | 0.452 | -0.509 | -0.549 | 0.294 | -0.457 | 0.170 | -0.429 | -0.404 | -0.333 |
| $K^+$ | -0.455 | 0.416 | 0.547 | 0.412 | -0.363 | -0.774* | -0.740 | -0.233 | -0.422 | -0.278 | -0.588 | -0.209 |
| $Ca^{2+}$ | -0.924** | **0.885*** | 0.247 | -0.481 | 0.377 | 0.244 | 0.646 | 0.458 | 0.594 | 0.737 | 0.080 | 0.344 |
| $Mg^{2+}$ | -0.845* | **0.883*** | 0.538 | 0.012 | 0.251 | 0.209 | 0.529 | 0.422 | 0.521 | **0.804*** | -0.066 | 0.262 |

590 In bold: The content of water-soluble component is related to each indicator level; *

591 and**: the correlation level is statistically significant ($P<0.05$, $P<0.01$).

592 **Table 2** Pearson correlation coefficients between cellular responses and heavy metal

593 contents in $PM_{2.5.}$

| | Urban area | | | Industrial area | | | Suburban area | | | Rural area | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ROS | TNF-α | IL-6 | ROS | TNF-α | IL-6 | ROS | TNF-α | IL-6 | ROS | TNF-α | IL-6 |
| As | -0.556 | 0.574 | 0.582 | -0.515 | 0.573 | **0.811*** | 0.610 | 0.039 | **0.801*** | 0.643 | -0.457 | 0.423 |
| Cd | 0.121 | 0.372 | 0.293 | 0.258 | 0.136 | -0.408 | 0.429 | -0.556 | 0.409 | 0.698 | -0.433 | 0.055 |
| Co | -0.788* | 0.717 | 0.606 | -0.719 | 0.310 | **0.927**** | 0.688 | 0.588 | 0.445 | 0.585 | 0.059 | 0.470 |
| Cr | -0.491 | 0.366 | 0.464 | -0.619 | 0.534 | **0.860*** | 0.563 | 0.269 | 0.749 | 0.589 | -0.243 | 0.535 |
| Cu | 0.113 | 0.441 | 0.161 | -0.258 | -0.089 | 0.169 | 0.054 | -0.792* | 0.532 | 0.612 | -0.414 | 0.665 |
| Mn | -0.587 | 0.397 | 0.633 | -0.884** | 0.185 | 0.702 | 0.308 | 0.372 | 0.348 | 0.422 | 0.142 | 0.359 |
| Ni | -0.729 | 0.726 | 0.595 | -0.078 | 0.201 | 0.399 | 0.279 | 0.089 | 0.637 | 0.554 | -0.213 | 0.537 |
| Pb | 0.571 | -0.146 | 0.301 | 0.235 | -0.280 | -0.585 | 0.086 | -0.735 | 0.115 | 0.431 | -0.194 | -0.363 |
| Sb | 0.183 | -0.113 | 0.433 | -0.571 | 0.348 | 0.472 | 0.538 | -0.191 | 0.445 | 0.246 | -0.587 | 0.327 |
| Sr | -0.740 | 0.678 | 0.549 | -0.684 | 0.501 | **0.855*** | **0.765*** | 0.387 | **0.757*** | 0.681 | -0.114 | 0.553 |
| V | -0.679 | **0.799*** | 0.496 | -0.657 | 0.476 | **0.882**** | 0.701 | 0.346 | **0.755*** | 0.453 | -0.091 | 0.442 |
| Zn | -0.424 | 0.220 | 0.696 | -0.352 | -0.589 | 0.033 | 0.489 | 0.291 | 0.436 | 0.664 | -0.168 | 0.085 |

594 In bold: The content of trace metal component is related to each indicator level; *

595 and**: the correlation level is statistically significant ($P<0.05$, $P<0.01$).