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RESEARCH ARTICLE

Physicochemical properties, in vitro cytotoxic and genotoxic effects of $PM_{1.0}$ and $PM_{2.5}$ from Shanghai, China

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Abstract Exposure to ambient particulate matter (PM) links with a variety of respiratory diseases. However, compared with coarse particles (PM_{10}) and fine particles ($PM_{2.5}$), submicrometer particles ($PM_{1.0}$) may be a more important indicator of human health risks. In this study, the cytotoxic and genotoxic effects of $PM_{1.0}$ samples from Shanghai were examined using A549 cells, and compared with the effects of $PM_{2.5}$, to better understand the health effects of $PM_{1.0}$ in this area. The $PM_{1.0}$ and $PM_{2.5}$ samples were characterized for morphology, water-soluble inorganic ions, organic and elemental carbon, and metal elements. The cytotoxicity of PMs was measured using cell viability and cell membrane damage assays. The genotoxic effects of PMs were determined using the comet assay, and DNA damage was quantified using olive tail moment (OTM) values. The physicochemical characterization indicated that $PM_{1.0}$ was enriched in carbonaceous elements and hazardous metals (Al, Zn, Pb, Mn, Cu, and V), whereas $PM_{2.5}$ was more abundant in large, irregular mineral particles. The biological results revealed that both $PM_{1.0}$ and $PM_{2.5}$ could induce significant cytotoxicity and genotoxicity in A549 cells, and that exposure to $PM_{1.0}$ caused more extensive toxic effects than exposure to $PM_{2.5}$. The greater cytotoxic effects of $PM_{1.0}$ can be attributed to the combined effects of size and chemical composition, whereas the genotoxic effects of $PM_{1.0}$ may be mainly associated with chemical species.

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Introduction

Ambient particulate matter (PM) is a complex air pollutant that originates from both natural sources (e.g., dust, sea salt, and fungal spores) and anthropogenic emissions (e.g., diesel exhaust, coal burning, and cigarette smoke) (Kelly and Fussell 2012). The major components of PM include acid salts, heavy metals, organic chemical components, and biological contaminants (Hueglina et al. 2005). Daily exposure to PM is associated with a variety of cardiovascular and respiratory diseases, and even the increased risk of lung cancer (André 2005). Chronic lung disease is among the major problems that has elicited great public health concern (Vinikoor-Imler et al. 2011). Moreover, increasing researchers have recognized that the production of reactive oxygen species (ROS), immune deficiency, inflammation, and DNA degradation are involved in PM-mediated lung injury (Deng et al. 2013; Senthilkumara et al. 2014).

The relative ability of PMs to provoke adverse effects in target cells and tissues may depend greatly on their chemical constituents (Thomson et al. 2015). A number of studies have demonstrated significant associations between various health outcomes and the chemical components of PM, such as sulfate, ammonium, and nitrate (Bonetta et al. 2009; Pui et al. 2014). Furthermore, PM size is an important factor influencing biological responses (Lee et al. 2006; Velali et al. 2016). According to the size of the aerodynamic diameter (Ø), PM can be classified into coarse particles (PM_{10}, Ø between 2.5 and 10 μm), fine particles ($PM_{2.5}$, Ø < 2.5 μm), and submicrometer particles ($PM_{1.0}$, Ø < 1.0 μm) (Huang et al. 2003). The toxic effects of PM_{10} and $PM_{2.5}$ have been widely

examined; the coarse fraction was reported to be mainly associated with pro-inflammatory and cytotoxic effects, whereas the fine fraction was mainly related to genotoxic potential (Akhtara et al. 2014; Gualtieri et al. 2010; Osornio-Vargas et al. 2003).

Recently, the health threat of $PM_{1.0}$ has attracted growing interest, because $PM_{1.0}$ is more enriched in toxic and carcinogenic compounds and can penetrate deeper into the respiratory tract than fine and coarse particles (Lim et al. 2012; Longhin et al. 2013). Several studies have been conducted to explore the toxic effects of $PM_{1.0}$ in developed areas. For instance, Huang et al. (2003) reported that $PM_{1.0}$ in Taiwan could result in significantly higher interleukin-8 (IL-8) production and lipid peroxidation than $PM_{2.5–10}$ because of its higher contents of metals (Cr, Mn, and Fe) and carbon compounds. Perrone et al. (2010) found that $PM_{1.0}$ in Milan could induce a more significant reduction in cell viability and increase of IL-8 than the $PM_{2.5}$ fraction. In a more recent study, Longhin et al. (2013) concluded that $PM_{1.0}$ in Milan could elicit high ROS generation, and exhibited considerable DNA-damaging effects.

The mega-city of Shanghai in China suffers from severely deteriorated air pollution. During 2011–2012, the annual $PM_{2.5}$ mass concentration in Shanghai was 54.94 μg/m^3, five times higher than the global guidelines (10 μg/m^3) that set by the World Health Organization (Qiao et al. 2014). Particularly, $PM_{1.0}$ composing the dominant proportion of fine PM (Gong et al. 2015; Huang et al. 2012; Li et al. 2015) is presumed to play an important role in adverse health effects. However, studies on the toxic effects of $PM_{1.0}$ in Shanghai are scarce.

In the present study, airborne $PM_{1.0}$ and $PM_{2.5}$ from the Minhang District of Shanghai were used as model samples to elucidate the adverse biological effects of $PM_{1.0}$ in Shanghai. The PM samples were characterized in terms of their morphology, water-soluble inorganic ions, organic carbon (OC) and elemental carbon (EC) contents, and metal element contents. To investigate the in vitro biological effects of $PM_{1.0}$ and $PM_{2.5}$, which include cell viability, cell membrane disruption, and DNA damage, the human lung adenocarcinomatous cell line A549 was used. The A549 cell line, which is derived from the neoplastic transformation of type-II alveolar cells, shows high proliferative potential and possesses the typical features of type-I normal epithelial cells (Calcabrini et al. 2004). The A549 cell is one of the major in vitro cell lines used extensively in the study of airway particle toxicology, although this cell line is sensitive to minor genetic modifications (Alessandria et al. 2014; Berg et al. 2013; Zeng et al. 2016).

Materials and methods

PM sampling and extraction

Sampling was conducted on the roof of a six-floor building in the Minhang campus of Shanghai Jiao Tong University (N 31° 1′ 51″ E 121° 26′ 27″), Shanghai, China. $PM_{1.0}$ and $PM_{2.5}$ samples were collected simultaneously using two mid-volume samplers (Wuhan Tianhong TH Ⅲ, China) equipped with $PM_{1.0}$ and $PM_{2.5}$ cut-off inlets, respectively. Daily airborne PM samples were collected on quartz fiber filters (Ø 90 mm), with a pump flow rate of 100 L/min. Sampling was carried out over a continuous period of 24 h/day, from September 2 to November 19, 2014. Treatment of the filters before and after sampling was performed following the methods described in our previous work (Zou et al. 2016).

The suspensions of $PM_{1.0}$ and $PM_{2.5}$ used for toxic assays were prepared as follows. A portion of each sampled filters was cut and immersed in ultrapure water with sonication in water bath for 6 × 30 min. The extract was filtered with eight-layer sterile gauze, and transferred into a clean sterile tube (weighed before use). The suspension was freeze-dried in a vacuum, and the resulting pellet was weighed and stored in −80 °C until use.

Morphological characterization of $PM_{1.0}$ and $PM_{2.5}$

The morphological characteristics of the extracted particles ($PM_{1.0}$ and $PM_{2.5}$) were investigated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Prior to analysis, 1 cm^2 of the $PM_{1.0}$ and $PM_{2.5}$ filters were cut and sonicated in 1 mL of ethanol (analytical reagent grade) separately. For SEM observation, several drops of each suspension were dropped onto the surface of a double conductive adhesive fixed on an aluminum stub. After drying, each sample was coated with gold and put into the sample compartment of the SEM (FEI, Nano 230, USA) for further analysis. For TEM analysis, a portion of the resuspended particles was placed onto carbon-coated copper grids and air-dried. The grids were analyzed with a FEI TEM (Tecnai G2 spirit Biotwin, USA).

Analysis of chemical species in $PM_{1.0}$ and $PM_{2.5}$

$PM_{1.0}$ and $PM_{2.5}$ filter samples were analyzed for water-soluble inorganic ions, OC and EC, and metal elements. To determine the contents of water-soluble inorganic ions, one-eighth of the sampled filters were sonicated in 25 mL of ultrapure water for 45 min. The extract was then filtered (with 0.45 μm PTFE filters) to remove the insoluble components. NH_4^+, F^-, Cl^-, NO_3^-, and SO_4^{2-} ions in the extracts were analyzed via ionic chromatography (IC, Thermo Fisher, ICS-5000$^+$, USA), and Na^+, K^+, Ca^{2+}, and Mg^{2+} were quantified via inductively coupled plasma spectrometry (ICP, Thermofisher, iCAP7600, USA). The OC and EC fractions were analyzed with a thermal/optical carbon analyzer (Atmoslytic Inc., DRI2001A, USA) using the thermal optical transmission (TOT) method, following the EUSAAR2 temperature protocol (Cavalli et al. 2010). A 0.518 cm^2 punch

area of each filter was used for the measurement of eight carbon fractions. First, the filter was heated to 650 °C in a pure helium atmosphere (OC1, OC2, OC3, and OC4 at 200, 300, 450, and 650 °C, respectively). Subsequently, oxygen was added to the combustion atmosphere, and a pyrolyzed carbon fraction (OP) was determined when reflected laser light attained its original intensity. Lastly, the filter was brought to 850 °C in a 2% oxygen and 98% helium oxidizing atmosphere (EC1, EC2, and EC3 at 550, 700, and 850 °C, respectively). OC is defined as OC1 + OC2 + OC3 + OC4 + OP, and EC is calculated as EC1 + EC2 + EC3 − OP. Metal elements were measured via inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher, iCAPQ, USA). Before analysis, one-eighth of the sampled filters were digested using a mixture of HNO_3 and HF in a microwave digestion system (Anton Paar, Multiwave 3000, Austria). After cooling, the mixture was adjusted to a final volume of 25 mL with distilled water. A total of 15 elements (Al, Zn, Fe, Pb, Mn, Cu, V, Cr, As, Se, Ni, Sb, Mo, Cd, and Co) were measured. For all analyses, field blank filters were measured together with each batch of samples to provide information about contaminants that may have been introduced during sample collection, storage, and transport.

Cell culture

In the current investigation, A549 cells were used to examine the biological endpoints of $PM_{1.0}$ and $PM_{2.5}$. A549 cells were grown in F-12K (Life Technologies, Thermo Fisher, USA) cell culture medium supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, Thermo Fisher, USA) and 1% antibiotics penicillin/streptomycin (100 U/mL). The cells were cultivated in plastic cell culture flasks at 37 °C in a humidified atmosphere containing 5% CO_2. After 70–80% cell confluency was reached, the monolayer cells were used for toxicity assays.

Cell viability assay

The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which mainly reflects the mitochondrial activity of cells, was employed to evaluate cell viability. For the MTT assay, A549 cells were seeded in a 96-well plate at a density of 8×10^3 cells/well and cultured for 24 h to allow cell adhesion. The cells were then exposed to $PM_{1.0}$ and $PM_{2.5}$ in the concentration ranges of 25, 50, 100, and 200 μg/mL. After 24-h incubation, freshly prepared MTT solutions were added to each well at a final concentration of 200 μg/mL, and incubated with cells for 4 h at 37 °C in darkness. Later, the cell culture medium was discharged, and 200 μL of DMSO was added to each well to dissolve the formazan compound. The optical density (OD) of the solution was recorded at a wavelength of 492 nm using a multi-scan plate reader (BioTek, Synergy H4, USA). For each concentration of PMs, six replicates were tested, including the untreated control cells. The OD values of PM-exposed cells were compared with those of the control cells to determine the percentage of cell viability.

Cell membrane damage assay

Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme that is rapidly released into the culture medium when cell membranes are damaged. In this study, LDH leakage in the cell culture medium was assayed to determine the cell membrane disruption of PM-treated A549 cells. For the LDH assay, the cell culture and PM exposure were conducted as for the MTT assay described above. The activity of LDH in the cell-free culture supernatant was detected following the manufacturer's protocol (CytoTox 96, Promega, USA). Briefly, the cell-free culture supernatants were obtained using centrifugal separation (800 rpm, 10 min), and 50 μL of the clean supernatant in each well was transferred to a new 96-wells plate. Then, 50 μL of the pre-prepared substrate mixture was added to each well and incubated with the supernatant for 30 min at room temperature in darkness. Next, 50 μL of stop solution was added to each well, and the OD values of the final solution were recorded on the multi-scan plate reader (BioTek, Synergy H4, USA) at a wavelength of 490 nm. The culture medium without cells served as the background control, the supernatants from lysis solution-treated cells were used as positive controls. Each concentration of PMs was tested in six replicates, including the untreated control cells and the positive control. The results were calculated as fold of LDH release to control.

DNA damage assay

The comet assay, a gel electrophoresis-based method, is widely used for measuring chemical and environmental contaminant-induced DNA damage in individual cells. In this study, the alkaline version of the comet assay, which is sensitive to DNA strand breaks, alkali-labile, and incomplete excision repair sites, was used to detect the DNA damage potential of $PM_{1.0}$ and $PM_{2.5}$. For the comet assay, A549 cells were seeded in a 12-well plate at a density of 2×10^4 cells/well. After 24 h of seeding, cells were exposed to different concentrations of $PM_{1.0}$ and $PM_{2.5}$ (25, 50, 100, and 200 μg/mL) for 24 h. The cells were then harvested with 0.25% Trypsin-EDTA and washed with phosphate-buffered saline (PBS) three times. Then, 15 μL of the cell suspension (1×10^5 cells/mL in PBS) was mixed with 110 μL of 0.65% low-fusion-point agarose and applied to the prepared slides. The cells were stained with GelRed (Biotium, USA) and lysed under alkaline conditions (2.5 M NaCl, 10 mM Tris, 100 mM Na_2EDTA, 1% sodium sarcosinate, pH = 10), to which 1% Triton X-100 and 10% DMSO were added, for at

least 1 h. Then, the samples were heated with Protease K buffer solution (0.5 mg/L Protease K, 2.5 M NaCl, 5 mM Tris, 0.05% sodium sarcosinate, pH = 7.4) in a water bath at 55 °C for 2 h. The slides were then placed in a single-cell gel electrophoresis chamber (Trevigen; Maryland, USA), positioned close to the anode and covered with an alkaline buffer solution (300 mM NaAC, 100 mM Tris, 100 mM Na_2EDTA, pH = 10). After the DNA was unwound for 30 min, it was electrophoresed at 15 V and 130 mA for 60 min. After the slides were rinsed and soaked in 75% ethanol for 5 min, they were examined under a DMLB microscope (Olympus, Japan) with an adapted charge-coupled device camera (Cohu Inc.; San Diego, USA). Images from 100 random cells were analyzed for each experiment as described by the guidelines. To quantify the level of DNA damage, the extent of DNA migration was determined based on the olive tail moment (OTM), which is the relative amount of DNA in the tail of the comet multiplied by the median migration distance. Statistical analysis was based on the mean OTMs of scored cells.

Statistical analysis

Values are given as means ± standard deviations (SD). Data were analyzed using one-way analysis of variance (ANOVA) to determine significance relative to the unexposed controls. In all cases, $P < 0.05$ was considered significant.

Results and discussion

Morphology of $PM_{1.0}$ and $PM_{2.5}$

The morphological features of $PM_{1.0}$ and $PM_{2.5}$ particles obtained from SEM and TEM observations are shown in Fig. 1. As depicted in Fig. 1a, b, regular and irregular mineral particles were observed in both $PM_{1.0}$ and $PM_{2.5}$. According to Murillo et al. (2012), irregular mineral particles were related to natural sources, such as feldspar, clay, and limestone. In contrast, regular mineral particles might be generated from high-temperature processes of anthropogenic origin or biomass burning. Compared with $PM_{1.0}$, there were greater numbers of large irregular mineral particles in $PM_{2.5}$, which was consistent with previous research that reported that mineral particles of dust tended to be found in PMs of larger size fractions (Wang et al. 2013). Despite the slight difference observed between SEM images of the two kinds of particles, TEM images of the ultrafine particles in $PM_{1.0}$ (Fig. 1c) and $PM_{2.5}$ (Fig. 1d) showed great similarity in particle morphology, which indicated that the majority of ultrafine particles in $PM_{1.0}$ originated from the same sources as $PM_{2.5}$. These round-shaped nanoparticles, with average sizes of approximately 30–40 nm, were agglomerated in clusters and interconnected with each other in various ways. In addition, the appearance of these ultrafine particles is similar to the morphology of vehicle-emitted particles (Barone and Zhu 2008), which demonstrates that the ultrafine particles from the sampling site may have been generated from burning of gasoline and diesel fuel.

Mass fractions of main chemical components in $PM_{1.0}$ and $PM_{2.5}$

The chemical composition of size-fractionated ambient PM aerosols is an important factor affecting their health effects. Therefore, in the present study, the chemical species in $PM_{1.0}$ and $PM_{2.5}$, samples which include water-soluble inorganic ions, OC, EC, and metal elements, were quantified to better assess their toxicity. The relative mass fractions of these chemical components are depicted in Fig. 2. As shown in Fig. 2a, b, water-soluble inorganic ions (SO_4^{2-}, NO_3^-, and NH_4^+) were the most abundant constituents in $PM_{1.0}$ and $PM_{2.5}$, followed by carbonaceous elements (OC and EC). According to Perrone et al. (2010), inorganic ions such as SO_4^{2-}, NO_3^-, and NH_4^+ that resulted from the transformation of their gaseous precursors SO_2, NOx, and NH_3 represented secondary pollution. Therefore, the high mass fraction of inorganic ions (SO_4^{2-}, NO_3^-, and NH_4^+) in $PM_{1.0}$ and $PM_{2.5}$ indicates pronounced secondary contamination in this region. Moreover, Perronea et al. (2014) and Salameh et al. (2015) have demonstrated that an OC/EC ratio exceeding 2.0–2.2 can be used as an indicator of secondary contamination, because EC is mainly derived from primary anthropogenic combustion, whereas OC is produced either from direct emission or gas-to-particle reactions involving atmospheric oxidation or oligomerization. Therefore, in addition to the high contents of water-soluble secondary ions, the relatively high OC/EC ratios of PMs (2.35 for $PM_{1.0}$ and 3.09 for $PM_{2.5}$) from the sampling site further confirms severe secondary pollution in this area. The present results are broadly consistent with previous findings that secondary aerosols dominated the fine particulate mass in this region (Feng et al. 2009). Regarding to the mass fraction of chemical species in $PM_{1.0}$ and $PM_{2.5}$, no significant differences were found for water-soluble ions in the two kinds of PMs (Fig. 2a). In contrast, significantly higher proportions of carbonaceous and toxic metal elements (Al, Zn, Pb, Mn, Cu, and V) were observed in $PM_{1.0}$ (Fig. 2b). Carbonaceous aerosols were reported to be major contributors to fine aerosols smaller than 1 μm, and typically constitute significant, sometimes dominant, fractions of the total mass of submicron particles (Huang et al. 2012). Similarly, hazardous metal elements with notable health impacts emitted from anthropogenic sources were found to be mainly accumulated in the smaller particles ($Ø < 1$ μm) (Espinosa et al. 2001). The size distribution of the chemical species in $PM_{1.0}$ and $PM_{2.5}$ is consistent with their morphological characters; $PM_{2.5}$ is rich in large, irregular mineral particles from natural sources, whereas

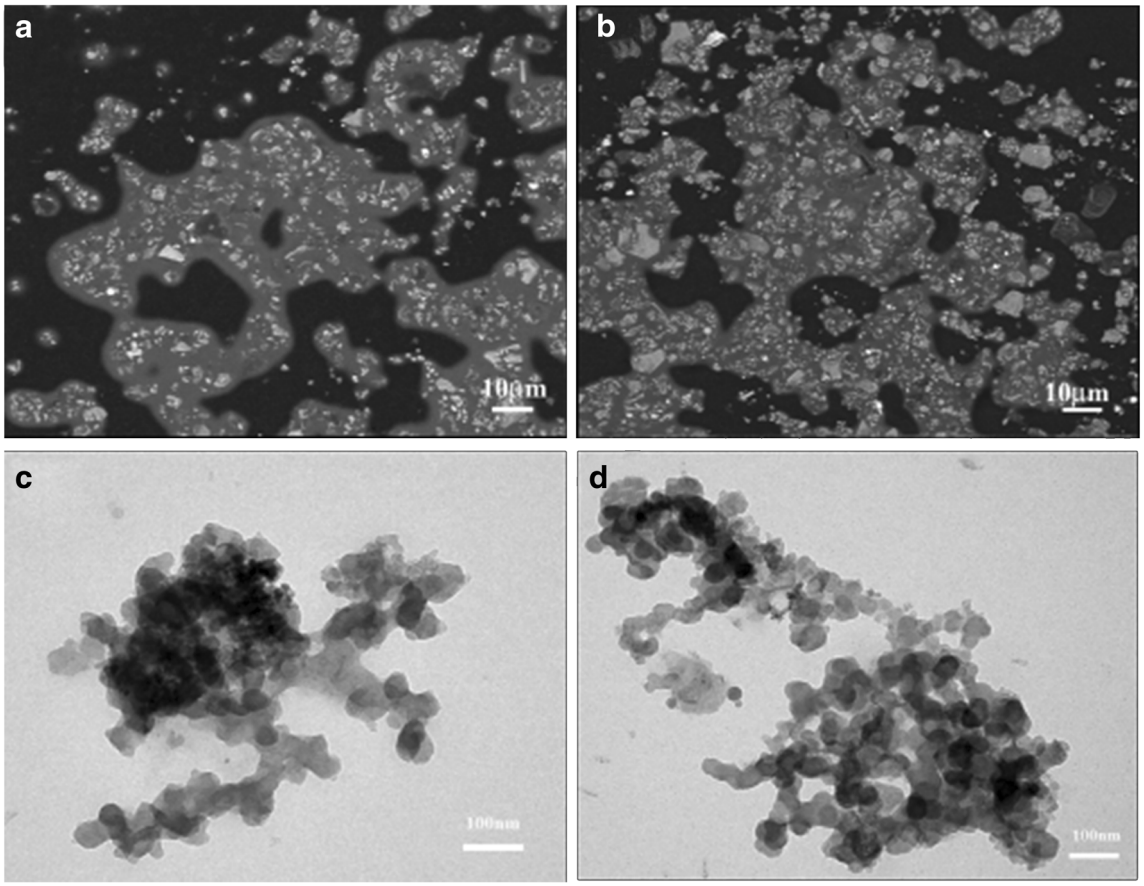

Fig. 1 Morphological characteristics of $PM_{1.0}$ and $PM_{2.5}$. SEM image of $PM_{1.0}$ (**a**) and $PM_{2.5}$ (**b**), *scale bar* = 10 μm; TEM image of $PM_{1.0}$ (**c**) and $PM_{2.5}$ (**d**), *scale bar* = 100 nm

$PM_{1.0}$ is enriched in chemicals emitted from anthropogenic processes. Consequently, $PM_{1.0}$ may be analyzed to minimize the interference from natural sources, and provide better information for human health than $PM_{2.5}$ (Lee et al. 2006).

Cytotoxic effects of $PM_{1.0}$- and $PM_{2.5}$-treated A549 cells

To obtain more reliable results in evaluating the potential cytotoxic effects of $PM_{1.0}$ and $PM_{2.5}$, both MTT viability assay and LDH release assay were employed to examine the toxic effects of $PM_{1.0}$- and $PM_{2.5}$-treated A549 cells; the results are presented in Fig. 3. As illustrated in Fig. 3a, for the MTT assay, cell viability decreased in $PM_{1.0}$- and $PM_{2.5}$-treated A549 cells, and significant decrease in cell viability relative to the control cells occurred at PM mass concentrations of 25 μg/mL. Moreover, LDH release was increased in $PM_{1.0}$- and $PM_{2.5}$-exposed A549 cells, with significant increase of LDH release from PM concentrations of 50 μg/mL (Fig. 3b). These results indicate that the amount of LDH release from cells exposed to PMs is related to the decrease of cell viability induced by the particles. The data in our study are consistent with previous observations that the increase of LDH release, as an indicator

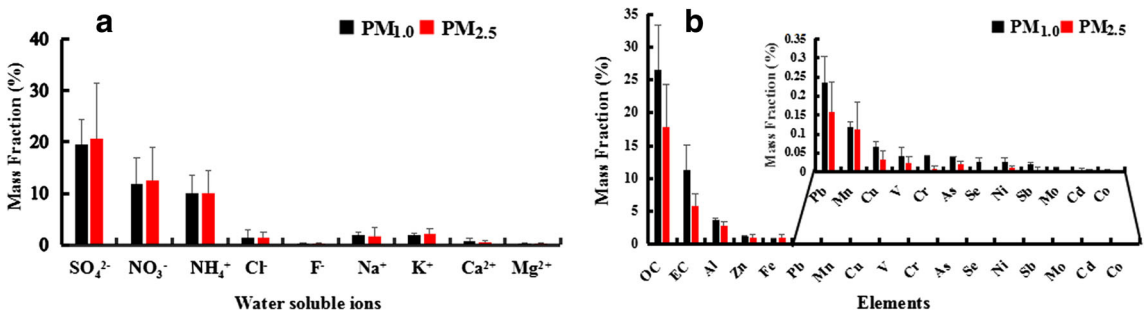

Fig. 2 Mass fractions of chemical components in $PM_{1.0}$ and $PM_{2.5}$ samples. **a** Water-soluble inorganic ions. **b** Carbonaceous elements (OC and EC) and metal elements

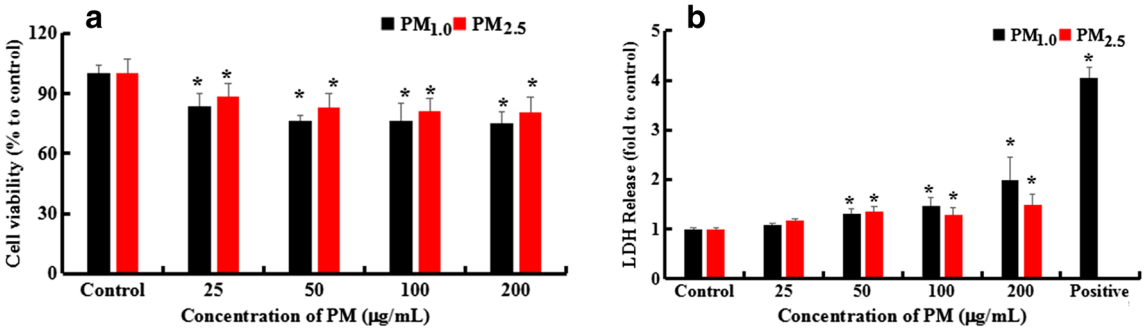

Fig. 3 The cytotoxic effects of A549 cells treated with $PM_{1.0}$ and $PM_{2.5}$ at concentrations of 25, 50, 100, and 200 μg/mL for 24 h. **a** MTT assays of cell viability; data are shown as percentages relative to the control (untreated A549 cells). **b** Content of LDH release in cell culture medium; data are shown as fold to control. *$P < 0.05$ (one-way ANOVA with Dunnett post-test)

of PM exposure, caused cell membrane disturbance, and was highly correlated with decreases of cell viability and other biological effects (Billet et al. 2007; Shang et al. 2014). Although both assay methods showed great similarity in $PM_{1.0}$- and $PM_{2.5}$-induced cytotoxic effects, differences were also detected for the two size fractions of PMs. The results clearly revealed that $PM_{1.0}$ was more potent in inducing cytotoxic effects than was $PM_{2.5}$, as $PM_{1.0}$ could induce significantly lower cell viability at each exposure concentration, as well as significantly higher LDH release, relative to $PM_{2.5}$ at exposure doses of 100 and 200 μg/mL. For the different effects observed for $PM_{1.0}$ and $PM_{2.5}$, both particle size and source-linked chemical composition are considered to play dominant roles. Massolo et al. (2002) and Velali et al. (2016) have reported a strong correlation between cell cytotoxicity and the finest PM fraction, and Thomson et al. (2015) showed that particle toxicity might be related to the contents of metals and organic compounds. Carbon-containing exhaust and some transition metals were found to be associated with enhanced intracellular oxidants and the resulting cell injuries (Huang et al. 2003). Accordingly, Perrone et al. (2010) found that $PM_{1.0}$ could induce a greater reduction of cell viability in A549 cells than $PM_{2.5}$, and that the MTT response appeared to be related to As, Zn, Cr, Cu, and Mn contents. More recently, Perrone et al. (2013) reported that the LDH response in $PM_{1.0}$- and $PM_{2.5}$-treated cells was associated with metals from anthropogenic sources and organic compounds from biogenic and biomass-burning origins. Therefore, the significantly higher cytotoxicity of $PM_{1.0}$ compared with $PM_{2.5}$ reported in our study can mainly be attributed to the combined effects of its smaller particle size and higher contents of carbonaceous components and metal elements.

DNA damage in $PM_{1.0}$- and $PM_{2.5}$-treated A549 cells

In the present study, DNA damage in PM-treated A549 cells was investigated, using the comet assay, to evaluate the genotoxic effects of $PM_{1.0}$ and $PM_{2.5}$. Images of assayed cells are presented in Fig. 4. As shown in Fig. 4, both $PM_{1.0}$ and $PM_{2.5}$ demonstrated DNA-damaging potential, which was indicated by relatively smaller comet heads and increased lengths of tails in the exposed cells, compared with control cells. The results of our study are in accordance with general observations demonstrating that inhaled particles can cause the breakage of DNA strands and oxidative damage to DNA (Upadhyay et al. 2003; Zeng et al. 2016). The chemical compositions of PMs have been reported to be the main factor in PM-induced oxidative stress and DNA damage. For instance, Lu et al. (2008) found that the plasmid DNA damage induced by Shanghai $PM_{2.5}$ was highly correlated with heavy metal content. Other studies have shown that PM samples collected in high-traffic locations, which greatly enriched in metal elements, such as Cu, Ni, and Cd, were able to inhibit DNA repair pathways (Bonetta et al. 2009; Wessels et al. 2010). Moreover, Shang et al. (2014) and Velali et al. (2016) have demonstrated that oxidative damage of DNA caused by PMs is mainly associated with the organic fractions. In addition to the considerable importance of chemical composition, the insoluble particles in PMs may also play a critical role in inducing DNA damage. As demonstrated in our previous study (Zou et al. 2016), smaller nanoparticles that were either already present in $PM_{2.5}$ or were generated from decomposition processes were able to penetrate into the nuclear core of A549 cells, and might attack crucial cellular macromolecules including the genomic DNA.

In addition to the above information, there are several specific characteristics of $PM_{1.0}$- and $PM_{2.5}$-induced DNA damage (Fig. 4). First, the analysis revealed significant cellular DNA damage even in the presence of low concentrations of $PM_{1.0}$ and $PM_{2.5}$ (25 μg/mL), indicated by the presence of DNA tails in Fig. 4b, f. This finding can mainly be attributed to the fact that the comet assay can provide sensitive and reliable results for DNA damage detection. In contrast to the traditional chromosome aberration and micronuclei tests, the comet assay allows rapid detection of DNA damage shortly after the injury, before the DNA is repaired, and without any need to wait for progression into mitosis (Liao et al. 2009). Secondly, $PM_{1.0}$ and $PM_{2.5}$ may induce more extensive DNA

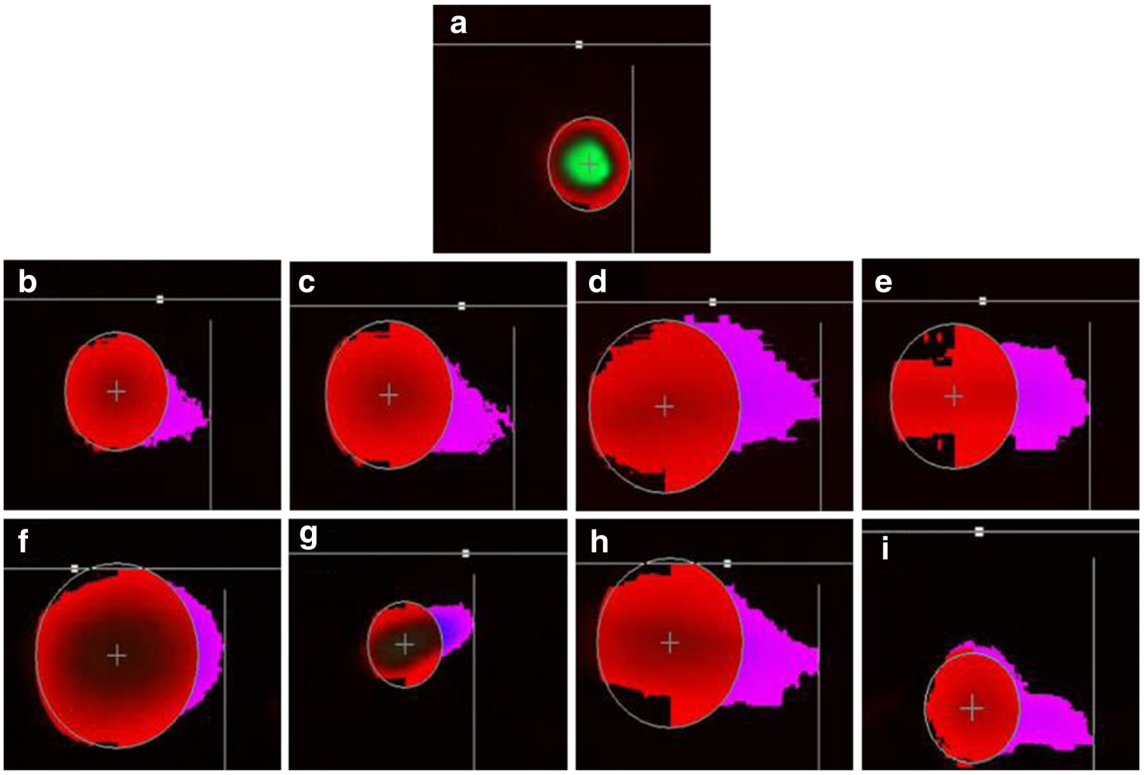

Fig. 4 Comet assay of A549 cells exposed to $PM_{1.0}$ and $PM_{2.5}$ for 24 h. **a** Control A549 cells. **b**–**e** $PM_{1.0}$-treated A549 cells in the concentration ranges of 25, 50, 100, and 200 μg/mL, respectively. **f**–**i** $PM_{2.5}$-treated A549 cells in the concentration ranges of 25, 50, 100, and 200 μg/mL

damage in cells treated with a higher concentration of PMs, as demonstrated by DNA tails with greater areas and lengths (distance from DNA head to the end of DNA tail) in cells exposed to higher doses of PMs. These findings are in good agreement with previously reported data, which indicated that particle exposure can cause increased DNA damage in a dose-dependent manner (Bonetta et al. 2009; Shang et al. 2014). Thirdly, $PM_{2.5}$ was found to be less potent than $PM_{1.0}$ in inducing oxidative DNA damage in A549 cells. Because there are no agreeable comments about the outcomes of DNA damage outcomes and particle size fractions, the greater DNA damage induced by $PM_{1.0}$ can be attributed to the abundant chemicals species, such as metals and organic compounds. For example, some studies have showed that $PM_{2.5}$ has the ability to induce stronger DNA damage than $PM_{1.0}$ in the comet assay (Perrone et al. 2010), whereas Osornio-Vargas et al. (2003) reported that PM_{10} samples could induce higher DNA degradation than $PM_{2.5}$. Moreover, Wessels et al. (2010) have emphasized that the genotoxic effects of PMs depend more on the chemical composition of the PMs than on the size fractions.

To better compare the genotoxic effects induced by $PM_{1.0}$ and $PM_{2.5}$, quantitative data from comet assays were analyzed; the results are shown in Fig. 5. Tail length, percentage

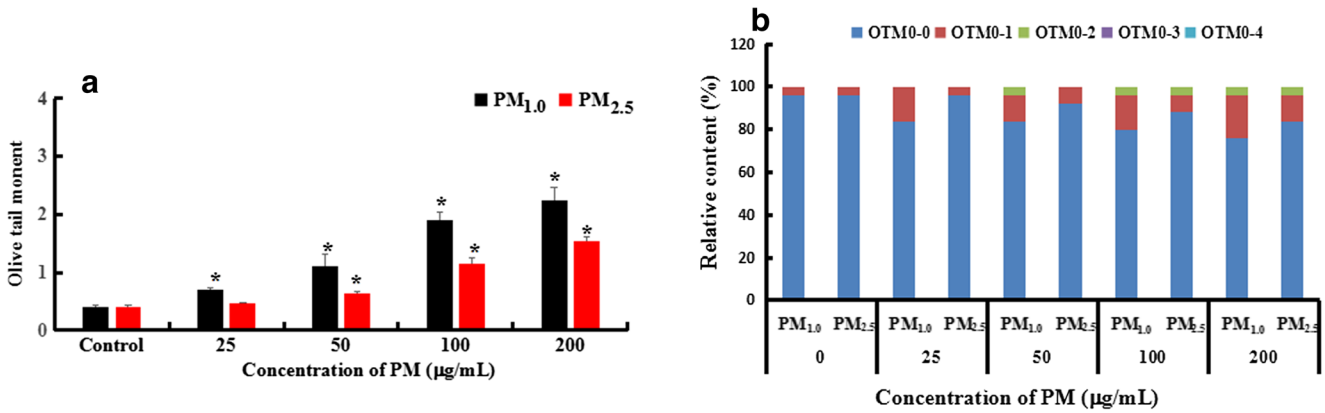

Fig. 5 The genotoxic effects of $PM_{1.0}$ and $PM_{2.5}$. A549 cells were treated with $PM_{1.0}$ and $PM_{2.5}$ at concentrations of 25, 50, 100, and 200 μg/mL for 24 h. **a** OTM values of assayed A549 cells. **b** Percentages of OTM damage classes in the exposed cells. *$P < 0.05$ (one-way ANOVA with Dunnett post-test)

of DNA in the tail, and OTM values were used as the main indicators for analysis of the comet assays (Collins 2004). Of these parameters, OTM as the fraction of DNA in the tail multiplied by the distance between the means of the head and tail distributions more comprehensively reflects the degree of DNA damage than do the tail length and percentage of DNA indicators (Olive and Banáth 2006). In the present study, DNA migration in assayed cells was quantified based on the OTM values, and five classes of OTM damage, ranging from 0 (no tail) to 4 (almost all DNA in tail) (Liao et al. 2009), were then used to provide high quantitative resolution of PM-induced DNA damage. As illustrated in Fig. 5a, exposure to $PM_{1.0}$ and $PM_{2.5}$ caused statistically significant dose-dependent increases of the OTM in A549 cells, and $PM_{1.0}$ was associated with higher OTM values than $PM_{2.5}$ at each exposure concentration, which is in qualitative agreement with the trends observed in Fig. 4. Furthermore, as shown in Fig. 5b, a shift from a low DNA damage category (OTM-1) to a high DNA damage category (OTM-2) was observed in $PM_{1.0}$- and $PM_{2.5}$-treated A549 cells, with increasing concentration of PMs. Moreover, $PM_{1.0}$ induced a higher portion of each OTM damage class than did $PM_{2.5}$ at each exposure concentration. These quantitative data provide a clear explanation for the qualitative observations of the comet assays shown in Fig. 4, which further confirms that both $PM_{1.0}$ and $PM_{2.5}$ can induce DNA damage in A549 cells in a dose-dependent manner, and that $PM_{1.0}$ is a more potent genotoxic inducer.

Conclusions

These results indicate that $PM_{1.0}$ and $PM_{2.5}$, collected from the same sampling site and in a specific season, had similar morphological features and chemical properties. $PM_{1.0}$ was enriched in chemicals emitted from anthropogenic processes, whereas $PM_{2.5}$ consisted more of large, irregular mineral particles from natural sources. The biological results revealed that both $PM_{1.0}$ and $PM_{2.5}$ could induce significant cytotoxicity and genotoxicity in A549 cells, and that $PM_{1.0}$ induced more extensive toxic effects. The higher cytotoxic effects of $PM_{1.0}$ may be attributed to the combined effects of size and chemical composition, whereas the genotoxic effect of $PM_{1.0}$ may be mainly associated with chemical species. To better understand how sources and specific constituents impact fine PM toxicity, long-term investigations of the chemical composition and in vitro biological activity of $PM_{1.0}$ are needed.

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References

Akhtar US, Rastogi N, McWhinney RD, Urch B, Chow CW, Evans GJ, Scott JA (2014) The combined effects of physicochemical properties of size-fractionated ambient particulate matter on in vitro toxicity in human A549 lung epithelial cells. Toxicology Reports 1:145-156

Alessandria L, Schilirò T, Degan R, Traversi D, Gilli G (2014) Cytotoxic response in human lung epithelial cells and ion characteristics of urban-air particles from Torino, a northern Italian city. Environ Sci Pollut Res 21:5554–5564

André N (2005) Air pollution-related illness: effects of particles. Science 308:804–806

Barone T, Zhu Y (2008) The morphology of ultrafine particles on and near major freeways. Atmos Environ 42:6749–6758

Berg J, Romoser A, Figueroa D, West C, Sayes C (2013) Comparative cytological responses of lung epithelial and pleural mesothelial cells following in vitro exposure to nanoscale SiO_2. Toxicol in Vitro 27: 24–33

Billet S, Garcon G, Dagher Z, Verdin A, Ledoux F, Cazier F, Courcot D, Aboukais A, Shirali P (2007) Ambient particulate matter ($PM_{2.5}$): physicochemical characterization and metabolic activation of the organic fraction in human lung epithelial cells (A549). Environ Res 105:212–223

Bonetta S, Gianotti V, Bonetta S, Gosetti F, Oddone M, Gennaro M, Carraro E (2009) DNA damage in A549 cells exposed to different extracts of $PM_{2.5}$ from industrial, urban and highway sites. Chemosphere 77:1030–1034

Calcabrini A, Meschini S, Marra M, Falzano L, Colone M, Berardis B, Paoletti L, Arancia G, Fiorentini C (2004) Fine environmental particulate engenders alterations in human lung epithelial A549 cells. Environ Res 95:82–91

Cavalli F, Viana M, Yttri K, Genberg J, Putaud J (2010) Toward a standardised thermal-optical protocol for measuring atmospheric organic and elemental carbon: the EUSAAR protocol. Atmos Meas Tech 3:79–89

Collins A (2004) The comet assay for DNA damage and repair. Mol Biotechnol 26:249–261

Deng X, Zhang F, Rui W, Long F, Wang L, Feng Z, Chen D, Ding W (2013) $PM_{2.5}$-induced oxidative stress triggers autophagy in human lung epithelial A549 cells. Toxicol in Vitro 27:1762–1770

Espinosa A, RodrmHguez M, Rosa F, SaHnchez J (2001) Size distribution of metals in urban aerosols in Seville (Spain). Atmos Environ 35:2595–2601

Feng Y, Chen Y, Guo H, Zhi G, Xiong S, Li J, Sheng G, Fu J (2009) Characteristics of organic and elemental carbon in $PM_{2.5}$ samples in Shanghai, China. Atmos Res 92:434–442

Gong W, Zhang T, Zhu Z, Ma Y, Ma X, Wang W (2015) Characteristics of $PM_{1.0}$, $PM_{2.5}$, and PM_{10}, and their relation to black carbon in Wuhan, central China. Atmosphere 6:1377–1387

Gualtieri M, Ørevik J, Holme J, Perrone M, Bolzacchini E, Schwarze P, Camatini M (2010) Differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells. Toxicol in Vitro 24:29–39

Huang S, Hsu M, Chan C (2003) Effects of submicrometer particle compositions on cytokine production and lipid peroxidation of human bronchial epithelial cells. Environ Health Perspect 111:478–482

Huang X, He L, Xue L, Sun T, Zeng L, Gong Z, Hu M, Zhu T (2012) Highly time-resolved chemical characterization of atmospheric fine particles during 2010 Shanghai World Expo. Atmos Chem Phys 12: 4897–4907

Hueglina C, Gehriga R, Baltenspergerb U, Gyselc M, Monnd C, Vonmont H (2005) Chemical characterisation of $PM_{2.5}$, PM_{10} and coarse particles at urban, near-city and rural sites in Switzerland. Atmos Environ 39:637–651

Kelly F, Fussell J (2012) Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. Atmos Environ 60:504–526

Lee S, Cheng Y, Ho K, Cao J, Louie P, Chow J, Watson J (2006) $PM_{1.0}$ and $PM_{2.5}$ characteristics in the roadside environment of Hong Kong. Aerosol Sci Technol 40:157–165

Li Y, Chen Q, Zhao H, Wang L, Tao R (2015) Variations in PM_{10}, $PM_{2.5}$ and $PM_{1.0}$ in an urban area of the Sichuan basin and their relation to meteorological factors. Atmosphere 6:150–163

Liao W, McNutt M, Zhu W (2009) The comet assay: a sensitive method for detecting DNA damage in individual cells. Methods 48:46–53

Lim S, Lee M, Lee G, Kim S, Yoon S, Kang K (2012) Ionic and carbonaceous compositions of PM_{10}, $PM_{2.5}$ and $PM_{1.0}$ at Gosan ABC superstation and their ratios as source signature. Atmos Chem Phys 12:2007–2024

Longhin E, Pezzolato E, Mantecca P, Holme J, Franzetti A, Camatini M, Gualtieri M (2013) Season linked responses to fine and quasi-ultrafine Milan PM in cultured cells. Toxicol in Vitro 27:551–559

Lu S, Yao Z, Chen X, Wu M, Sheng G, Fu J, Daly P (2008) The relationship between physicochemical characterization and the potential toxicity of fine particulates ($PM_{2.5}$) in Shanghai atmosphere. Atmos Environ 42:7205–7214

Massolo L, Muller A, Tueros M, Rehwagen M, Franck U, Ronco A, Herbarth O (2002) Assessment of mutagenicity and toxicity of different-size fractions of air particulates from La Plata, Argentina, and Leipzig, Germany. Environ Toxicol 17(3):219–231

Murillo J, Ramos A, García F, Jiménez S, Cárdenas B, Mizohata A (2012) Chemical composition of $PM_{2.5}$ particles in Salamanca, Guanajuato Mexico: source apportionment with receptor models. Atmos Res 107:31–41

Olive P, Banáth J (2006) The comet assay: a method to measure DNA damage in individual cells. Nat Protoc 1(1):23–29

Osornio-Vargas A, Bonner J, Alfaro-Moreno E, Martínez L, García-Cuellar C, Ponce-de-León Rosales S, Miranda J, Rosas I (2003) Proinflammatory and cytotoxic effects of Mexico City air pollution particulate matter in vitro are dependent on particle size and composition. Environ Health Perspect 111:1289–1293

Perrone M, Gualtieri M, Ferrero L, Porto C, Udisti R, Bolzacchini E, Camatini M (2010) Seasonal variations in chemical composition and in vitro biological effects of fine PM from Milan. Chemosphere 78:1368–1377

Perrone M, Gualtieri M, Consonni V, Ferrero L, Sangiorgi G, Longhin E, Ballabio D, Bolzacchini E, Camatini M (2013) Particle size, chemical composition, seasons of the year and urban, rural or remote site origins as determinants of biological effects of particulate matter on pulmonary cells. Environ Pollut 176:215–227

Perronea M, Dinoia A, Becaglib S, Udisti R (2014) Chemical composition of PM_1 and $PM_{2.5}$ at a suburban site in southern Italy. Intern J Environ Anal Chem 94(2):127–150

Pui D, Chen S, Zuo Z (2014) $PM_{2.5}$ in China: measurements, sources, visibility and health effects, and mitigation. Particuology 13:1–26

Qiao L, Cai J, Wang H, Wang W, Zhou M, Lou S, Chen R, Dai H, Chen C, Kan H (2014) $PM_{2.5}$ constituents and hospital emergency-room visits in Shanghai, China. Environ Sci Technol 48:10406–10414

Salameh D, Detournay A, Pey J, Pérez N, Liguori F, Saraga D, Bove M, Brotto P, Cassola F, Massabò D, Latella A, Pillon S, Formenton G, Patti S, Armengaud A, Piga D, Jaffrezo J, Bartzis J, Tolis E, Prati P, Querol X, Worthama H, Marchand N (2015) $PM_{2.5}$ chemical composition in five European Mediterranean cities: a 1-year study. Atmos Res 155:102–117

Senthilkumara S, Manjub A, Muthuselvama P, Shalinia D, Indhumathia V, Kalaiselvib K, Palanivelb M, Chandrasekarc P, Rajagurua P (2014) Characterization and genotoxicity evaluation of particulate matter collected from industrial atmosphere in Tamil Nadu State. J Hazard Mater 274:392–398

Shang Y, Zhang L, Jiang Y, Li Y, Lu P (2014) Airborne quinones induce cytotoxicity and DNA damage in human lung epithelial A549 cells: the role of reactive oxygen species. Chemosphere 100:42–49

Thomson E, Breznan D, Karthikeyan S, MacKinnon-Roy C, Charland J, Dabek-Zlotorzynska E, Celo V, Kumarathasan P, Brook J, Vincent R (2015) Cytotoxic and inflammatory potential of size-fractionated particulate matter collected repeatedly within a small urban area. Part Fibre Toxicol:12–24

Upadhyay D, Panduri V, Ghio A, Kamp D (2003) Particulate matter induces alveolar epithelial cell DNA damage and apoptosis. Am J Respir Cell Mol Biol 29:180–187

Velali E, Papachristou E, Pantazaki A, Choli-Papadopoulou T, Argyrou N, Tsourouktsoglou T, Lialiaris S, Constantinidis A, Lykidis D, Lialiaris T, Besis A, Voutsa D, Samara C (2016) Cytotoxicity and genotoxicity induced in vitro by solvent-extractable organic matter of size-segregated urban particulate matter. Environ Pollut 218: 1350–1362

Vinikoor-Imler L, Davis J, Luben T (2011) An ecologic analysis of county-level $PM_{2.5}$ concentrations and lung cancer incidence and mortality. Int J Environ Res Public Health 8:1865–1871

Wang J, Hu Z, Chen Y, Chen Z, Xu S (2013) Contamination characteristics and possible sources of PM_{10} and $PM_{2.5}$ in different functional areas of Shanghai, China. Atmos Environ 68:221–229

Wessels A, Birmili W, Albrecht C, Hellack B, Jermann E, Wick G, Harrison R, Schins R (2010) Oxidant generation and toxicity of size-fractionated ambient particles in human lung epithelial cells. Environ Sci Technol 44:3539–3545

Zeng Y, Deng J, Huo T, Dong F, Wang L (2016) Assessment of genetic toxicity with major inhalable mineral granules in A549 cells. Appl Clay Sci 119:175–182

Zou Y, Jin C, Su Y, Li J, Zhu B (2016) Water soluble and insoluble components of urban $PM_{2.5}$ and their cytotoxic effects on epithelial cells (A549) in vitro. Environ Pollut 212:627–635