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Atmospheric levels and cytotoxicity of polycyclic aromatic hydrocarbons and oxygenated-PAHs in $PM_{2.5}$ in the Beijing-Tianjin-Hebei region☆

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ABSTRACT

The chemical composition of $PM_{2.5}$ and cellular effects from exposure to fine aerosol extracts were studied for samples collected in Beijing, Tianjin, Shijiazhuang, and Hengshui, China in winter 2015. Effects of priority polycyclic aromatic hydrocarbons (PAHs) and their oxygenated derivatives (OPAHs) in $PM_{2.5}$ on cell cultures were a major focus of the study. Total quantified PAHs and OPAHs at Shijiazhuang and Hengshui were higher than at Beijing and Tianjin, and benz(*a*)anthracene, chrysene and 1,8-naphthalic anhydride were the most abundant species. Exposure to $PM_{2.5}$ extracts caused a concentration-dependent decline in cell viability and a dose-dependent increase in nitric oxide production. Two cytokines, tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6), also increased when A549 test cells were exposed to $PM_{2.5}$ extracts. PAHs and OPAHs in $PM_{2.5}$ can potentially cause cell damage and induce cytotoxicity and pro-inflammatory responses: benzo(*a*)anthracene-7,12-dione was highly correlated with NO production, dibenz(a,h)anthracene and 1,4-chrysenequinone were correlated with TNF-α production, and 1-naphthaldehyde was significantly correlated with IL-6 production. The study provides a new approach for evaluating relationships between air-quality and cell toxicity with respect to specific chemicals.

1. Introduction

Air pollution in China is mainly the result of coal combustion, biomass burning, motor vehicle emissions, and fugitive dust. The number of hazy days in China increased from 9.4 d/10 y in 1961 to 38.4 d/10 y in 2000 (Su et al., 2015). It has been estimated that more than two million premature deaths occur around the world each year as a result of cardiopulmonary diseases and lung cancer caused by exposure to anthropogenic particulate matter (PM) (Silva et al., 2013); even more concerning is the fact that these types of health problems are increasing (Chen et al., 2013). Epidemiological and clinical evidence also indicates that there are strong correlations between exposures to PM and adverse health effects (Brunekreef and Holgate, 2002), especially cardiopulmonary diseases, including pulmonary inflammation, oxidative stress, blood coagulation, etc. (Brook et al., 2010).

Physical and chemical properties of ambient PM can cause pro-inflammatory reactions and oxidative stress in cellular systems (Kroll et al., 2013). Many studies of the health effects of air pollution have focused on particles with aerodynamic diameters ≤2.5 μm ($PM_{2.5}$) because these fine particles can penetrate the alveoli and terminal bronchioles of the lungs and cause cell damage and

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cardiopulmonary effects (Boldo et al., 2011). Both inorganic substances, especially trace metals, and organic matter can cause cellular oxidative stress (Li et al., 2010); and some polycyclic aromatic hydrocarbons (PAHs) are important for health because they cause pro-inflammatory effects (Lin et al., 2013).

PAHs can be cytotoxic, mutagenic, teratogenic, or carcinogenic to humans (IPCS, 1998), and they can be generated from natural sources and processes; for example from forest fires, volcanic eruptions, and diagenesis. Anthropogenic sources include coal combustion, biomass burning, petroleum and diesel combustion, industrial processes and waste incineration (Okuda et al., 2010; Xu et al., 2015). Delfino et al. (2010) observed that for both indoor and outdoor environments, particulate PAHs are the most important biomarkers of inflammation while hopanes were the next most important.

Oxygenated-PAHs (OPAHs) can be derived from parent-PAHs through reactions with atmospheric oxidants, including ozone (O_3), nitrogen oxides (NO_x), and hydroxyl radicals (Shen et al., 2012). Some OPAHs are more toxic than their corresponding parent-PAHs even at low concentrations, and they also can be involved in the production of reactive oxygen species (ROS), which in turn can induce inflammatory reactions in human cells (Benbrahim-Tallaa et al., 2012).

The Beijing-Tianjin-Hebei region, located on the North China Plain, is the cultural and political center of China. Rapid economic and industrial development combined with sustained population growth has led to frequent and heavy atmospheric pollution episodes in the region. Even though the levels and chemical composition of PM from various sites in the area have been studied in considerable detail, the cytotoxicity of the particles and the constituents that cause the effects are largely unknown. In the "Technical Regulation on Ambient Air Quality Index (on trial)" promulgated by the Chinese Ministry of Environmental Protection in 2012, heavy pollution is defined as times when the air quality index (AQI) > 200, and this corresponds to a $PM_{2.5}$ concentration of >150 μg m^{-3}. In the toxicity study described here, the ambient $PM_{2.5}$ samples were divided into two groups: those collected on non-hazy ($PM_{2.5} \leq 150$ μg m^{-3}) versus hazy ($PM_{2.5} > 150$ μg m^{-3}) days; this was done to assess possible relationships between the $PM_{2.5}$ loadings and health effects. The objectives of this study were (1) to measure the concentrations PAHs and OPAHs in $PM_{2.5}$ from the Beijing-Tianjin-Hebei region and (2) to investigate possible relationships between the fine particles and *in vitro* toxicity.

2. Methodology

2.1. Sampling sites and $PM_{2.5}$ collection

Four representative sites in the Beijing-Tianjin-Hebei region were selected for the study, and these are shown in Fig. S1. The locations include: (i) Beijing (BJ), the national capital city and economic center in China—the sampling site there was in an educational and residential area that was far from industrial districts; (ii) Tianjin (TJ), a municipality with international ports and a well-developed economy—sampling was conducted in the center of the old city with only universities and scenic spots nearby; (iii) Shijiazhuang (SJZ), the provincial capital city of Hebei with heavy industries—the sampling site was in the city center, relatively close to the industrial zone; and (iv) Hengshui (HS), a less-developed city in Hebei province—the sampling site was near Hengshui Lake with small industries in nearby villages. All sampling equipment and devices were set up on the rooftops of buildings 6–20 m above ground level.

Twenty-four hour integrated daily $PM_{2.5}$ samples were collected with mini-volume samplers (Airmetrics, Oregon, USA) which operated at a flow rate of 5 L min^{-1}. The samples were collected from January 23 to February 23, 2015, which is during traditional winter heating period. The $PM_{2.5}$ samples were collected on 47 mm Teflon® membrane filters (Whatman QM/A, Maidstone, UK). Field blanks were collected at each sampling site by mounting filters in the samplers but not drawing air through them. $PM_{2.5}$ mass loadings were determined by a gravimetric method for which all filters were equilibrated in a controlled chamber at a temperature of 20–23 °C and relative humidity (RH) of 35–45% for 24 h. Each sample was weighed at least twice (weighing differences were <15 and < 20 μg before and after sampling, respectively) using a MC5 electronic microbalance (±1 μg sensitivity, Sartorius, Gottingen, Germany).

2.2. PAH and OPAH extraction and analysis

Seven deuterated-PAHs (naphthalene-D8, acenaphthene-D10, phenanthrene-D10, pyrene-D10, chrysene-D12, perylene-D12, and benzo[g,h,i]perylene-D12), and two deuterated-OPAHs (benzophenone-D10 and anthraquinone-D8) were spiked onto the sample and blank filters for use as internal standards (IS). One half of each filter was extracted with high-purity dichloromethane and methanol (2:1, v/v) under ultrasonication for 15 min. The extraction procedure was repeated three times to ensure the completeness of extraction. Water and debris in the combined extracts were then removed by passing them through Pasteur pipettes filled with sodium sulfate ($NaSO_4$) and glass wool. The extracts were finally concentrated to 1 ml by a rotary evaporator under vacuum.

The samples were analyzed with a gas chromatography/mass spectrometer (GC/MS) (Model 7890A/5975C, Agilent Technology, CA, USA). The extract was injected through a GC injection port at 275°CGC in an auto-sampler, and the GC separation made use of a DB-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technology). The MS was operated in the selective ion monitoring mode (SIM) with two ions monitored for each compound and dwell times ranging between 25 and 50 ms. The GC oven was programmed to increase from room temperature to 50 °C in 2 min, ramped to 120 °C (at a rate of 15 °C min^{-1}), then to 300 °C (at 5 °C min^{-1}), and finally held at 300 °C for 16 min. The target compounds were identified by comparison of their retention times and ratios of qualifier ions with those in standards. An Internal standard (IS) was added into the samples to qualify the actual amounts of the target compounds. Calibration curves were constructed over concentration ranges of 10–1000 ng ml^{-1} for the PAHs and 10–500 ng ml^{-1} for the OPAHs. All data recording and processing was done with the Agilent MSD ChemStation software package.

The recoveries for each target PAH and OPAH from the extraction procedure were determined using standard mixtures of the analytes and the extraction ISs that were spiked onto pre-cleaned blank filters. The extraction procedures and GC–MS analyses of the spiked filters proceeded in the same fashion as those used for the sample filters. The recovery of each analyte was computed by comparing the peak-area ratios of the analyte in the spiked filter sample and the injection IS with the corresponding standard sample that was not processed through any of the sample treatment steps. The recoveries of PAHs and OPAHs ranged from 76 to 114% and all concentrations reported in this paper were corrected for the recovery efficiencies and blanks.

2.3. Filter extraction for cell toxicity studies

The halves of the particle-laden Teflon® filters not used for chemical analyses were immersed in 2 ml of high-purity methanol,

and then they were ultrasonicated in an ice-cooled water bath for 30 min. The extraction procedure was repeated twice, and the combined extracts of each site were purged under a gentle stream of nitrogen (N_2 > 99.995%) for 2 h to completely remove the solvent. The extracts after weighting were dissolved in phosphate-buffered saline (PBS) and diluted to levels of 10, 50 and 100 mg ml^{-1}; and the PBS diluent alone was used as a control. A small amount of dimethyl sulfoxide (<0.05% v/v) was added to the samples and controls to dissolve any water-insoluble matter.

2.4. Cell cultures

Human alveolar epithelial cells (A549) were obtained from the American Type Culture Collection (ATCC, Maryland, USA), and they were cultured using F-12 cell culture medium (Thermo Fisher Scientific Inc., MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Biowest, MO, USA) and 1% antibiotics penicillin/streptomycin (100 U ml^{-1}) in a humidified incubator supplied with 5% carbon dioxide at 37 °C. The A549 cells were seeded into inserts for 24-well transwells (ThermoFisher, Waltham, MA, USA) at a density of 1×10^5 cells ml^{-1} and incubated for 24 h. The bottom media was then replaced with fresh media, and the upper media was removed and replaced with 300 μl of the prepared samples in media for 4 h. Each experiment was conducted in quadruplicate. The bottom layer of media was taken to determine concentrations of tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and nitric oxide (NO).

2.5. MTT assay and ELISA

Metabolic activities of the cells in the extract exposure experiments were determined by the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay. For these studies, A549 cells from the 10, 50 and 100 mg ml^{-1} exposures were detached from the inserts using trypsin and seeded into 96-well transwells at a final volume of 200 μl of F-12 cell culture medium and incubated in 5% CO_2 at 37 °C for 24 h. Then, 200 μl of a 10% MTT solution (Sigma Aldrich, St. Louis, MO, USA) was added to each well and the cultures were incubated in the dark at 37 °C for 4 h for color development. After that, the medium was discarded and 150 μl DMSO was added. Optical density was measured at 540 nm with an absorbance microplate reader (ELx800, BioTek, VT, USA). The results are presented as the percentage of the absorbance relative to control cells.

Cytokines and nitrogen oxide levels were determined for the supernatants collected from the cell cultures from the $PM_{2.5}$ extract exposure studies after 24 h of incubation. Enzyme-linked immunosorbent assays (ELISA) kits (R&D systems, Inc., MN, USA) were used to determine the TNF-α and IL-6 concentrations. NO production was measured by a Nitric Oxide Synthase Assay Kit (Calbiochem, USA). All ELISA experiments were performed using manufacturer's instructions.

2.6. Statistical analysis

All averages in this study are expressed as arithmetic mean values along with standard deviations (SD). One-way analyses of variance (ANOVA) were used to test the statistical significance of between-group differences. Pearson's correlation coefficients were calculated to assess the associations between PAHs/OPAHs, vasoactive function (i.e., NO), and pro-inflammatory cytokines (i.e., TNF-α and IL-6). The level of significance for all statistical tests was set as $p < 0.05$. The statistical analyses were done using SPSS software (version 12.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. $PM_{2.5}$ mass, PAHs and OPAHs concentrations

The mean $PM_{2.5}$ mass and total measured PAH and OPAH concentrations (∑PAHs and ∑OPAHs) at the four cities are shown in Fig. 1. The mean $PM_{2.5}$ masses in SJZ (173.3 μg m^{-3}) and HS (120.4 μg m^{-3}) were 20–90% higher than those in BJ (90.0 μg m^{-3}) and TJ (99.1 μg m^{-3}) ($p < 0.01$). Moreover, the∑PAHs and ∑OPAHs at SJZ and HS were also 20–30% and 30–90%, respectively, higher than those measured at BJ and TJ ($p < 0.01$). These differences can be explained by some of the cities' characteristics: that is, BJ and TJ are mainly supported by industries with low environmental impact, such as finance, service, culture and tourism, while there are many manufacturing facilities and electricity and heating power plants in SJZ and HS. Emissions from heavy industries probably caused in more severe pollution. The factories in Shijiazhuang are close to some residential and commercial areas, and the downtown area is often affected by pollutants that presumably come from those factories. In Hengshui, many factories were built without careful planning, and the large quantities of pollutants they emit can affect the whole city. In addition, in northern China, the burning of natural gas, coal, and biomass to satisfy the large heating demands in winter is a well known source for pollutants. This is reflected in high levels of PAHs and OPAHs in the PM organic carbon fractions due to incomplete combustion processes (Wang et al., 2011). The HYSPLIT model was used to generate 24 h air-mass back-trajectories for Beijing (Fig. S2), and the results showed that the air masses sampled on hazy days mainly originated from Hebei Province and therefore passed over the highly industrialized areas. In contrast, on non-hazy days, cleaner air was transported from Mongolia and Inner Mongolia.

Table 1 lists mean concentrations of individual PAHs and OPAHs, and the results are presented graphically in Fig. S3. The abundances of low molecular weight (LMW) PAHs were relatively low, presumably due at least in part to their partitioning into the gas phase, whereas high molecular weight (HWM) PAHs, which tend to be more carcinogenic, were mainly particle bound and at higher concentrations (Chang et al., 2006). The most abundant PAH was benz(*a*)anthracene (BaA), which showed concentrations as high as 35.4 and 31.1 ng m^{-3} at SJZ and HS, respectively, and it had the highest contributions to the ∑PAHs (11.0–17.0%). Chrysene (CHR) was the next most abundant PAH, contributing 9.4–11.7% to the ∑PAHs, and it was followed by fluoranthene (FLT), benzo(*b*)fluoranthene (BbF), and indeno(1,2,3-*cd*)pyrene (IPY). It should be noted that these compounds were relatively more abundant in SJZ and HS, which are the two more polluted cities in our study.

The most abundant OPAH was 1,8-naphthalic anhydride (1,8-NAP), which accounted for > 30% of the ∑OPAHs at SJZ and HS and also was at high levels at TJ and BJ where the contributions were 30.6% and 25.7%, respectively. 6H-Benzo(*c*,*d*)pyrene-6-one (6H-BcdP) was the next most abundant OPAH, and it contributed 16.5–20.5% of the ∑OPAHs, but its highest contribution was at BJ. Benzo(*a*)pyrene (BaP) is the most potent carcinogen of the PAHs we studied, it is listed as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) (Baek et al., 1991), and it is also a well-known mutagen. The levels of BaP at all of our sites were higher than the limits in the air-quality guidelines for $PM_{2.5}$ recommended by the World Health Organization (WHO) (1.0 ng m^{-3}) (WHO, 2010) and China National Ambient Air Quality Standards (2.5 ng m^{-3}). The daily BaP concentrations at SJZ and HS were more than ten times those in the WHO standard, and therefore this compound represents a clear and serious health concern for citizens in the region.

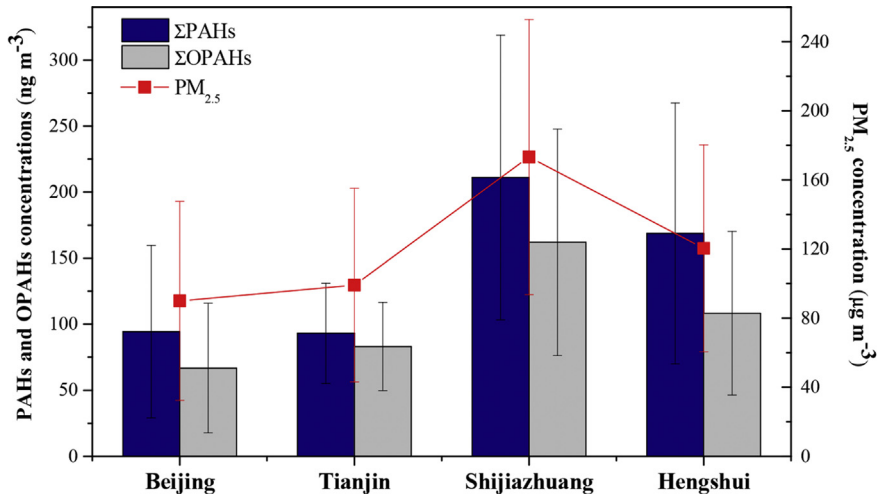

Fig. 1. $PM_{2.5}$ mass loadings and total polycyclic aromatic hydrocarbons (∑PAHs) and total oxygenated polycyclic aromatic hydrocarbons (∑OPAHs) concentrations in $PM_{2.5}$ from the Beijing-Tianjin-Hebei region.

Table 1
Concentrations of PAHs and OPAHs in four Chinese cities (ng m^{-3}).

	Abbreviation	Beijing	Tianjin	Shijiazhuang	Hengshui
		Ave.± Std.	Ave. ± Std.	Ave. ± Std.	Ave. ± Std.
PAHs					
Naphthalene	NAP	3.44 ± 1.88	2.76 ± 2.53	1.30 ± 1.18	4.04 ± 1.51
Acenaphthylene	ANY	0.69 ± 0.28	0.94 ± 0.41	1.32 ± 0.46	0.86 ± 0.26
Acenaphthene	ANA	1.78 ± 0.17	1.51 ± 0.51	1.41 ± 0.31	1.77 ± 0.22
Fluorene	FLU	5.27 ± 1.46	5.71 ± 1.56	5.85 ± 2.00	5.65 ± 2.03
Phenanthrene	PHE	6.29 ± 2.12	8.25 ± 2.06	11.56 ± 4.25	7.38 ± 2.51
Anthracene	ANT	1.26 ± 0.31	1.84 ± 0.68	2.42 ± 0.49	1.78 ± 0.54
Fluoranthene	FLT	7.13 ± 5.04	8.55 ± 3.12	20.89 ± 11.38	12.58 ± 8.19
Pyrene	PYR	6.42 ± 4.58	7.21 ± 2.75	18.29 ± 9.60	12.22 ± 8.25
Benz(*a*)anthracene	BaA	13.58 ± 11.92	11.32 ± 7.36	35.44 ± 22.92	31.05 ± 21.81
Chrysene	CHR	10.47 ± 9.42	9.47 ± 5.48	25.82 ± 15.17	21.06 ± 14.32
Benzo(*b*)fluoranthene	BbF	9.51 ± 8.10	8.71 ± 4.90	21.54 ± 12.02	17.44 ± 11.13
Benzo(k)fluoranthene	BkF	5.76 ± 5.09	5.13 ± 3.02	12.73 ± 6.95	10.41 ± 6.62
Benzo(*a*)pyrene	BaP	7.92 ± 6.84	6.87 ± 3.92	18.30 ± 10.52	15.44 ± 9.69
Indeno(1,2,3-*cd*)pyrene	IPY	8.55 ± 7.00	8.50 ± 5.01	20.21 ± 11.19	15.85 ± 10.01
Dibenz(a,h)anthracene	DBA	0.49 ± 0.31	0.58 ± 0.22	1.14 ± 0.71	0.97 ± 0.53
Benzo(g,h,i)perylene	BghiP	5.76 ± 4.45	5.77 ± 3.17	12.84 ± 6.80	10.29 ± 6.09
∑PAHs		94.33 ± 65.25	98.00 ± 36.80	211.07 ± 107.79	168.77 ± 98.67
OPAHs					
1,4-Naphthoquinone	1,4-NQ	0.55 ± 0.35	0.82 ± 0.60	1.09 ± 0.56	0.84 ± 0.56
1-Naphthaldehyde	1-NAD	0.90 ± 0.31	0.99 ± 0.48	0.99 ± 0.16	0.91 ± 0.28
1-Acenaphthenone	1-ACP	5.68 ± 1.92	16.03 ± 3.46	14.43 ± 2.30	5.63 ± 1.07
9-Fluorenone	9-FLU	4.79 ± 2.65	5.31 ± 1.48	8.30 ± 3.75	4.82 ± 1.94
1,2-Acenaphthenequinone	1,2-ACQ	1.71 ± 0.78	3.63 ± 1.79	5.25 ± 2.46	3.44 ± 1.84
9,10-Anthraquinone	9,10-ANQ	8.29 ± 7.51	7.52 ± 3.33	18.91 ± 10.51	11.58 ± 6.91
1,8-Naphthalic anhydride	1,8-NAP	28.60 ± 19.00	27.39 ± 16.96	61.99 ± 49.68	41.33 ± 30.09
Benzo(*a*)anthracene-7,12-dione	BANTdione	4.88 ± 4.15	4.74 ± 2.35	10.58 ± 5.61	7.62 ± 3.75
5,12-Naphthacenequinone	5,12-NACQ	1.47 ± 1.28	2.08 ± 1.72	3.89 ± 2.61	4.18 ± 2.61
1,4-chrysenequinone	1,4-CRQ	3.60 ± 2.35	3.40 ± 1.68	7.20 ± 3.44	6.10 ± 3.03
6H-Benzo(*c,d*)pyrene-6-one	6H-BcdP-6-one	14.01 ± 11.11	13.99 ± 6.68	29.32 ± 15.25	21.91 ± 13.34
∑OPAHs		84.70 ± 53.83	96.17 ± 34.18	180.01 ± 91.66	121.30 ± 67.26

3.2. Source characterization

Diagnostic ratios have been widely used to characterize the sources for atmospheric PAHs (Lin et al., 2015; Tobiszewski and Namiesnik, 2012), and we calculated several ratios for this purpose. A scatterplot of the FLU/(FLU + PRY) versus ANT/(ANT + PHE) ratios is shown in Fig. 2: the ANT/(ANT + PHE) ratios in all samples were ≥0.10, suggesting large contributions from pyrogenic sources (e.g., wood and coal) and petrogenic sources (e.g., petroleum from industries) (Wang et al., 2014). The distribution of the FLU/(FLU + PRY) ratios in Fig. 2 indicates that the pollution at BJ was mainly caused by coal combustion and biomass burning. For TJ and HS, most of the data points were concentrated in the center of the plot [i.e., in a range of 0.4–0.5 for FLU/(FLU + PRY)] suggesting that petroleum combustion sources had the greatest impact on those two cities (Xu et al., 2013). At SJZ, the FLU/(FLU + PRY) ratio ranged from 0.2 to 0.4, and that can be ascribed to influences from both burned and unburned petroleum emitted by the manufacturing and textile industries and power plants in and around the city (Ringuet et al., 2012).

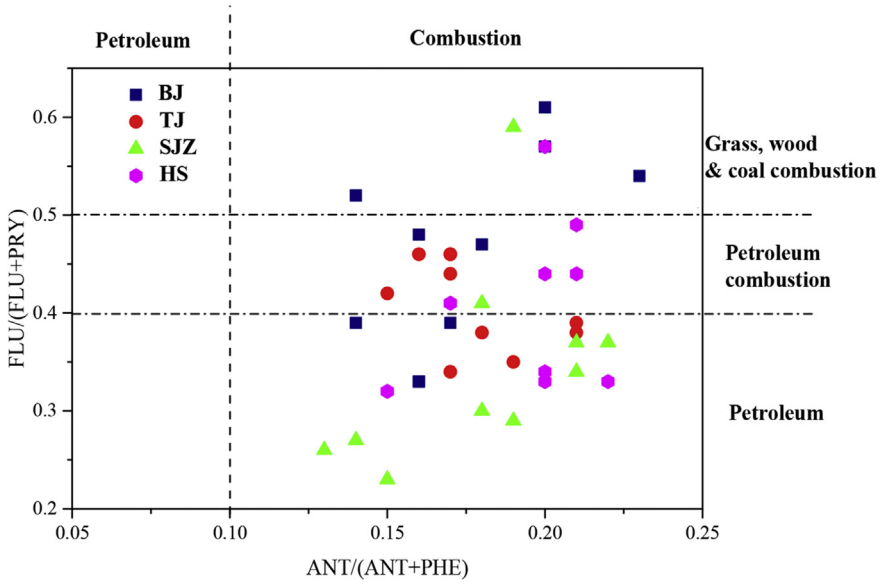

Fig. 2. Correlations between PAHs diagnostic ratios (ANT stands for Anthracene, PHE is Phenanthrene, FLU is Fluorene, PRY is Pyrene) for source identification in Beijing (BJ), Tianjin (TJ), Shijiazhuang (SJZ)and Hengshui (HS).

The mass ratio of ∑OPAHs/∑PAHs in TJ was the highest (0.85), followed by SJZ (0.77), BJ (0.71), and HS (0.64). Those ratios were high compared with the results from previous studies that average ratio of 0.8 and 0.5 were found in summer and winter, respectively. The patterns in these ratios imply that there was more photochemical activity and a greater degradation of parent-PAHs at TJ and SJZ relative to the other two sites (Walgraeve et al., 2010; Albinet et al., 2007). Fig. 3 shows positive correlations between ANT and 9,10-ANQ ($r = 0.63$) and BaA and BANTdione ($r = 0.86$), and these were statistically significant at $p < 0.05$. The extensive burning of natural gas and coal for heating in winter leads to large quantities of the primary parent-PAHs (e.g., ANT and BaA) (Chang et al., 2006; Shen et al., 2011), and evidently some related OPAHs are emitted at the same time. In contrast, the correlations of 9-FLU and 1,4-NQ with their related parent-PAHs (i.e., FLU and NAP, respectively) were not significant, suggesting that more of these two OPAHs would rather present in the gas-phase following heterogeneous reactions of their more volatile parent-PAHs. It should be noted that any interference of other trace OPAHs cannot be ignored which could lead to uncertainties in the correlation analysis (Bandowe et al., 2014).

3.3. Cell viability experiments

Plots of cell viabilities of A549 cells after 24 h exposure to the $PM_{2.5}$ sample extracts (Fig. 4) show that the treatments induced a concentration-dependent decrease in MTT-reduction activity; this implies that exposure to $PM_{2.5}$ could lead to a decline in cell viability. For the experiments with $PM_{2.5}$ extracts at concentrations of 100 μg ml^{-1}, the cell viabilities had a significant decrease of 0.7–5.7%. Moreover, there were distinct differences in cytotoxic effects of the $PM_{2.5}$ extracts with respect to where the samples were collected. For example, at SJZ and TJ, there were 37% and 6% greater declines in cell viability, respectively, with exposure to 50 and 100 μg ml^{-1} of $PM_{2.5}$, compared with the average level of the other two sites. As the cell exposures were adjusted to test the same $PM_{2.5}$ levels, the effects on cell viabilities can be explained by variations in the toxicity of the particles themselves (Michael et al., 2013).

Our data thus indicate that the $PM_{2.5}$ from SJZ and TJ would have greater cytotoxic effects than those from BJ and HS. Furthermore, slightly lower cell viabilities were found for the samples collected in hazy days than non-hazy days; that is, for the $PM_{2.5}$ extracts of 100 μg ml^{-1}, the cell viabilities between the haze and non-haze samples were statistically different. This result implies that differences in the amounts of specific substances associated with the particles during the haze episodes could lead to greater cytotoxic effects.

3.4. NO expression

Fig. 5 shows the results of the NO expression tests with A549 after 24 h exposure to $PM_{2.5}$ extracts prepared from samples collected on hazy and non-hazy days. The NO production showed a dose-dependent response, which increased with the $PM_{2.5}$ contents of the extracts. Higher NO levels were more often seen for the hazy days' samples than the non-haze ones. With the exposures to $PM_{2.5}$ extracts of 100 μg ml^{-1}, extremely high NO values were measured for the haze samples collected at TJ (366.1 μmol L^{-1}) and SJZ (311.6 μmol L^{-1}).

NO is a signal-transducing free radical that is produced by NO synthase (NOS), and it plays important roles in mediating inflammatory reactions and tissue cytotoxicity (Diociaiuti et al., 2001; Sun et al., 2006). Alvarez and Evelson (2007) showed that NO influenced a variety of inflammatory responses, ranging from the production of immune competent cells to the recruitment of leukocytes, which then resulted the generation of a stable peroxynitrite anion ($ONOO^-$) (Magnani et al., 2011).

In our study, comparatively high NO was induced by exposure of the test cells to PM extracts from SJZ and TJ; and this suggests a stronger potential for inflammatory reactions or damage to the lung cells compared with the other sites—the lowest risk was found at BJ. Differences in NO production may have been related to the chemical constituents of the particles; that is, compared with the non-hazy days, one would expect stronger physical and chemical atmospheric reactions during the hazy periods owing to the higher pollutants levels, and this could favor the formation of more toxic particles. Our findings are thus consistent with the

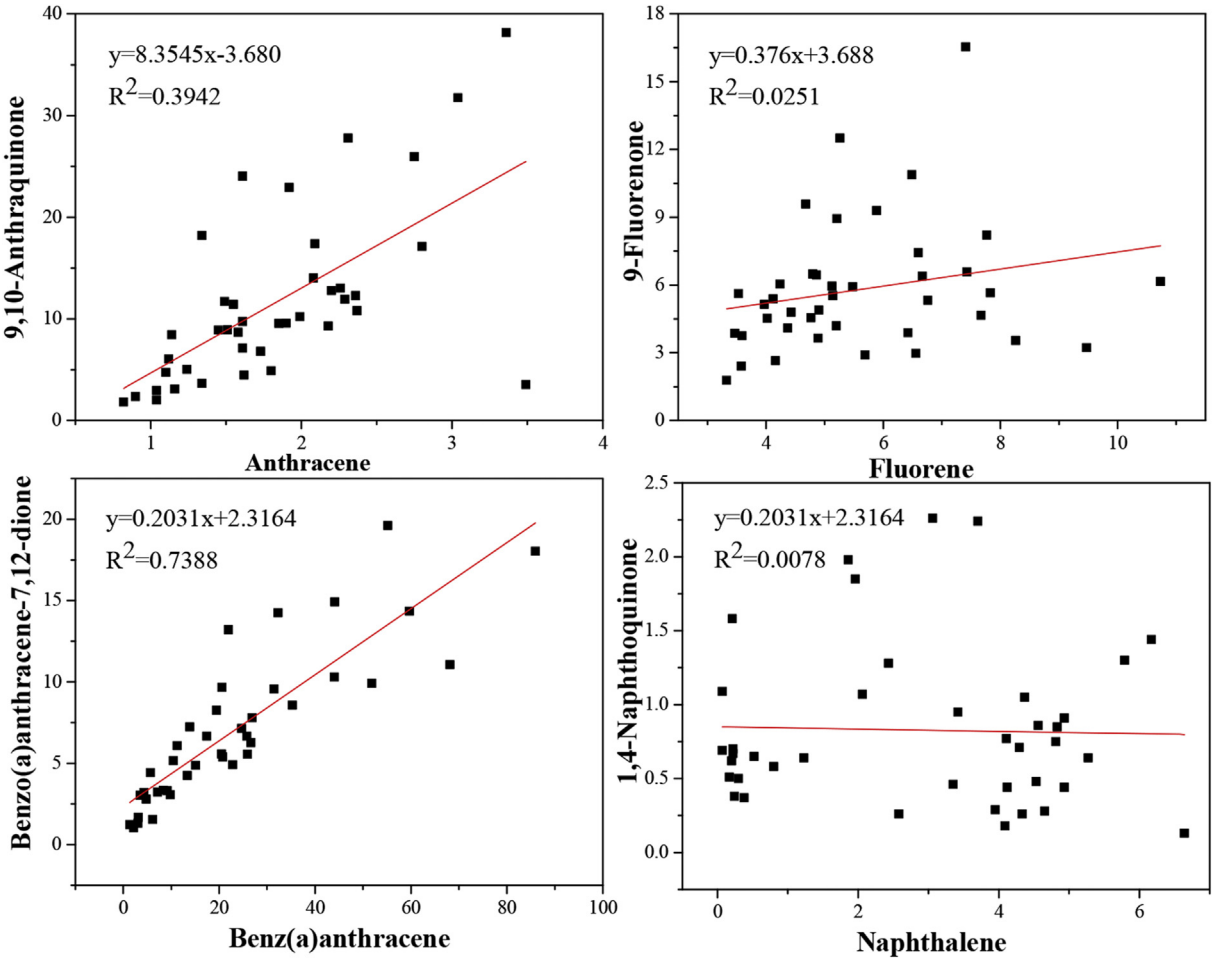

Fig. 3. Relationship between parent PAHs and their related OPAHs concentrations in $PM_{2.5}$.

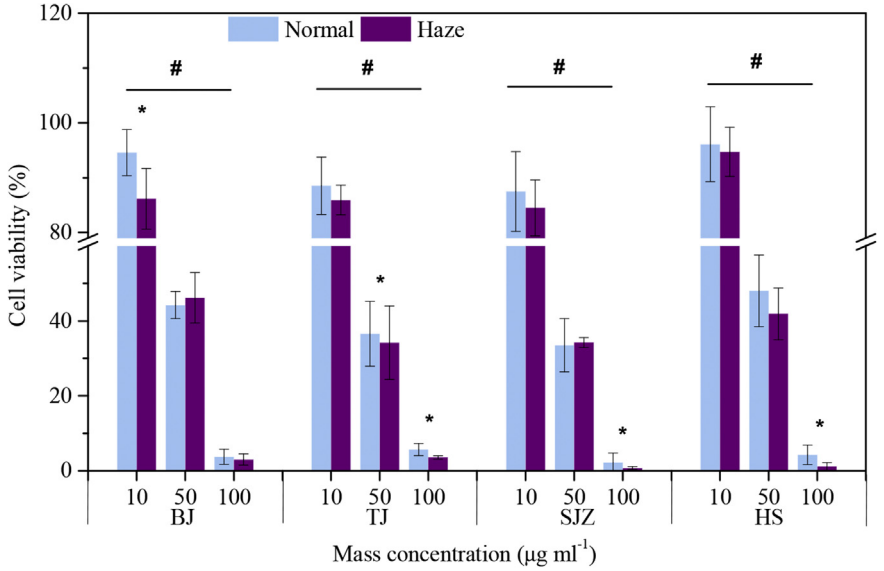

Fig. 4. Cell viability following exposure to different mass concentrations of $PM_{2.5}$ extracts from hazy and non-hazy days. *$p < 0.05$ compared between haze and non-haze groups; #$p < 0.05$ compared between concentration groups.

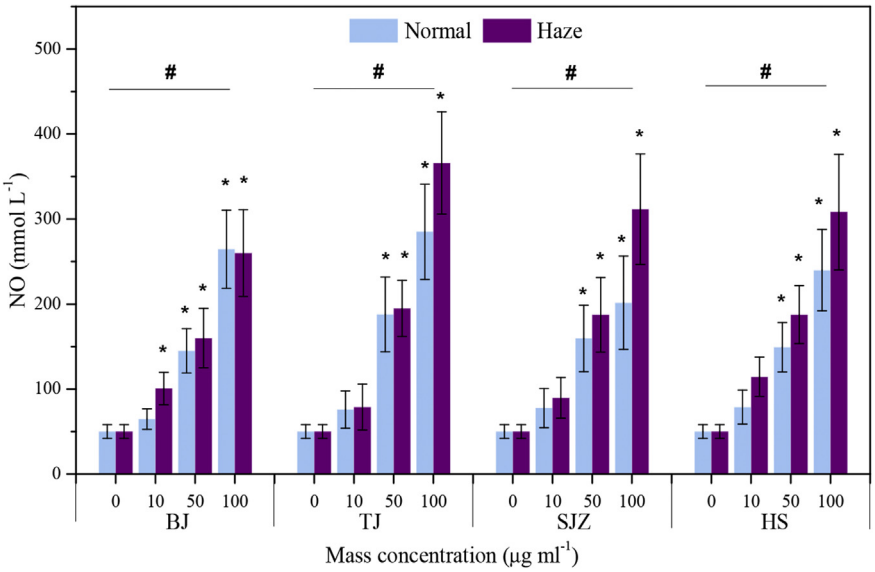

Fig. 5. Nitrogen oxide (NO) production of A549 cells exposed to different mass concentrations of $PM_{2.5}$ extracts from hazy and non-hazy days. *p < 0.05 compared with control group; #p < 0.05 compared between concentration groups.

studies of Castranova et al. (1998) and Chen et al. (2004) who reported that *in vivo* and *in vitro* exposures of mononuclear cells and macrophages to $PM_{2.5}$ extracts could stimulate NO production.

3.5. Inflammatory cytokines

The production of pro-inflammatory cytokines TNF-α and IL-6 was determined from the cell culture supernatants after 24 h of incubation with $PM_{2.5}$ extracts. The results for each of the four sites are shown separately in Fig. 6. The synergistic effects of oxidative stress and inflammation are underlying causes for cell toxicity and thus they are important in the context of environmental health effects (Castell et al., 2005; Happo et al., 2010). Generally, redox-active metals and organic compounds (i.e., PAHs and OPAHs) are both directly and indirectly associated with reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxyl radicals) production (Øvrevik et al., 2010). Indeed, PM can induce ROS in human epithelial lung cells, and this in turn, can lead to oxidative stress when the levels of ROS exceed the capacity of antioxidant defenses (DiStefano et al., 2009; Janssen et al., 2014). As the concentrations and composition of the PM from different cities varies, it is likely that the ability of PM to influence the levels of ROS also differed among cities.

Moreover, oxidative stress can activate signaling pathways that cause inflammatory responses, for which the pro-inflammatory mediators [e.g. IL-6, interleukins 8 (IL-8) and TNF-a] have been used as biomarkers (Gerlofs-Nijland et al., 2009). The TNF-α and IL-6 production in the A549 epithelial cell culture studies showed a concentration-dependent increase for both hazy and non-hazy days. The maximum production of TNF-α and IL-6 was found for the exposure to 100 μg ml^{-1} of $PM_{2.5}$ extract for hazy day samples from SJZ, and this was followed by the matching exposure test at HS. Variations in levels of pro-inflammatory cytokines produced at the sites may be explained by chemical composition of the $PM_{2.5}$ because SJZ is affected by anthropogenic emissions that not only increase the $PM_{2.5}$ mass loadings but also the concentrations of specific chemicals that could trigger the release of inflammation cytokines (Liu et al., 2014). These toxic substances, especially organic compounds from combustion process are discharged and emitted from factories and power plants in large quantities, are thus they are critical concerns for environmental health. In addition, higher pro-inflammatory cytokines levels were measured for the hazy days than the non-hazy ones ($p < 0.05$).

$PM_{2.5}$ particles are small enough to penetrate into the lower respiratory tract of humans, and the PAHs and OPAHs are toxins and carcinogens that induce cell damage when inhaled deep into the respiratory system (Ho et al., 2016). Once inhaled, PAHs and OPAHs can be converted into their hydroxyl derivatives (i.e., quinones) in biological systems (Xia et al., 2004), and this can lead to inflammation and vasoactive dysfunction (Chuang et al., 2012). Bostrom et al. (2002) found that some PAHs can be retained in bronchial tissues due to their lipophilic properties, and therefore localized high concentrations in some tissues may occur even at low environmental exposure levels, and this, too, can lead to inflammatory responses. The contributions of BaA, CHR and BbF to ∑PAHs and 1,8-NAP to ∑OPAHs were 20–50% higher on hazy days than non-hazy days, and these compounds are potentially key components with regard to the inflammatory cytokines.

3.6. Relationships between specific chemicals and bioreactive responses

Pearson's correlation coefficients were calculated and used to determine whether the concentrations of PAHs or OPAHs extracted from the fine aerosol were related to the production of NO, TNF-α, or IL-6. The bioreactive results for all the four cities were calculated and grouped together, and the $PM_{2.5}$ extracts of 100 μg m^{-3} were selected for the evaluation and comparison. Among the quantified PAHs and OPAHs, BANTdion ($r^2 = 0.71$, $p < 0.05$) was highly correlated with NO production while DBA and 1,4-CRQ were significantly correlated with TNF-α ($r^2 = 0.64$ and 0.71, respectively, both $p < 0.05$) and 1-NAD ($r^2 = 0.69$, $p < 0.05$) was correlated with IL-6 production. This is evidence that links specific chemical compounds in the aerosol to adverse effects on cellular systems—and by inference, to human health.

TNF-α and IL-6 are two major factors involved in the mediation of airway inflammation (Verma et al., 2014), and pro-inflammatory responses involving these cytokines can be induced by the exposure to heavy metals, endotoxins, and some carbonaceous species (Daher et al., 2014; den Hartigh et al., 2010). Transition metals and

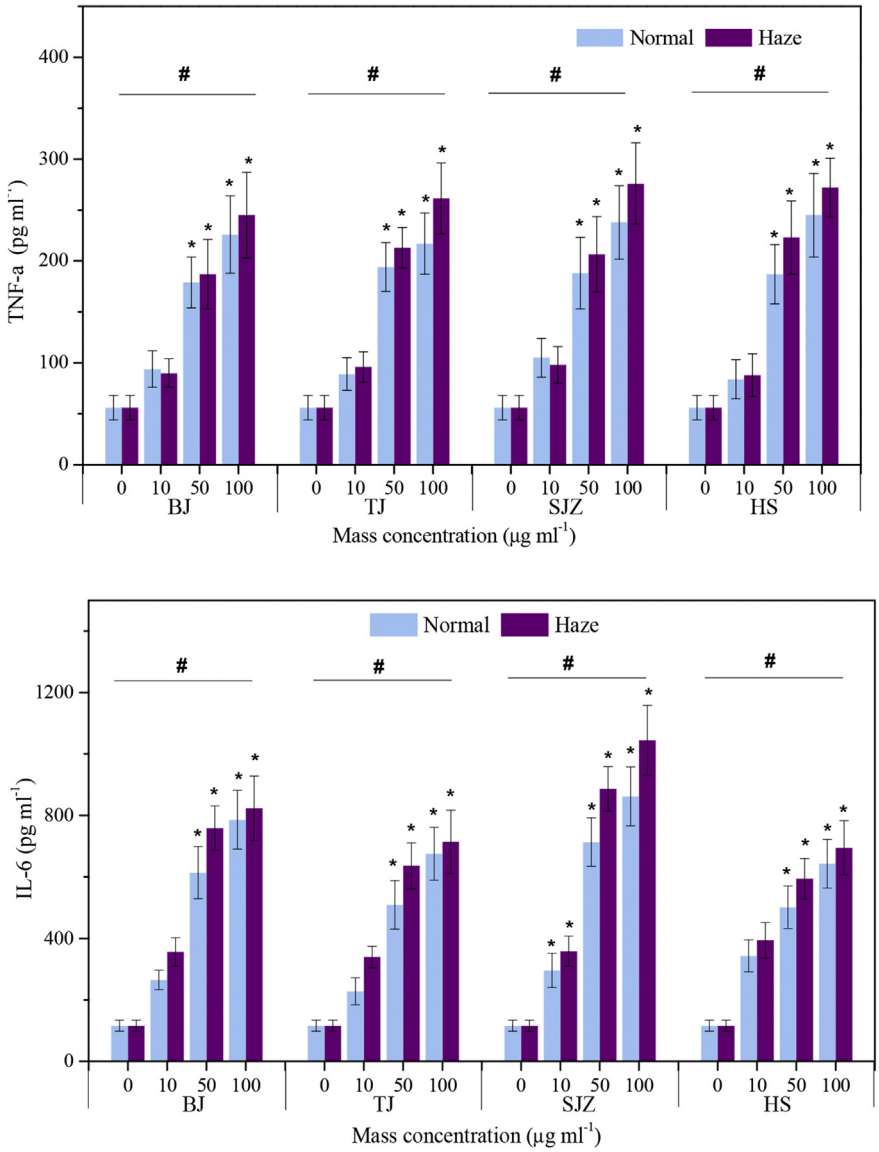

Fig. 6. Tumor necrosis factor αTNF-α and interlukin-6 (IL-6) production of A549 exposed to different mass concentrations of $PM_{2.5}$ extracts from hazy and non-hazy days. *p < 0.05 compared with control group; #p < 0.05 compared between concentration groups.

PAHs adsorbed onto the surface of carbonaceous matter evidently play more important roles in inflammation than elemental carbon or other organic fractions (Gualtieri et al., 2009; Jalava et al., 2009). Chuang et al. (2012) showed that PAHs in PM, particularly the congeners with 4–6 rings, were associated with the production of NO and IL-6, whereas more recent studies have demonstrated that PAHs associated with the carbonaceous fraction of aerosol particles can lead to cell damage and induce cytotoxicity and pro-inflammatory responses (Cachon et al., 2014; Gualtieri et al., 2012). In this study, both PAHs and OPAHs in $PM_{2.5}$ were well correlated with the oxidative-inflammatory responses; in particular, DBA and 1,4-CRQ were significantly correlated with TNF-α, and 1-NAD was significantly correlated with IL-6 production.

A study conducted in the New York City region showed that the aerodynamic sizes of ambient PM and the locations where the samples were collected could affect the production of ROS and inflammatory responses in pulmonary cells in controlled exposure studies (Mirowsky et al., 2013). More important, the responses were highly dependent upon the chemical composition of the PM. Some biological effects of PAHs and urea in $PM_{2.5}$ from various cities in China have been investigated, and both the oxidative potentials and pro-inflammatory cytokines showed higher expressions in samples from Beijing compared with other megacities (i.e., Xi'an, Xiamen, Guangzhou, Hong Kong), and this implies that the migratory activities of cells and tumorigenicity associated with PM will also vary with the particles' origins (Ho et al., 2016; Leung et al., 2014). Ho et al. (2016) reported that sulfate, nitrate, ammonium, organic carbon, urea, and levoglucosan in PM are associated with oxidative-inflammatory responses as well. Our results are consistent with these studies, but further research is needed to investigate the toxicity of specific components as well as representative mixtures of substances; the underlying mechanisms of $PM_{2.5}$ oxidative and inflammatory responses also deserve further study.

4. Conclusions

Priority PAHs and OPAHs were quantified in the $PM_{2.5}$ samples collected at four cities in the Beijing-Tianjin-Hebei region, and the

toxicity of extracts prepared from the fine particles was investigated *in vitro*. The atmospheric levels of BaP in Beijing, Tianjin, Shijiazhuang, and Hengshui all exceeded the guidelines of the WHO and China National Ambient Air Quality Standards, and they were particularly high in the industrial cities of SJZ and HS. Lipids in tissues can bind certain organic pollutants, and in this way, PAHs can accumulate to high levels, even at low environmental exposure levels; this in turn could lead to cell damage and induce cytotoxicity and pro-inflammatory responses. More aerosol toxicity studies should be conducted in China due to the frequent and severe air pollution episodes that affect the more industrialized parts of the country.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.08.099.

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