

Chemical composition and bioreactivity of PM_{2.5} during 2013 haze events in China



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H I G H L I G H T S

- Significant increases of sulfate, nitrate and ammonium were observed during episodes.
- High contributions of biomass burning emissions to organic carbon (OC) were estimated in this study.
- BJ PM_{2.5} samples has the highest bioreactivity although PM_{2.5} levels are not the highest.
- The OC, urea and levoglucosan are associated with oxidative-inflammatory responses.

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Chemical composition and bioreactivity of PM_{2.5} samples collected from Beijing (BJ), Xi'an (XA), Xiamen (XM) and Hong Kong (HK) in China during haze events were characterized. PM_{2.5} mass concentrations in BJ, XA, XM and HK in the episodes were found to be $258 \pm 100 \mu\text{g m}^{-3}$, $233 \pm 52 \mu\text{g m}^{-3}$, $46 \pm 9 \mu\text{g m}^{-3}$ and $48 \pm 13 \mu\text{g m}^{-3}$, respectively. Significant increase of sulfate, nitrate and ammonium concentrations in northern cities were observed. High contributions of biomass burning emissions to organic carbon (OC) in northern cities were estimated in this study implying frequent biomass burning during the haze periods. The urea concentrations in PM_{2.5} were $1855 \pm 755 \text{ ng m}^{-3}$ (BJ), $1124 \pm 243 \text{ ng m}^{-3}$ (XA), $543 \pm 104 \text{ ng m}^{-3}$ (XM) and $363 \pm 61 \text{ ng m}^{-3}$ (HK) suggesting higher or close to upper limits compared to other regions in the world. Dose-dependent alterations in oxidative potential, IL-6, IFN- γ and TNF- α levels were also investigated. The oxidative potential levels are BJ > XM > XA > HK, whereas levels of IL-6, IFN- γ and TNF- α were BJ > XA > XM > HK. The sulfate, nitrate, ammonium, OC, urea and levoglucosan

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are associated with oxidative-inflammatory responses. These experimental results are crucial for the policymakers to implement cost-effective abatement strategies for improving air quality.

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1. Introduction

Very recent studies report that more than 2 million premature deaths around the world each year are associated with anthropogenic PM_{2.5} (particulate matter with an aerodynamic diameter <2.5 μm) related cardiopulmonary diseases and lung cancer (Silva et al., 2013). Numerous epidemiological and toxicological studies have also shown correlation between exposure to particulate matter (PM) and adverse health effects (Brunekreef and Holgate, 2002; Pope et al., 2002; Pun et al., 2014). The underlying mechanism of particle-induced health effects is believed to be driven by the production of reactive oxygen species (ROS, e.g. superoxide ($\cdot\text{O}_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot\text{OH}$)) and the interaction of PM with epithelial cells and macrophages in the lung environment (Li et al., 2003; Nel, 2005). Adverse human health effects occur when an overproduction of ROS impacts on the body's anti-oxidative defenses (oxidative stress, OS) leading to cell dysfunction, inflammation and cardio-pulmonary disease (BeruBe et al., 2007). The oxidative stress can activate signaling pathways leading to the release of pro-inflammatory mediators (e.g. interleukin 6 (IL-6), interleukins 8 (IL-8), tumor necrosis factor α (TNF- α) and interferon γ (IFN- γ)) (Mitschik et al., 2008). Previous studies have focused on airborne particles with an aerodynamic diameter smaller than 2.5 μm (PM_{2.5}) because these fine particles can penetrate into the airways of the respiratory tract, reaching the alveoli and diffusing to other extrapulmonary target organs (Semmler et al., 2004). It is found that the size of particles is an important parameter in inducing cardiovascular and respiratory effects (Boldo et al., 2011). The ultrafine and fine particles are more potent than coarse particles on per mass basis (Cho et al., 2005; Ntziachristos et al., 2007). Besides particle size, there is growing evidence that the chemical composition of particles is also an important factor mediating cellular oxidative stress (Daher et al., 2014; Yang et al., 2014).

The PM_{2.5} concentrations observed in Chinese urban air are often one to two orders of magnitude higher than those observed in urban areas in the US and European countries (Huang et al., 2014b). Despite such high level of particulate pollution, studies related to particle toxicities are still very scarce in China (Deng et al., 2013; Huang et al., 2014a; Wei et al., 2011; Xu and Zhang, 2004). Especially, the toxicity of particles during haze events (visibility range is less than 10 km, RH < 80%) is not clear. The objective of this study was to investigate the toxicity of particles collected during severe haze events in China during January–February 2013. These haze events received worldwide media attention, with daily PM_{2.5} concentration higher than 700 μg m⁻³. The PM_{2.5} samples were collected in four Chinese megacities with the total mass, organic and elemental carbon (OC and EC) content being analyzed in order to determine the PM_{2.5} cytotoxicity. Certain organic compounds such as levoglucosan, water soluble organic nitrogen (WSON) species (e.g. amino acids, amines and urea) were also measured in the aerosol samples. WSON consists of a broad array of nitrogen-containing organic species that are derived from both anthropogenic and natural emissions (Cornell et al., 2003; Neff et al., 2002). There are concerns about toxicity of organic nitrogen compounds such as nitrophenols (Natangelo et al., 1999), nitrated polycyclic aromatic hydrocarbons and other N-containing combustion

products or industrial emissions which are present in the atmosphere (Albinet et al., 2008; Cheng et al., 2006) and how all of these compounds have effects on human health. Therefore, the PM_{2.5} toxicity was tested in vitro in human alveolar epithelial A549 cells that are considered as the relevant target cells. PM biological activities were characterized by measuring the expression of a panel of biomarkers. The IL-6, TNF- α and IFN- γ were further used as effective biomarkers for investigating PM exposure to the occurrence of oxidative stress and pro-inflammatory responses. The above biological results were combined with chemical analysis to elucidate the difference in aerosol bioactivities during haze episodes in four Chinese megacities.

2. Materials and methods

2.1. PM_{2.5} collection

Beijing (BJ), Xi'an (XA), Xiamen (XM) and Hong Kong (HK) were chosen for sample collection and were classified as northern (BJ and XA) and southern (XM and HK) Chinese cities in order to represent high and low PM_{2.5} exposure levels during the haze periods in this study (Fig. 1). The PM_{2.5} samples were collected over a range of six to eight days sampling campaign during the haze pollution period from the end of January until the beginning of February in 2013 (26th January to 2nd February, 2013). The PM_{2.5} samples were collected on quartz fiber filters (QM/A, Whatman Inc., Clifton, NJ, USA, 8 inch × 10 inch) using a high-volume sampler at a flow rate of 1.05–1.16 m³ min⁻¹ for bulk chemical analysis. Quartz filters were pre-baked at 800 °C for 3 h prior to sampling. The PM_{2.5} particulates were collected on Teflon filters (Pall Life Sciences, Ann Arbor, MI, 47-mm) using mini-volume samplers equipped with PM_{2.5} impactors (Airmetrics, OR, USA) at a flow rates of 5 L/min and underwent the biological testings described below. A preliminary sampling test was conducted using PM_{2.5} high-volume and PM_{2.5} mini-volume samplers in parallel at Xi'an city sampling location prior to the field study. The test results showed no significant mass differences in using high-volume and mini-volume samplers. The mass concentration data collected by the mini-volume samplers were used throughout this study. Samples were collected starting at 10:00 am. each day over a 24 h interval at the four sampling locations. All PM_{2.5} Teflon filters were equilibrated on a temperature of 25 ± 1 °C and 40 ± 5% relative humidity for 48 h before and after PM_{2.5} mass concentration analysis. A microbalance (Sartorius Model MC5 Microbalance, Göttingen, Germany) with 1 μg precision was used for the PM_{2.5} mass concentration measurements. All of the filters were stored at -20 °C and in the dark prior to the analysis. Sample blanks were collected and analyzed along with the samples.

2.2. Extraction procedures

Teflon filters were extracted with methanol for biological analysis and quartz filters were extracted with either methanol or ultrapure water for different chemical analysis.

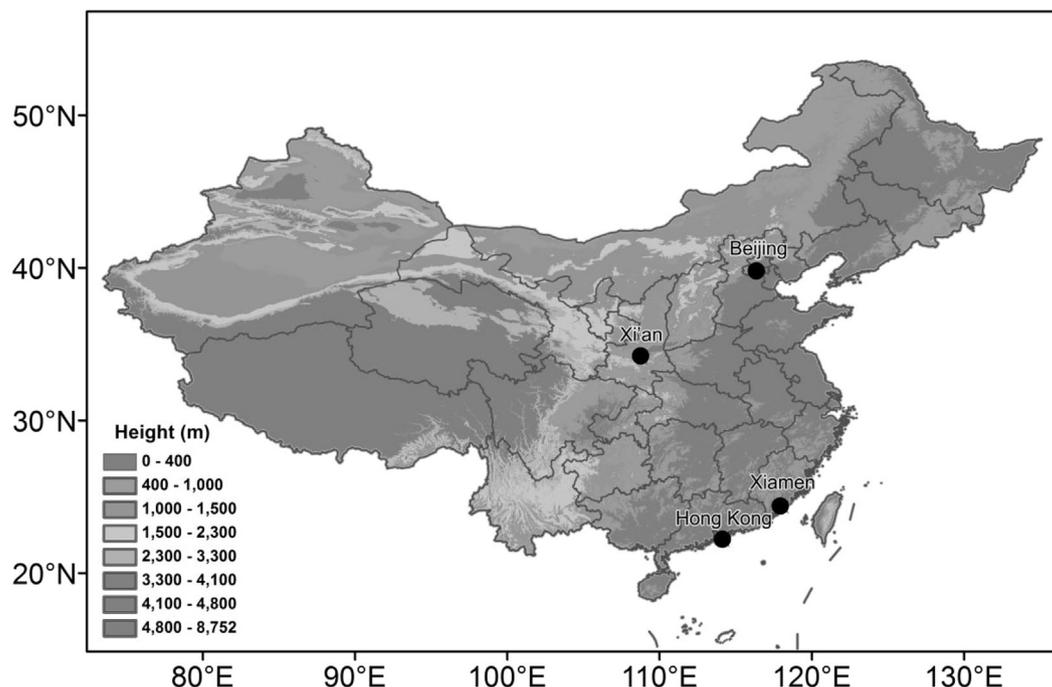


Fig. 1. PM_{2.5} samples were taken in two southern China cities: Hong Kong (HK) and Xiamen (XM); and two northern China cities: Beijing (BJ) and Xi'an (XA).

2.3. Methanol extraction

Each quartz sample filter (4.3 cm²) was extracted with 20 ml methanol and ultrasonicated in a constant temperature (25 °C) water bath for 20 min. A few drops of toluene were added to the extractant as a volatile compound trapping agent subsequently after the methanol addition. The extractant was transferred to a round bottom flask and evaporated by the rotary evaporator until 5 ml of sample was conserved. The aerosol extractants were stored at –20 °C before amine analysis.

2.4. Water extraction

Each quartz sample filter (4.3 cm²) was extracted with 5 mL of Milli-Q water (18 MΩ cm) and ultrasonicated in a constant temperature (25 °C) water bath for 30 min. The extraction procedure was repeated. The first and second extractant was combined and subsequently filtered by a syringe filters (0.45 μm, Pall Corporation, NY, USA) to remove insoluble materials. The filtered solution was stored at 4 °C before water-soluble ions and anhydrosugars analysis. The filtered water extractant was pre-concentrated to 0.5 ml before amino acids and urea analysis (Yang et al., 2005).

2.5. Methanol extraction for biological analysis

Each Teflon sample filter was extracted with 20 ml methanol and ultrasonicated in a constant temperature (25 °C) water bath for 20 min. The procedure was repeated and the first and second extractant were combined. The combined methanol extractant was purged by nitrogen gas (N₂ ≥ 99.995%) for 60 min until all the methanol solvent was removed (Lee et al., 2014). The residual particulate matter was re-dissolved in dimethyl sulfoxide (DMSO) [$<0.01\%$ vol in phosphate-buffered saline (PBS)] at 0 (control), 50 and 150 μg/ml for biological assays. Near-pure, manufactured, carbon black (CB), with an average diameter of 65 nm (Monarch 120; Cabot Corporation, UK), was selected as a control particle

(Chiang et al., 2013; Chuang et al., 2011b). The chemical characteristics of CB have been described previously (Chuang et al., 2011a; Zhu et al., 2004).

2.6. Chemical analysis

Water-soluble ions and anhydrosugars (e.g., levoglucosan and mannosan) were separated and identified by high-performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). The detailed description of the analytical method is found in (Engling et al., 2006; Iinuma et al., 2009). The urea compounds in the water extractant was analyzed by the HPLC (Agilent 1200 system) coupled with a photodiode array detector (DAD). The absorbance wavelength 210 nm was applied for the identification and quantification of the urea samples. After using dansyl chloride (in acetone) as the derivatization reagent, twenty-two amines derivatized samples were determined by HPLC coupled to an ion trap mass spectrometer for the detection.

OC and EC were analyzed (on a 0.526 cm² punch) by thermal analysis with optical detection following the IMPROVE protocol on a Desert Research Institute (DRI) Model 2001 Thermal/Optical Carbon Analyzer (Atmoslytic Inc., Calabasas, CA, USA) (Cao et al., 2003; Chow et al., 2005). The MDL for the carbon analysis were 0.8 and 0.4 μgC cm⁻² for the OC and EC, respectively, with a precision better than 10% for total carbon (TC).

2.7. DCFH assay

ROS production was determined by using a 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma–Aldrich, UK) probe with serum-containing DMEM, which resulted in the cleavage of the diacetate groups by esterase enzymes to produce the relatively lipid insoluble and non-fluorescent dichlorodihydrofluorescein (DCFH). The fluorescent 2',7'-dichlorofluorescein (DCF) was produced after oxidation of DCFH with ROS, which was measured using a FLUOstar fluorescence plate reader (BMG, Germany) at an

excitation wavelength of 485 nm and an emission wavelength of 530 nm. The result was presented as fluorescence intensity (AU) (Chuang et al., 2011b).

2.8. Cell culture and treatment

A549 cells were obtained from the American Type Culture Collection and cultured in RPMI (10% foetal bovine serum, penicillin and streptomycin) under the conditions of 37 °C, 95% humidity and 5% CO₂. A549 cells were seeded onto surface-treated, 24-well transwells (1 × 10⁵ cells/ml; BD Biosciences, UK) and incubated for 24 h. The cells were then incubated with 300 μl of sample at particle concentrations of 0 (control), 50 and 150 μg/ml for 4 h. Each experiment was conducted in quadruplicate. The concentrations were chosen to produce oxidative and inflammatory effects (>80% cell viability), according to criteria described previously (Chuang et al., 2012; Wilson et al., 2002).

2.9. Determination of cytokines

An ELISA (BD OptEIA™ set, BD Biosciences, USA) was used to determine IL-6, TNF-α and IFN-γ levels according to the manufacturer's instructions.

2.10. Statistical analysis

The samples analyzed in the experimental Sections 2.2–2.6 were repeated in triplicate. The data were expressed in the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for multiple values comparison. Pearson's correlation coefficient analysis was performed to identify the correlation between (1) oxidative stress and inflammation and (2) chemical species with oxidative-inflammation cytokines. The statistical analyses were conducted using GraphPad Prism software (Version 5 for Windows). The significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Major components of PM_{2.5}

Table 1 shows the chemical components of the PM_{2.5} samples collected during the haze events from the 26th January to 2nd February in 2013. Significant differences in PM_{2.5} concentration was observed between north and south China (4–5 fold). The average PM_{2.5} mass concentrations in BJ, XA, XM and HK were found to be 258 ± 100 μg m⁻³, 233 ± 52 μg m⁻³, 46 ± 9 μg m⁻³ and 48 ± 13 μg m⁻³, respectively. Most of the results measured at northern cities are higher than observable mass concentration in the previous roadside and ambient studies in China (Cao et al., 2007; Jahn et al., 2013; Yang et al., 2011). Sulfate, nitrate and OC are the major constituents of PM_{2.5} at the BJ, XA and XM sampling locations, contributing up to 74% of total PM_{2.5} mass. In HK, sulfate, OC and EC are the major constituents of PM_{2.5} (Ho et al., 2003). The detailed OC and EC results were reported previously at elsewhere. The sulfate concentrations in BJ and XA are on average 3.3 to 9.5 times higher than those in XM and HK, indicating a significant sulfate source in the northern cities (e.g., residential coal combustion). Similar trends were observed for the nitrate and ammonium between the northern and southern cities. The concentrations of nitrate in BJ and XA are 3.3–24 times higher than in XM and HK, while the concentrations of ammonium in BJ and XA are 5–83 times higher than in XM and HK. The gas-to-particle partition of nitrate and ammonium depends strongly on temperature (T), relative humidity (RH), and gaseous precursor concentrations. Relative low concentrations of nitrate and ammonium in the

southern cities could be a consequence of thermal decomposition of ammonium nitrate at relatively high temperature and hence the release of gaseous nitric acid and ammonium.

3.2. Levoglucosan and organic nitrogen species in PM_{2.5}

Levoglucosan, the thermal degradation product of cellulose (Simoneit et al., 1999), has been used as an organic tracer for biomass burning derived aerosols for many years (Ho et al., 2014). The concentrations of levoglucosan range from 6 to 870 ng m⁻³ and follow an ascending order HK < XM < BJ < XA at these four cities. High levoglucosan concentrations at the northern cities (i.e., 653 ± 191 ng m⁻³ at XA and 359 ± 119 ng m⁻³ at BJ) indicate strong local biomass burning activities and/or regional transport of biomass burning particles. The relatively low values in Hong Kong could be explained by the lack of such activities in the city. The levoglucosan to OC ratio (LG/OC) is used to estimate contributions from biomass burning to OC mass (Ho et al., 2014; Puxbaum et al., 2007; Zhang et al., 2010). A simplified receptor-based approach was used to estimate biomass burning contributions at these four cities:

$$\begin{aligned} & \text{contribution of biomass burning emissions to OC} \\ & = (\text{LG/OC})_{\text{measured}} / (\text{LG/OC})_{\text{reference}} \end{aligned}$$

while “(LG/OC)_{reference}” refers to the emission factor of levoglucosan based on OC emissions derived from biomass burning chamber studies (Zhang et al., 2007). The contributions of biomass burning emissions to OC at XA (28.2%) and BJ (14.5%) were significantly higher than those at XM (9.0%) and HK (5.5%), consistent with the more common and intensive biomass burning activities in northern cities. In previous studies, the effects of short-term exposure to biomass burning emission on respiratory symptoms, lung function, asthma and daily mortality were observed (Boman et al., 2003; Saffari et al., 2013).

Table 1 shows the concentrations of 21 organic nitrogen species in PM_{2.5} samples. The concentrations of urea are much higher in northern China (1855 ± 755 ng m⁻³ at BJ and 1124 ± 243 ng m⁻³ at XA) than in southern China (543 ± 104 ng m⁻³ at XM and 363 ± 61 ng m⁻³ at HK). The levels of urea measured in this study are consistent with previous studies. For example, Shi et al. (2010) measured 1188 ng m⁻³ and 636 ng m⁻³ of urea in total suspended particulate during dust episodes and non-dust periods at Qingdao in North China. This shows a worrying high level of urea in urban atmosphere of China when compared with other countries, e.g. the average concentration of urea in TSP at Hawaii was 78–378 ng m⁻³ in dirty samples and 42–132 ng m⁻³ in clean samples (Cornell et al., 2001). The use of agricultural fertilizers could be a key anthropogenic source of urea (Glibert et al., 2005) with high urea concentration being found in BJ and XA sampling locations. China is a global player of manufacturing and consuming urea with production of 1.9 × 10⁷ tons in 2004. However, only 30–35% of the urea fertilizer was efficiently utilized compared to 70–80% in Europe and in North America (Wang, 2004). Urea of agricultural origin can enter the atmosphere by wind-blown and biomass burning. The possible primary emission source of urea in China could be due to active agricultural activities, soil dust and the sea surface through cavitation processes (Shi et al., 2010; Violaki and Mihalopoulos, 2010). The secondary production of urea from atmospheric reactions could also be additional major contributing sources. All of these possible pathways could lead to high urea concentrations in the four sampling locations.

The total concentration of 20 amines measured in PM_{2.5} range from 35 to 212 ng m⁻³. The BJ samples contain the highest levels (133 ± 53 ng m⁻³) whereas the HK samples contain the lowest

Table 1
Average (\pm standard error) mass concentration and chemical composition of PM_{2.5} collected at four megacities in China during the haze events.

	Beijing (BJ)	Xi'an (XA)	Xiamen (XM)	HongKong(HK)
PM_{2.5} mass ($\mu\text{g}/\text{m}^3$)^a	257.7 \pm 100.2	232.6 \pm 52.4	45.9 \pm 8.9	48.0 \pm 13.0
Sulfate	50.2 \pm 21.2 (19%)	47.9 \pm 24.2 (22%)	14.5 \pm 2.8 (32%)	5.3 \pm 1.0 (11%)
Nitrate	41.8 \pm 15.9 (16%)	52.1 \pm 20.6 (23%)	10.4 \pm 5.7 (22%)	2.2 \pm 0.7 (5%)
Ammonium	24.5 \pm 10.7 (9%)	25.6 \pm 12.1 (12%)	5.3 \pm 2.0 (11%)	0.3 \pm 0.5 (1%)
OC	47.9 \pm 18.2 (19%)	40.7 \pm 10.7 (17%)	9.2 \pm 2.5 (20%)	7.9 \pm 1.6 (17%)
EC	6.3 \pm 1.7 (3%)	7.6 \pm 1.9 (3%)	3.2 \pm 1.3 (7%)	4.7 \pm 0.9 (10%)
Amine (ng/m^3)^a				
4-Aminophenol	0.6 \pm 0.2	0.6 \pm 0.2	0.4 \pm 0.1	0.2 \pm 0.0
2-Amino-1-butanol	0.1 \pm 0.1	0.1 \pm 0.1	0.2 \pm 0.0	0.1 \pm 0.0
N-Methylformamide	56.8 \pm 25.4	40.3 \pm 14.7	25.6 \pm 7.2	17.5 \pm 4.7
Methylamine	31.0 \pm 10.8	24.7 \pm 9.7	10.2 \pm 2.0	12.1 \pm 2.4
Ethylamine	14.8 \pm 6.5	12.6 \pm 5.6	5.3 \pm 1.4	4.5 \pm 1.1
Ethanolamine	6.7 \pm 3.0	6.6 \pm 3.3	2.4 \pm 0.3	2.5 \pm 0.7
Dipropylamine	2.8 \pm 1.4	2.7 \pm 1.3	1.3 \pm 0.2	1.1 \pm 0.3
Tripropylamine	2.4 \pm 1.0	2.0 \pm 1.1	1.2 \pm 0.3	1.0 \pm 0.3
Iso-Butylamine	0.5 \pm 0.2	0.2 \pm 0.1	0.4 \pm 0.1	0.3 \pm 0.1
Sec-Butylamine	1.2 \pm 0.5	1.1 \pm 0.6	0.5 \pm 0.1	0.5 \pm 0.1
Dimethylamine	4.3 \pm 1.7	3.8 \pm 1.6	1.7 \pm 0.4	1.5 \pm 0.5
Diethylamine	2.1 \pm 0.9	2.0 \pm 0.8	0.9 \pm 0.2	0.9 \pm 0.2
Ethylenediamine	0.5 \pm 0.2	0.4 \pm 0.2	0.3 \pm 0.1	0.2 \pm 0.1
Phenylamine	5.7 \pm 2.2	5.1 \pm 2.2	2.5 \pm 0.8	2.0 \pm 0.6
4-Ethylphenylamine	0.5 \pm 0.2	0.3 \pm 0.1	0.1 \pm 0.0	0.2 \pm 0.0
N-Methylphenylamine	1.0 \pm 0.5	0.9 \pm 0.4	0.3 \pm 0.1	0.4 \pm 0.1
Iso-Propylphenylamine	0.4 \pm 0.2	0.4 \pm 0.2	0.2 \pm 0.0	0.2 \pm 0.0
2-Methylphenylamine	0.3 \pm 0.1	0.2 \pm 0.1	0.1 \pm 0.0	0.1 \pm 0.0
4-Methylphenylamine	0.6 \pm 0.3	0.6 \pm 0.2	0.2 \pm 0.0	0.2 \pm 0.0
3-Propylphenylamine	0.8 \pm 0.4	0.6 \pm 0.3	0.2 \pm 0.0	0.2 \pm 0.1
Total amines	133.2 \pm 52.8	105.3 \pm 41.0	53.7 \pm 13.2	45.7 \pm 9.8
Urea	1854.7 \pm 754.9	1124.0 \pm 242.8	542.8 \pm 103.5	362.6 \pm 61.3
Levogluconan	359.3 \pm 130.2	653.3 \pm 191.4	53.5 \pm 13.4	30.6 \pm 26.9

concentrations ($46 \pm 10 \text{ ng m}^{-3}$) during the haze periods. N-methylformamide, methylamine and ethylamine are the three most abundant amines in all of the PM_{2.5} samples at the four locations. Amines are strong bases in the atmosphere and can rapidly react with nitric acid (HNO₃) and sulfuric acid (H₂SO₄) via acid–base reactions leading to secondary aerosol formation. The atmospheric reaction pathways of amines include gas-phase reactions with oxidants such as OH \cdot , NO₃ and O₃, to form amides, nitramines, and imines, which can also partition to the particle phase (Murphy et al., 2007; Schade and Crutzen, 1995; Silva et al., 2008). For example, N-methylformamide is formed from the OH \cdot and O₃ reaction with dimethylamine, and possibly OH \cdot reaction with methyldiethanolamine. More than one hundred amine species have been observed in the aerosol particles (Ozel et al., 2010), contributing 10–20% of the organic content in ambient particles (Docherty et al., 2011; Hildebrandt et al., 2011). Amines originate from various primary sources including animal husbandry, industry and combustion, composting operations, automobiles, and natural sources such as biomass burning and biodegradation of organic matter that contains proteins or amino acids (Ge et al., 2011). The total emissions of methylamine, dimethylamine and trimethylamine are estimated to be $\sim 300 \text{ Gg N a}^{-1}$ globally (Ge et al., 2011).

Although large numbers of organic nitrogen species in the atmosphere were determined, only limited studies of their health effects were investigated, e.g. the potential symptoms of exposure to aliphatic and aromatic amines include: respiratory irritation; drowsiness and headache; cough, sneezing, wheezing, and dyspnea etc (Ge et al., 2011). Moreover, amines 2-naphthylamine, benzidine, and 4-aminobiphenyl are proven human carcinogens (Pinches and Walker, 1980).

3.3. Oxidative potential and inflammatory response

Fig. 2a shows the dose-dependent alterations in oxidative

potential, IL-6, IFN- γ and TNF- α levels. The levels of oxidative potential are in descending order BJ > XM > XA > HK at 150 $\mu\text{g}/\text{ml}$. The levels of IL-6, IFN- γ and TNF- α is in descending order BJ > XA > XM > HK in at 150 $\mu\text{g}/\text{ml}$. The results show that BJ PM_{2.5} samples have the highest bioreactivity although the PM_{2.5} levels are not the highest among the four cities. Pearson's correlation coefficient test was applied to elucidate the correlation between oxidative potential and inflammation induced by the PM_{2.5} exposure. The test shows the oxidative potential is highly correlated with the IL-6 ($r^2 = 0.78$, $p < 0.05$) (Figure 2b) and TNF- α production ($r^2 = 0.78$, $p < 0.05$) (Fig. 2 (b)), while the correlation between oxidative stress and IFN- γ is moderate ($r^2 = 0.47$, $p < 0.05$). Oxidative stress is recognized to be an important factor in the regulation of inflammatory response (Danielsen et al., 2010; Luo et al., 2009). The oxidative stress induced by the PM depends on the physical properties and chemical composition of the particles. For example, reactive oxygen species (ROS) can be generated from the surface of particles or by the particle itself. The PM can provide a platform for intermixing with various ROS produced, for example by the organics (Chuang et al., 2012) and metals. Such exogenous ROS from PM can alter functions of mitochondria or NADPH-oxidase, leading to inflammation (Risom et al., 2005).

3.4. Correlation between chemicals and oxidative-inflammatory responses

The correlations between chemical composition per unit μg of the haze PM_{2.5} and oxidative-inflammatory responses were calculated using the Pearson's correlation coefficient test and the results are shown in Fig. 3. Good correlations are observed for the oxidative potential (determined by DCFH) against sulfate ($r^2 = 0.70$, $p < 0.05$), nitrate ($r^2 = 0.57$, $p < 0.05$), ammonium ($r^2 = 0.59$, $p < 0.05$), OC ($r^2 = 0.76$, $p < 0.05$), urea ($r^2 = 0.61$, $p < 0.05$) and levogluconan ($r^2 = 0.60$, $p < 0.05$). IL-6 is positively correlated with sulfate

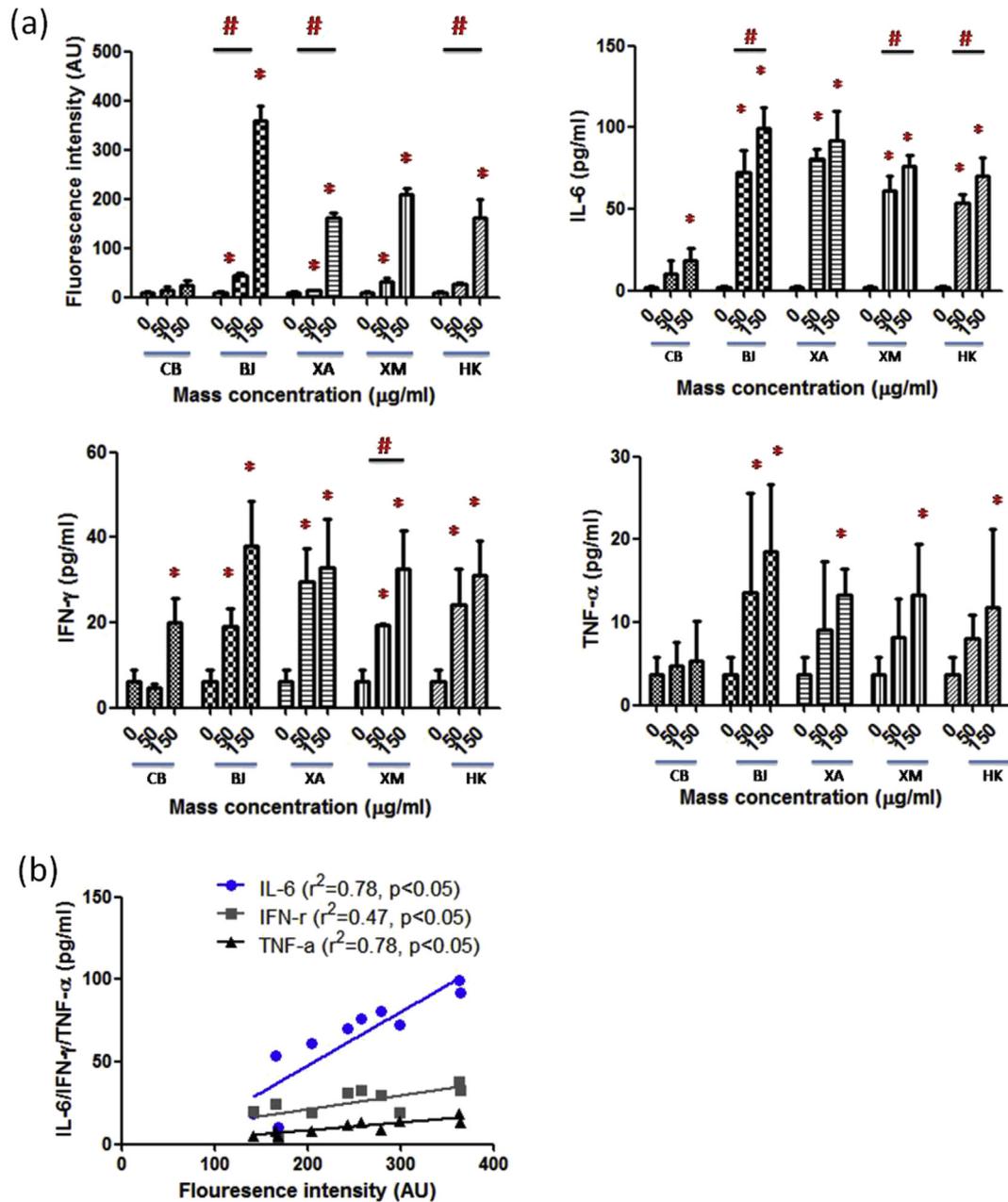


Fig. 2. (a) Oxidative potentials, IL-6, IFN- γ and TNF- α production of the haze PM_{2.5} collected from BJ, XA, XM and HK. (b) Correlation of oxidative potentials of the haze PM_{2.5} to IL-6, IFN- γ and TNF- α . Carbon black (CB) was served as negative control. * $p < 0.05$ compared with control; # $p < 0.05$ compared between groups.

($r^2 = 0.68, p < 0.05$), nitrate ($r^2 = 0.69, p < 0.05$), ammonium ($r^2 = 0.75, p < 0.05$) and levoglucosan ($r^2 = 0.76, p < 0.05$). IFN- γ is positively correlated with sulfate ($r^2 = 0.72, p < 0.05$), nitrate ($r^2 = 0.60, p < 0.05$), ammonium ($r^2 = 0.58, p < 0.05$), urea ($r^2 = 0.60, p < 0.05$) and levoglucosan ($r^2 = 0.62, p < 0.05$). TNF- α is positively correlated with sulfate ($r^2 = 0.58, p < 0.05$), ammonium ($r^2 = 0.54, p < 0.05$) and OC ($r^2 = 0.59, p < 0.05$). According to the Windrose plot in Fig. 3, the sulfate, nitrate, ammonium, OC, urea and levoglucosan are statistically associated with oxidative-inflammatory responses. Since PM_{2.5} has the capability of penetrating deep into the lung and even entering the blood circulation the chemical components carried by the PM_{2.5} are important parameters in revealing the cytotoxicity and bioreactivity (Chuang et al., 2012). Atmospheric carbonaceous PM is an important component of PM_{2.5}, and its major fractions are EC and OC. EC is

emitted directly from combustion sources and has been considered an indicator for the primary anthropogenic air pollutants. However, OC is produced from volatile organic compound gas-to-particle conversion processes occurring in the atmosphere. Previous studies have shown that diesel exhaust particles contain a solid carbon core (soot/black carbon) that can intermix with chemical compounds and lead to adverse health effects. Physicochemical-specific relationships in inflammatory initiation were observed. A blunting of the endothelium-dependent and -independent vasodilation has been reported in relation to black carbon and sulfate (O'Neill et al., 2005). Sulfate and OC have also been reported to be capable of initiating endothelial dysfunction (Lei et al., 2005). Changes in the cardiovascular reaction in response to PM_{2.5}, NO₃⁻, OC and EC have been revealed in human (Chuang et al., 2007) and *in vivo* studies (Rohr et al., 2011), which could be associated with

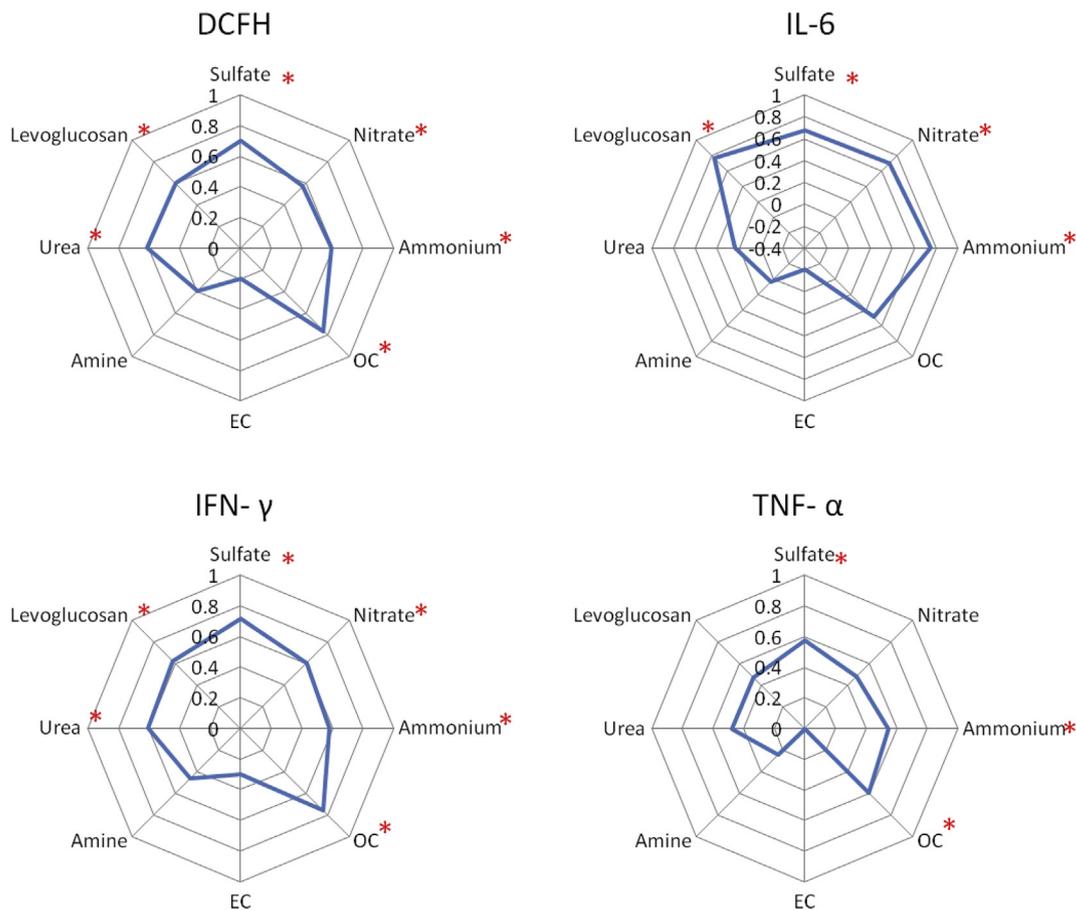


Fig. 3. Correlations of DCFH (oxidative potential), IL-6, IFN- γ and TNF- α to the chemical compositions (sulfate, nitrate, ammonium, OC, EC, amine, urea and levoglucosan). * $p < 0.05$.

the oxidative-inflammatory reaction resulted from the particle. Urea is associated with oxidative stress and IFN- γ production, which is in line with previous findings (D'Apolito et al., 2010; Zhang et al., 1999). Urea-induced ROS has been associated with mitochondrial ROS production and the mitochondrial manganese superoxide dismutase (MnSOD) pathway (D'Apolito et al., 2010). Levoglucosan has been used as a urinary biomarker after the exposure to wood smoke (Hinwood et al., 2008). However, its potential toxicity in response to pulmonary exposure remains to be determined. The haze PM_{2.5}-containing levoglucosan correlates with oxidative stress, IL-6 and IFN- γ production. A previous study demonstrated that ROS-induced by PM_{2.5} emitted from biomass burning in related to levoglucosan levels (Saffari et al., 2013), which is in agreement with our results. Levoglucosan is commonly generated from biomass burning with other co-emitted species; therefore, the toxicity of levoglucosan requires further investigation.

The chemical composition of PM_{2.5} during the haze events in 2013 at four Chinese megacities and the relevant PM_{2.5} toxicity with the different chemical species were investigated. Oxidative potential and inflammation induced by the haze PM_{2.5} exposure demonstrates that oxidative potential is highly correlated with different biomarkers of pro-inflammatory responses. Several inorganic and organic species are associated with oxidative-inflammatory responses. Further research should focus on identifying the cytotoxic and carcinogenic mechanisms of PM_{2.5} in human organs (e.g. lung). Our study therefore supports the development of more effective and source-specific regulations for mitigating PM production.

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