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Oxidative capacities of size-segregated haze particles in a residential area of Beijing

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ABSTRACT

The frequent haze days around the Chinese capital of Beijing in recent years have aroused great attention owing to the detrimental effects on visibility and public health. To discover the potential health effects of the haze, oxidative capacities of airborne particles collected in Beijing during haze and clear days were comparably assessed by a plasmid scission assay. Eleven water-soluble trace elements (As, Cd, Cr, Cu, Mn, Ni, Pb, V, Se, Tl, and Zn) in the size-segregated airborne particles were quantitatively analyzed by inductively coupled plasma mass spectrometry, and most of the water-soluble trace elements were found to mainly concentrate in the fine particle size of 0.56–1.0 μm . In comparison with clear days, the mass concentrations of 11 analyzed water-soluble trace elements remarkably increased during haze days, and the oxidative capacities determined by the plasmid scission assay were markedly elevated accordingly during the haze days under the same dosage of particles as for those during clear days. Water-soluble trace elements in airborne particles, such as Cu, V, and particularly Zn, were found to have significantly positive correlations with the plasmid DNA damage rates. Because Cu, V, and Zn have been considered as bioavailable elements, the evident increase of these elements during haze days may be greatly harmful to human health.

Introduction

Owing to the adverse health effects, airborne particles, particularly those that accumulate during haze days, have drawn serious concern from the public and the government in the recent years. Exposure to airborne particles has been associated with increases in emergency admissions to hospitals and in mortality (Dockery et al., 1993; Borja-Aburto et al., 1998; Ostro, 1996). Beeson et al. (1998) suggest that exposure to airborne particles increases the risk of developing lung cancer. There is also evidence that PM_{10} and its soluble fractions can induce alterations in DNA (Villalobos et al., 1995; Shao et al., 2006, 2007; Lü

et al., 2006).

The plasmid scission assay is an *in vitro* method for studying the oxidative damage of supercoiled DNA induced by free radicals generated from particles (Greenwell et al., 2002; Shao et al., 2006, 2007; Chuang et al., 2011, 2013; Reche et al., 2012). This method has been used to study the bioreactivity of airborne particles (Donaldson, et al., 1996; Lü et al., 2006).

Most studies have indicated that the oxidative damage of plasmid DNA is caused by free radicals produced by particle components such as metals (Villalobos et al., 1995; Li et al., 1996; Prahalad et al., 2001; Lü et al., 2006; Wei et al., 2009). Costa and Dreher (1997) reported that bioavailable transition metals on the particle surface may produce free radicals. Donaldson et al. (1996) and Greenwell et al. (2002) proposed that the oxidative damage

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to key cells caused by free radicals on the particle surface may be the main cause of lung damage. Cuellar et al. (2002) suggested that DNA degradation occurs as a result of the metal content in airborne particles and the generation of reactive oxygen species participating in the Fenton reaction. Richard et al. (1989) pointed out that soluble Zn, and not insoluble Zn, causes pulmonary damage. Adamson et al. (2000) also identified soluble Zn in airborne particles as the reactive species that causes pulmonary cell damage. Other bioavailable transition metals, particularly iron, are also often thought to be associated with the oxidative damaging of particles (Ambroz et al., 2000; Valavanidis et al., 2000; Kim et al., 2001; Shao et al., 2006, 2007). Water-soluble trace elements of airborne particles are believed to be responsible for adverse health effects owing to their easy absorption by the human body (Shao et al., 2006), and As, Se, Pb, Cd, Ni, Cr, and Mn are well-known hazardous air pollutants (US EPA, 1998). Recently, Xu et al. (2007) and Duan et al. (2012) noticed that the different sizes of airborne particles have different trace element compositions, which implies that size-segregated airborne particles may have different oxidative capacities. Shao et al. (2006) researched the plasmid DNA damage induced by PM_{2.5}, PM₁₀ and dust-storm particles in Beijing and found that the oxidative capacity of PM_{2.5} is generally higher than that of PM₁₀ in Beijing. Considerable research work has been performed on plasmid DNA damage induced by airborne particles in Beijing by considering PM₁₀ and PM_{2.5} collected from different sites during different seasons (Shi et al., 2004; Shao et al., 2006, 2007; Lü et al., 2006). However, research on plasmid DNA damage induced by size-segregated airborne particles in Beijing, particularly during haze and clear days, has not been reported so far.

In this study, we collected airborne particles in a residential area of Beijing during haze and clear days using an eleven-stage cascade impactor and analyzed the oxidative capacity of particles of different size ranges using a plasmid scission assay. The size distribution of the water-soluble trace elements in these size-segregated airborne particles collected during haze and clear days was also analyzed. In addition, the relationship between the concentration of trace elements in the water-soluble fraction and the plasmid DNA damage rates was investigated. Our objective is to determine the size range of the particles that shows the highest oxidative capacity and associated negative health effects.

1 Materials and methods

1.1 Filter sampling

Airborne particles during haze days and clear days were collected on polycarbonate filters (47 mm diameter, Millipore, UK, pore size 0.6 µm) using an eleven-stage cascade

impactor with a flow rate of 30 L/min (MOUDI 110, MSP, USA) on the roof (ca. 15 m high) of a building at the Beijing Academy of Science and Technology. The site is located about 120 m away from the West 3rd Ring traffic road. The MOUDI 110 sampler segregated particles in the following size ranges: > 18, 10–18, 5.6–10, 3.2–5.6, 1.8–3.2, 1.0–1.8, 0.56–1.0, 0.32–0.56, 0.18–0.32, 0.1–0.18, and 0.056–0.1 µm. In this study, 0.1–0.18 µm particles and 0.056–0.1 µm particles have been grouped to represent one sample, having particle sizes in the range 0.056–0.18 µm. The filters were kept in vacuum desiccators for 24 hr to remove any moisture content before mounting them on the MOUDI sampler. After sampling, the filters were immediately placed in the vacuum desiccators again to remove moisture in the same manner. All of these filters were weighed before and after sampling with an analytical balance (0.01 mg, Sartorius CP225D, Germany). A total of 7 sets of samples were collected by the MOUDI sampler during haze and clear days in autumn and spring. Blank filters (seven in number) were also used during each sampling day. Wind speed and direction, relative humidity, barometric pressure, and ambient temperature were recorded using a Kestral 4500 Pocket Weather Tracker (Nielsen-Kellermann Inc., USA). Visibility was monitored using a Belfort Model 6000 Visibility Sensor (USA). The main meteorological parameters and visibilities recorded during the sampling days are listed in **Table 1**. The mass concentrations of particles in the different size ranges during haze and clear days have been given elsewhere (Sun et al., 2013).

1.2 Plasmid scission assay

The experimental protocol for the plasmid scission assay is as follows: (1) each filter (along with a corresponding blank filter) was cut into half, and the mass of each sample was calculated. The sample was immersed in sterile HPLC-grade water (resistivity ca. 18.2 MΩ) to achieve a 100 µg/mL dosage of particles in the water. The sample was gently shaken for 20 hr and then sonicated for 2 min. This released the particulate matter from the filter substrate into the solution; (2) the sample was centrifuged to deposit particles at the bottom of the tube, then, the supernatant of each sample was separated carefully into two Eppendorf (EP) tubes, of which one was used for the plasmid scission assay and the other was used for the water-soluble trace elements analysis. The volume of solution in each EP tube used for the plasmid scission assay was adjusted to 41 µL; (3) an amount of 1.8 µL (200 ng/µL) of φX174-RF DNA (Promega, USA) was added to each of these EP tubes to obtain a total volume of 42.8 µL; the tubes were sealed and placed in a vortex in which they were gently vibrated for 6 hr, and then, 7 µL of bromide phenol blue/glycerin stains was added to each tube; (4) agarose gel was prepared with 0.6% agarose and 0.25% ethidium bromide; the details have been given elsewhere (Lü et al.,

Table 1 Meteorological parameters and visibility during sampling days

Sample	SD (hr)	Wind speed (m/sec)			Temperature (°C)			Relative humidity (%)			Visibility (km)			Weather type
		Min	Ave	Max	Min	Ave	Max	Min	Ave	Max	Min	Ave	Max	
2010-10-07	18	0.5	1.1	1.8	15	17.9	23.3	48.2	79.0	90.1	1.1	1.8	2.7	Haze
2010-10-08	36	0.4	1.3	2.1	14	19.1	23.9	41.8	72.0	91.0	0.9	1.8	3.7	Haze
2010-10-30	45	0.3	1.8	4.2	7.1	13.7	21.9	16.6	38.8	69.8	12.7	18.7	30.0	Clear
2010-11-01	48	0.3	2.1	5.7	3.8	9.4	16.1	14.6	36.3	80.8	9.4	22.5	30.0	Clear
2011-04-13	19	1.5	3.4	5.6	13.2	20.3	31.2	6.0	41.9	70.9	5.0	6.4	8.4	Haze
2011-04-14	25.5	2.1	3.6	6.4	19.3	24	31.8	6.3	13.4	23.5	10.8	18.3	25.8	Clear
2011-10-31	8	0.3	1.5	5.3	12	14	15	73	76.0	84.0	1.0	1.48	1.8	Haze

SD: sampling duration; Min: minimum, Ave: average, Max: maximum.

2006); (5) the gel was placed in electrophoresis apparatus filled with TBE buffer (1X Tris-Borate-EDTA); (6) the sample solution prepared in step (3) was loaded onto the agarose gel with each 49.8 μL sample split into two 20 μL wells; (7) the electrophoresis apparatus was run for 16 hr at 30 V; (8) the Syngene Genetools ultraviolet imaging system (Synoptics Ltd., Cambridge, UK) was used to view and image the gel; (9) Syngene Genetools programs (Synoptics Ltd.) were used to quantify the optical density of the DNA streaks and to calculate the bioreactivity of a sample at a certain concentration (the so-called oxidative DNA damage, represented by the percentage of linearized and relaxed DNAs to all DNA).

The plasmid scission assay was performed at the State Key Laboratory of Coal Resources and Safe Mining based in the China University of Mining and Technology in Beijing.

1.3 ICP-MS analysis

The soluble samples were chemically analyzed by inductively coupled plasma mass spectrometry (ICP-MS, 7700x, Agilent Ltd.). As, Cd, Cr, Cu, Mn, Ni, Pb, V, Se, Tl, and Zn, were measured; the values of these elements were verified to be above the detection limits. The final results

were reported as $\mu\text{g/g}$ of each element in its soluble form in the analyzed particle sample. The mass concentration of the soluble elements was obtained by multiplying the $\mu\text{g/g}$ values of these soluble elements with the mass concentration of the corresponding particle samples, and the final results are given as ng/m^3 .

2 Results and discussion

2.1 Variation of plasmid DNA damage induced by the water-soluble fraction in size-segregated airborne particles

Previous investigations on the PM_{10} and $\text{PM}_{2.5}$ in Beijing have confirmed that the oxidative capacity of airborne particles is mainly derived from its water-soluble fractions (Shao et al., 2006, 2007; Reche et al., 2012). In our study, the analysis of plasmid DNA damage induced by the water-soluble fraction in the size-segregated airborne particles (the dosage of particles in water was 100 $\mu\text{g/mL}$) during haze and clear days was performed, and the results are shown in **Table 2** and **Fig. 1**. The DNA damage rates for all size ranges of the three haze day particles were between

Table 2 Percentage (%) of plasmid DNA damage induced by size-segregated airborne particles during haze and clear days at 100 $\mu\text{g/mL}$ particle dosage

Size range (μm)	2010-10-7 Haze	2010-10-8 Haze	2011-4-13 Haze	2010-10-30 Clear	2010-11-1 Clear	2011-4-14 Clear
>18	7.47	6.35	24.94	3.01	5.10	9.71
10–18	8.93	11.43	22.32	8.72	6.46	11.64
5.6–10	10.41	7.38	21.29	5.01	7.63	9.77
3.2–5.6	17.83	10.60	21.27	5.88	3.72	9.38
1.8–3.2	66.85	59.89	30.18	2.31	3.16	9.59
1.0–1.8	55.93	27.37	70.92	6.61	10.79	11.22
0.56–1.0	28.09	16.20	35.30	10.12	19.83	14.63
0.32–0.56	28.28	20.00	33.67	8.79	16.69	19.91
0.18–0.32	24.15	6.50	23.74	16.09	37.80	21.77
0.056–0.18	–	2.33	21.04	6.61	2.78	15.17
Average	27.55	16.80	30.47	7.31	11.40	13.28

Fig. 1 Plasmid DNA damage induced by the water-soluble fraction of size-segregated airborne particles during a haze day (13 April, 2011) and a clear day (14 April, 2011).

2.33% and 70.92%, and the average damage rates for these three samples were 27.55%, 16.80%, and 30.47%. The DNA damage rates for all size ranges of clear day particles were between 2.31% and 37.80%, and the average damage rates for these three clear day samples were 7.31%, 11.40%, and 13.28%. All these data showed that the overall oxidative capacities of the haze day samples were higher than the clear day samples at the same dosage of particles.

2.2 Correlation between DNA damage induced by airborne particles and content of water-soluble trace elements in size-segregated airborne particles

ICP-MS was used to investigate the content of water-soluble trace elements in size-segregated airborne particles (100 μg particles in 1 mL water) during haze and clear days. The correlation between water-soluble trace elements in the particles and the corresponding DNA damage rates at the particle dosage of 100 $\mu\text{g}/\text{mL}$ are shown in Fig. 2.

According to the correlation coefficients, for the number of samples $N = 58$, the linear regression coefficient (R^2) is higher than 0.113 at the 0.01 significance level, which implies that these two factors are related.

The content of the total analyzed water-soluble elements in the particles was found to have the most significant correlation with the percentage of DNA damage, with $R^2 = 0.350$. This indicates that these water-soluble elements were collectively responsible for the DNA damage of the particles. For individual elements, the water-soluble Zn showed the most significant correlation with the DNA damage rates with $R^2 = 0.370$. This demonstrated that water-soluble Zn is the most important element among the water-soluble trace elements; this is in agreement with the previous results obtained for Beijing (Shao et al., 2006) and those reported elsewhere (Richard et al., 1989; Ambroz et al., 2000; Valavanidis et al., 2000; Kim et al., 2001). Lü et al. (2006) pointed out that the contents of V and Cu in the water-soluble fraction also have a significant

correlation with the percentage of DNA damage in Beijing, and in our study, these two elements, in their soluble form, showed a definite positive correlation with the DNA damage rates. Therefore, it can be inferred that Cu, V, and Zn in airborne particles have high oxidative capacity.

2.3 Particle size distributions in terms of mass concentrations of water-soluble trace elements

The mass-size distributions of the particles in terms of their oxidative damage can be assessed by investigating the mass-size distributions of water-soluble trace elements in the particles. Figure 3 shows the mass-size distributions of water-soluble trace elements in haze and clear day particles. It can be seen that As, Cd, Pb, Se, Tl, and Zn exhibited a single mode peak in the range of 0.56–1.0 μm , which was in good agreement with previously obtained results (Allena et al., 2001; Xu et al., 2007; Hong et al., 2011; Duan et al., 2012). Ni, Cu, Cr, V, and Mn exhibited bimodal speaks, which were in the range of 0.56–1.0 μm for the fine mode and in the range of 3.2–5.6 μm for the coarse mode, and this is also in good agreement with the published results (Samara and Voutsas, 2005; Xu et al., 2007; Duan et al., 2012). For Cr, Cu, and Ni, the fine mode dominated the distribution on both haze and clear days. The mass concentration of V was dominated by the fine mode during haze days and dominated by the coarse mode during clear days. The mass concentrations of Mn both for 0.56–1.0 μm and for 3.2–5.6 μm were comparable. With the exception of the days in the spring (a haze day on April 13th, 2011, and a clear day on April 14th, 2011), the mass concentration of Mn for the size range of 0.56–1.0 μm was slightly higher than that for the size range of 3.2–5.6 μm .

Based on the above analyses, in airborne particles, Cu, V, and particularly Zn, which have a significant correlation with plasmid DNA damage, were mainly concentrated in 0.56–1.0 μm diameter particles. This may imply that the particles at 0.56–1.0 μm have the highest oxidative capacity.

Fig. 2 Correlation between water-soluble trace elements and the percentage of DNA damage.

Fig. 3 Mass-size distributions of water-soluble trace elements.

3 Conclusions

The plasmid scission assay revealed that particles during haze days caused an overall higher oxidative capacity than those during clear days.

The total analyzed water-soluble trace elements showed a significant positive correlation with the DNA damage rates, indicating that the particle-induced DNA damages were mainly from the water-soluble fractions. For the individual elements, the water soluble Cu, V and Zn showed a significant positive correlation with the DNA damages, in which Zn showed a most significant positive correlation.

The mass-size distributions of water-soluble trace elements showed that Cu, V and particularly Zn, were mainly concentrated in the size range of 0.56–1.0 μm , implying that the particles in this size range have the highest oxidative capacity.

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