



Heavy Metal Compositions and Bioreactivity of Airborne PM₁₀ in a Valley-Shaped City in Northwestern China

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ABSTRACT

Lanzhou, a valley-shaped city in northwestern China, experiences heavy air pollution. This study investigated the relationship between the oxidative capacity and heavy metal composition of the PM₁₀ in Lanzhou. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to examine the heavy metal element composition and an *in vitro* plasmid assay was employed to study the bioreactivity of airborne PM₁₀. The monitoring data revealed that the mass concentration of Lanzhou PM₁₀ exhibited seasonal variations at both the urban and suburban sites, with higher values in winter and spring, lower values in autumn, and the lowest ones in summer. The results of ICP-MS analysis showed that Zn was the metal present in the highest concentrations in both the whole and water-soluble fractions of PM₁₀ collected at both the urban and suburban sites, followed by Fe, Pb, and Mn. Furthermore, the results indicated that Zn, Cd, and As were present mostly in their soluble forms, while the Fe, Pb, and V were primarily in their insoluble ones. The plasmid DNA assay results indicated that the TD₂₀ values (toxic dosages of PM₁₀ causing 20% of plasmid DNA damage) of the Lanzhou PM₁₀ collected at both the urban and suburban sites ranged from 10 µg/mL to over 1000 µg/mL, and exhibited seasonal variations. The TD₂₀ values were high in spring and autumn, and thus the toxicities were low, while the TD₂₀ values were low in winter and summer, and thus the toxicities were high. For PM₁₀ collected at both the urban and suburban sites, the high concentrations of water-soluble Zn, Fe, Pb, and Mn displayed a strong negative correlation with the TD₂₀ values, suggesting that these metals were likely the major elements responsible for plasmid DNA damage. In addition, meteorological conditions (i.e., lower wind speed and higher relative humidity) during the sampling periods may have caused an indirect increase in oxidative damage to DNA.

Keywords: Lanzhou PM₁₀; Plasmid DNA assay; Oxidative damage; Meteorological conditions.

INTRODUCTION

An epidemiological association between exposure to ambient particulate matter (PM) and increased incidence of mortality and morbidity due to lung cancer and cardiovascular diseases has been demonstrated by recent studies (Pope *et al.*, 2002; Nawrot *et al.*, 2007; Gotschi *et al.*, 2008; Hsieh *et al.*, 2011; Hung *et al.*, 2012). However, the causes of and mechanisms responsible for the adverse health effects associated with air particulate pollution are still unknown. A widely accepted hypothesis is that oxidative damage to plasmid DNA induced by airborne PM₁₀ is caused by bioavailable metals on the particle surface (Squadrito *et al.*,

2001; Greenwell *et al.*, 2002; DiStefano *et al.*, 2009; Vidrio *et al.*, 2009; Zhong *et al.*, 2010). Extensive research into ambient particulate matter, combustion emission particulates, residual oil fly ash, and diesel exhaust particles has shown that soluble heavy metal components produce reactive oxygen species (ROS), which can induce oxidative stress and inflammation in the lungs and respiratory tract (Dreher *et al.*, 1997; Lambert *et al.*, 2000; Risom *et al.*, 2005; de Kok *et al.*, 2006; Park *et al.*, 2006; See *et al.*, 2007; DiStefano *et al.*, 2009; Vidrio *et al.*, 2009; Zhong *et al.*, 2010). Moreno *et al.* (2004) and Shao *et al.* (2006, 2007) suggested that soluble Zn might be the primary component responsible for oxidative damage of DNA. Furthermore, Lu *et al.* (2008) suggested that heavy metals in Shanghai PM_{2.5}, including Cu, Zn, Pb, Cd, Cr, Mn, and Ni, might have synergic effects on plasmid DNA damage.

Gaseous and particulate air pollution in Lanzhou is known to be a cause of public health concerns, since the

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concentrations of gaseous and particulate pollutants in Lanzhou have been reported to be highest of all urban regions of China (Ta *et al.*, 2004). The urban area of Lanzhou is located within a valley and is surrounded by mountains and hills rising to 200–600 m on three sides. The city center is 1530 m above sea level and the valley spans approximately 35 km east to west and 2–8 km north to south. Such valley topography results in special meteorological conditions such as high calm wind frequency, frequent temperature inversions, and a thick temperature inversion layer, especially in winter. The poor air quality in the region can be ascribed to these special geographical and meteorological conditions. It has been reported that the topography results in a long-term inversion for about 310 days per year, causing the pollutants to be trapped at ground level (Wei *et al.*, 1999). Daily mass concentration of Lanzhou PM₁₀ can reach 600 µg/m³ in winter and spring (Liu *et al.*, 2008). A number of epidemiological investigations in Lanzhou have shown that airborne PM has adverse effects on health (Wei and Hu, 2000; Wu *et al.*, 2001). However, few toxicological investigations regarding airborne PM have been conducted to date. Elucidation of sources of toxicity would be useful, helping to implement policies that could improve the respiratory health of Lanzhou residents.

The plasmid DNA assay is an *in vitro* method used to detect oxidative damage to plasmid DNA by free radicals. This method has been used to evaluate the bioreactivity of urban airborne particles and diesel exhaust particles in many cities (Greenwell *et al.*, 2002, 2003; Moreno *et al.*, 2004; Whittaker *et al.*, 2004; Shao *et al.*, 2006, 2007). The plasmid assay is based on the principle that any free

radicals on the particle surface can cause oxidative stress on the supercoiled DNA. The preliminary oxidative damage causes the supercoiled DNA to become relaxed, while further damage results in linearization of the DNA. The different forms of DNA can be separated on agarose gel by electrophoretic mobility and quantified using sensitive densitometry. Bioreactivity is referred as a percentage of linearized and relaxed DNA accounting for total DNA.

In this study, airborne PM₁₀ samples (including dust storm particles) were collected in Lanzhou. The oxidative capacities of these samples were measured by plasmid DNA assay. ICP-MS was used to measure heavy metal concentrations of whole and water-soluble fractions of the PM₁₀ samples in order to investigate the relationship between particle oxidative capacity and heavy metal concentration. In addition, the relationship between the bioreactivity of PM₁₀ and meteorological conditions during the sampling periods was discussed.

MATERIALS AND METHODS

Sampling

Two sampling sites were selected for collection of Lanzhou PM₁₀. The urban collection site was located in Chengguan District (Fig. 1), which is a commercial and residential area in the valley. The samplers were installed on the fourth floor of Gesanghua Hotel, about 15 m above ground level and 100 m away from the main road. The suburban collection site was located in Yuzhong County (Fig. 1), a small suburban town attached to Lanzhou City that lies to the east of the city and outside the valley. The

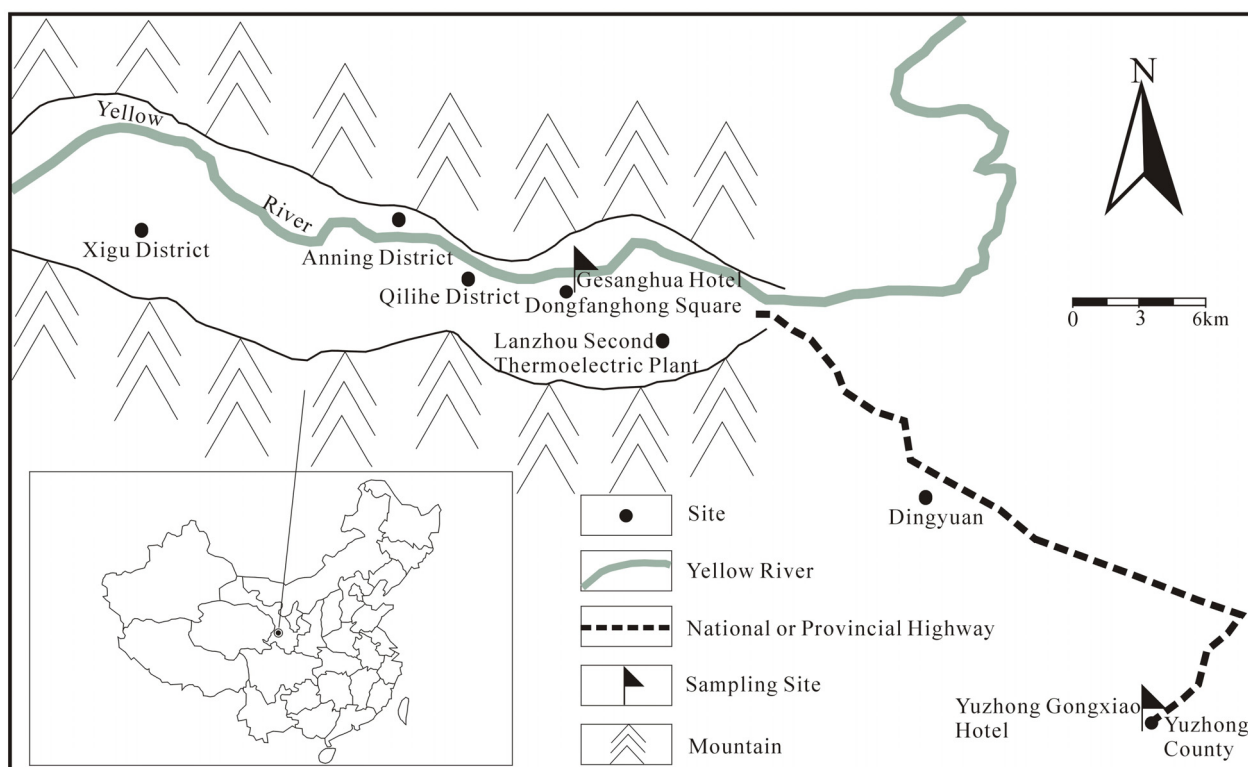


Fig. 1. Sketch diagram showing the sampling locations in Lanzhou, northwest China.

samplers were mounted on the sixth floor of Yuzhong Gongxiao Hotel, about 18 m above ground level. Although no large-scale industrial pollution sources were present in Yuzhong County, pollution relating to domestic coal burning in winter was still prevalent.

Two samplers with Negretti selective head (UK) were used synchronously to collect PM₁₀. Sampling flow of the sampler was 30L/min. In general, a-24 PM₁₀ was collected continuously 7 days per season at both the urban and suburban sites from December 2005 to October 2006. The PM₁₀ samples were collected on the Polycarbonate filters (47 mm diameter, 0.67 µm pore size, Millipore, UK). During sampling collection, meteorological parameters including temperature, humidity, wind direction/speed, and weather were recorded. In this study, twenty-two samples were selected for ICP-MS analysis and plasmid DNA assay, taking into account the meteorological characteristics and average mass concentrations for each season. The samples are detailed in Table 1.

ICP-MS Experiment

The PM₁₀ samples were separated into whole and soluble fractions and analyzed chemically using a Thermo Elemental X Series (X7) ICP-MS, as described by Jones *et al.* (2006). The mass of the polycarbonate filters was determined gravimetrically. First, the whole samples were diluted in chromatographically pure water and gently shaken in a

vortex mixer (Scientific Industries, Vortex-Genie 2) for 6 hours, followed by 13,000 × g centrifugation for another hour. Finally, the supernatant (soluble fraction) was removed carefully using a pipette to obtain the soluble sample. The whole samples (500 µg each) were prepared by digesting a quarter of filter using concentrated nitric acid (Fisher Primar grade, specific gravity: 1.48). Digestion was conducted in a CEM MDS-200 microwave system using CEM advanced composite vessels with Teflon liners. The digested samples were then concentrated by evaporating the nitric acid and redissolving in 2 mL of 10% nitric acid. Samples were diluted to a 20 mL volume using deionized (> 18 MΩ) water. One milliliter of each sample was combined with a 50 ppb thallium standard (1 mL), and this solution was made up to 10 mL with 2% nitric acid for analysis in the ICP-MS. The experimental results were transformed into concentrations of the analyzed total elements or soluble elements in the intact whole samples, expressed in ppm.

Plasmid DNA Assay

The procedure for the plasmid DNA assay was conducted in accordance with the methods reported by Merolla and Richards (2005) and Shao *et al.* (2006).

In this paper, particle samples were separated into intact whole particle solutions and soluble fractions. The mass on the polycarbonate filters was determined gravimetrically. Then, a quarter of cut filter (with particles) was placed in a

Table 1. Sample information for airborne PM₁₀ collected in Lanzhou.

Sampling season	Sampling site	Sampling No.	Sampling date	Sampling time	Mass concentration (µg/m ³)	Average temperature (°C)	Average wind speed (m/s)	Relative humidity (%)
winter	Urban site	No. 1	8–9 December, 2005	17:30–09:00	579.11	–1.9	0.2	51.9
		No. 2	12–13 December, 2005	09:30–09:30	219.44	–2.2	0.9	36.9
		No. 3	14–15 December, 2005	09:30–09:30	329.52	–5.4	0.2	42.3
	Suburban site	No. 4	9–10 December, 2005	19:00–19:00	140.68	–1.5	0.2	42.0
		No. 5	12–13 December, 2005	10:00–10:00	142.86	–6.6	0.2	54.3
spring	Urban site	No. 6	28–29 March, 2006	17:00–17:00	155.56	13.5	0.4	18.9
		No. 7	29–30 March, 2006	17:30–17:00	210.40	15.3	0.4	15.5
		No. 8	30–31 March, 2006	17:30–13:30	247.78	14.4	0.8	24
	Suburban site	No. 9	2–3 April, 2006	13:00–13:00	152.19	17.4	1	15.2
		No. 10	4–5 April, 2006	13:40–08:40	99.42	11.5	1.2	40.3
summer	Urban site	No. 11	14–15 July, 2006	10:00–09:00	118.84	30.6	1	50.8
		No. 12	16–17 July, 2006	09:00–08:30	141.84	32.7	0.7	42.4
		No. 13	17–18 July, 2006	09:00–09:00	157.85	28.6	0.8	45.8
	Suburban site	No. 14	19–20 July, 2006	13:30–11:30	56.82	27	1.2	44.7
autumn	Urban site	No. 15	21–22 October, 2006	16:00–08:30	181.83	14.1	0.5	61.6
		No. 16	22–23 October, 2006	20:30–08:30	114.30	8.4	1.3	40.5
		No. 17	24–25 October, 2006	23:00–10:30	165.65	15	0.5	62.6
	Suburban site	No. 18	25–26 October, 2006	19:30–08:30	112.64	7.9	1.1	62
		No. 19	26–27 October, 2006	21:00–09:00	86.54	13.3	1.6	69.3
		No. 20	27–28 October, 2006	20:30–09:00	101.15	7.6	0.7	54.7
Urban site	No. 21 ^a	31 March, 2006	13:30–21:30	556.94	17.0	8	25.6	
Suburban site	No. 22 ^b	22 July, 2006	11:50–20:50	117.42	18.8	0.3	67.7	

^a PM₁₀ sample collected during a dust storm.

^b PM₁₀ sample collected on a clean day after raining weather.

clean centrifuge tube (Scientific Industries, Vortex-Genie 2) with 15 mL HPLC-grade water, and intact whole particle solutions were obtained by gently shaking in a vortex mixer for 6 h to separate the particles as much as possible from the filter membrane. The soluble fraction of the PM₁₀ sample was obtained by shaking the intact whole particle solution in the vortex mixer (Scientific Industries, Vortex Genie 2) set at level 3 for 1 h followed by a 10,000 × g centrifugation for 1 h. At the end of this stage, the supernatant was removed carefully using a pipette; this supernatant represents the soluble fraction of the PM₁₀ samples.

The prepared intact whole particle solution and soluble fraction of each sample were incubated in chromatographically pure water at different concentrations. All incubations (n = 4) were carried out in a final volume of 20 μL, with each containing 200 ng φX174 RF DNA (Promega, London, UK). Incubations were gently agitated (in order to ensure maximum mixing of the sample and to avoid sedimentation) for 6 h at room temperature. Bromophenol blue/glycerol loading dye (3 μL) was added to each sample before loading. Gels comprising 0.6% agarose and 0.25% ethidium bromide were run in 1% tris-borate-EDTA (TBE) buffer, at an electrophoretic voltage of 30 V for 16 h and at room temperature. The finished gels were photographed and densitometric analysis was performed using the GeneTools program (Syngene Systems, UK). A semiquantitative protocol was established to measure the relative proportion of the damaged DNA (relaxed and linearized) in each lane of the gel (in terms of a percentage of the total DNA in each lane). The DNA damage rate induced by airborne particles was calculated by subtracting the damage caused by the negative control (water). Dose-response curves were generated from the resultant data and an arbitrary unit was generated to facilitate comparison between different PM₁₀ samples. This arbitrary unit was defined as the toxic dose of particles necessary to cause 20% of the DNA to become damaged (TD₂₀) and a lower TD₂₀ value means a higher oxidative capacity or toxicity. A regression equation for each plot facilitated the calculation of the TD₂₀.

Statistical Analyses

Statistical analyses were performed using the SPSS program (version 10.0). Correlation was conducted based on the Spearman correlation coefficient. A probability level of 0.05 was used as a cutoff for statistical significance.

RESULTS

Mass Concentration of Lanzhou PM₁₀

The mass concentrations of Lanzhou PM₁₀ at the urban and suburban sites during the sampling periods are presented in Table 1 and Fig. 2. The monitoring data revealed that mass concentration of Lanzhou PM₁₀ exhibited seasonal variation at both the urban and suburban sites, with higher values in winter and spring, lower values in autumn, and lowest values in summer. For the urban site, average seasonal mass concentration of PM₁₀ was as high as 376.02 μg/m³ in winter and as low as 139.51 μg/m³ in summer. The monitoring data also indicated that the average PM₁₀ mass concentrations were higher at the urban site than the suburban site. For winter 2006, the average mass concentrations of PM₁₀ at the urban and suburban sites were 376.02 μg/m³ and 141.77 μg/m³, respectively.

The daily mass concentration of Lanzhou PM₁₀ at the urban site varied greatly in winter, ranging from 219.44 to 579.11 μg/m³ (Table 1). Furthermore, the mass concentration of PM₁₀ collected during the dust storm episode was shown to exceed 500 μg/m³.

Heavy Metal Compositions

ICP-MS was used to detect the concentrations of heavy metals in Lanzhou PM₁₀. The analyzed heavy metals included Cr, Mn, Co, Cd, Cs, Pb, Zn, As, Ni, and Cu. The average concentrations of these heavy metals and their water-soluble fractions are presented in Fig. 3: Zn was the metal present at highest concentrations in both the whole and water-soluble fractions, followed by Pb and Mn. Generally, the concentrations of the analyzed heavy metals in the intact whole samples were higher than those in the soluble fractions. Of all the elements analyzed, Pb and V

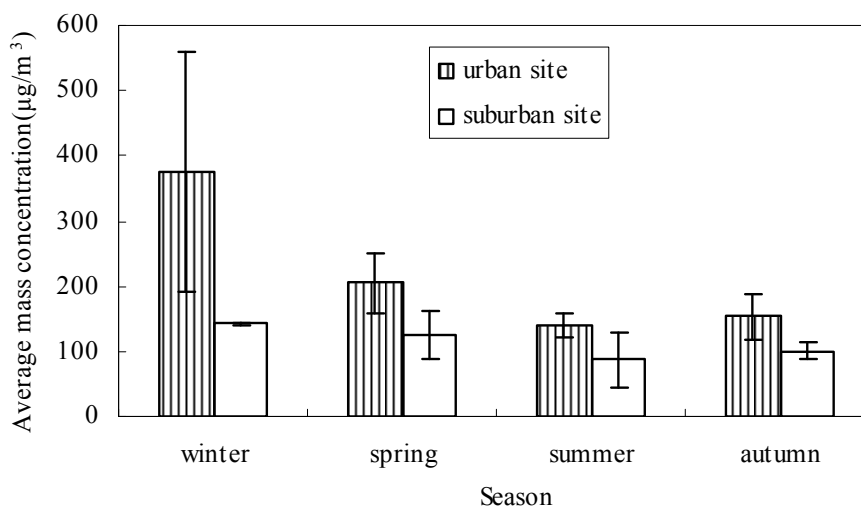


Fig. 2. Average seasonal mass concentration of PM₁₀ collected in Lanzhou urban and suburban atmosphere.

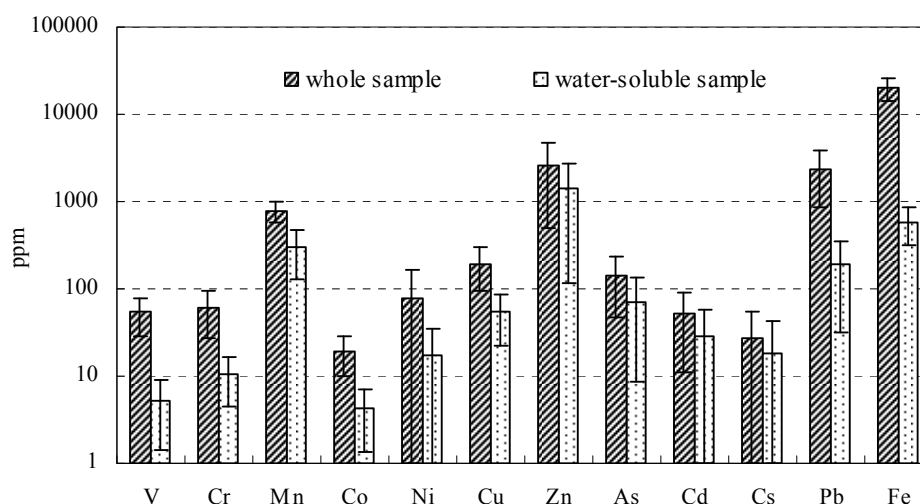


Fig. 3. Average ICP-MS values for concentrations of heavy metals and their soluble fractions in intact whole PM₁₀ samples in Lanzhou.

were the most insoluble: their concentrations were 90% higher in the whole sample than in the soluble fraction. Conversely, Zn, Cd, and As in Lanzhou PM₁₀ were present mostly in their soluble forms.

Whole and water-soluble Fe concentrations were analyzed for Lanzhou PM₁₀ samples collected in winter and spring (Fig. 3). The results indicate that total Fe in the whole samples was very high, compared to that in the soluble fraction, indicating that most of the Fe element in Lanzhou PM₁₀ was insoluble.

Oxidative DNA Damage by Lanzhou PM₁₀

A total of 22 samples were analyzed to investigate the oxidative capacity of Lanzhou PM₁₀ collected at the urban and suburban sites and their TD₂₀ values were calculated by linear regression. The results of oxidative damage of airborne particles are presented in Table 2. The urban samples generally exhibited lower TD₂₀ values, implying that the oxidative capacity of the PM₁₀ is highest in urban areas. Furthermore, the oxidative damage induced by Lanzhou PM₁₀ exhibited seasonal variation: TD₂₀ values were high (i.e., toxicities were low) in spring and autumn but low (i.e., toxicities were high) in winter and summer.

Additionally, the results indicated that the PM₁₀ samples collected during the Asian dust storm episodes and after rainy weather exhibited very low oxidative damage, with TD₂₀ values greater than 1000 µg/mL (Table 2).

DISCUSSION

Correlation between Heavy Metal Concentration and Bioreactivity

In order to examine the most probable source of the oxidative capacities of Lanzhou PM₁₀, the TD₂₀ values were correlated with the corresponding concentrations of heavy metals and their water-soluble fractions in the intact PM₁₀ samples. The TD₂₀ values were not correlated significantly with the total analyzed elements in the whole samples (correlation coefficient of -0.575 ; Fig. 4). In contrast, the

TD₂₀ values of PM₁₀ samples displayed a strong negative correlation with the total analyzed water-soluble metal concentrations (Pearson correlation coefficient of -0.854), implying that the oxidative capacity of Lanzhou PM₁₀ is derived mainly from its water-soluble fractions. This finding is consistent with other reports from Beijing (Shao *et al.*, 2006, 2007) and for UK particulate matter (Moreno *et al.*, 2004; Merolla and Richards, 2005).

In order to further elucidate the most probable sources of the oxidative capacities of airborne particles in Lanzhou, correlations were conducted between the TD₂₀ values and the analyzed water-soluble heavy metals in ambient particles (Table 3). The correlations between mass concentrations of water-soluble heavy metals (including Fe, V, Cr, Mn, Co, Cd, Cs, Pb, Zn, As, Ni, and Cu) and TD₂₀ values were conducted by means of Spearman's test. It can be seen that all heavy metals, except Ni and Cs, exhibit a negative association with TD₂₀ values. This is particularly true for Zn, Fe, Co, Mn, and Pb, which exhibit regression coefficients between -0.902 and -0.520 (Table 3), indicating that they were probably the heavy metals responsible for the plasmid DNA damage. Furthermore, it is interesting to note that high concentrations of water-soluble Zn, Fe, Pb, and Mn in the PM₁₀ samples exhibited relatively strong negative correlations with TD₂₀ values, suggesting that water-soluble Zn, Fe, Pb, and Mn may be the elements primarily responsible for the plasmid DNA damage.

Zn and Pb are defined as toxic elements by U.S. Environmental Protection Agency (EPA), and Zn is regarded as a bioreactive element (Adamson *et al.*, 2000). Previous studies have indicated that Zn is likely to be the major element responsible for plasmid DNA damage (Greenwell *et al.*, 2002; Shao *et al.*, 2006; Lu *et al.*, 2006; Shao *et al.*, 2007), which is in agreement with the results presented in this study.

Fe is often linked to the oxidative damage caused by particles (Valavanidis *et al.*, 2000; Han *et al.*, 2001; See *et al.*, 2007; Di Pietro *et al.*, 2009; Charrier and Anastasio, 2011), and soluble Fe affects oxidative capacity through

Table 2. Oxidative damage induced by airborne PM₁₀ collected at urban and suburban sites of Lanzhou.

Sampling Season	Sampling site	Sample No.	TD ₂₀ (µg/mL)	Sampling season	Sampling site	Sampling No.	TD ₂₀ (µg/mL)
Winter	Urban site	No. 1.	18	summer	Urban site	No. 11.	10
		No. 2.	21			No. 12.	10
		No. 3.	12			No. 13.	148
	Mean ± Stdev	17 ± 5	Mean ± Stdev		56 ± 80		
	Suburban site	No. 4.	80		Suburban site	No. 14.	400
No. 5.		150	Mean ± Stdev	40			
Spring	Urban site	Mean ± Stdev	115 ± 49	autumn	Urban site	No. 15.	40
		No. 6.	850			No. 16.	700
		No. 7.	730			No. 17.	41
		No. 8.	295		Mean ± Stdev	260 ± 381	
		Mean ± Stdev	625 ± 292		Suburban site	No. 18.	810
	Suburban site	No. 9.	950	No. 19.	410		
		No. 10.	980	No. 20.	80		
		Mean ± Stdev	965 ± 21	Mean ± Stdev	433 ± 366		
		spring	Urban site	No. 21.	1200		
		summer	Suburban site	No. 22.	1100		

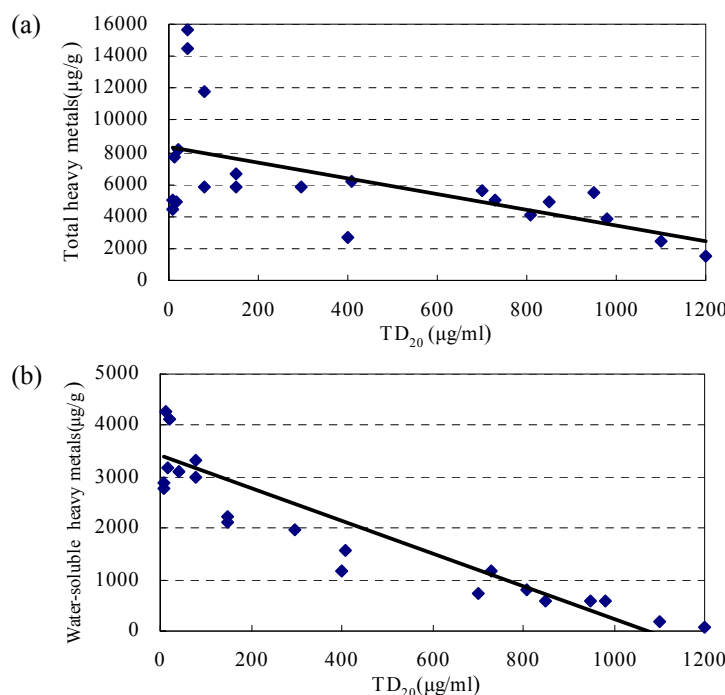


Fig. 4. The relationship between the TD₂₀ values and (a) the concentrations of the total heavy metal and (b) their water-soluble fraction in Lanzhou PM₁₀ samples.

Table 3. Correlation coefficients between the TD₂₀ values of PM₁₀ and the concentration of water-soluble fractions of heavy metals in Lanzhou PM₁₀.

	V	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Cs	Pb	Fe
TD ₂₀ values	-0.603*	-0.723*	-0.820*	-0.861*	-0.420	-0.616*	-0.902*	-0.520*	-0.685*	-0.442	-0.800*	-0.867*

Statistical analysis (Spearman's test) performed using SPSS 10.0, n = 22 (except Fe, n = 9)

* p < 0.05

the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + H_2O + OH^*$) (Shi et al., 2003; Risom et al., 2005; Vidrio et al., 2008; Nawrot et al., 2009). The ICP-MS analysis indicated that,

although total Fe concentration in the Lanzhou PM₁₀ samples was much higher than that in the soluble fraction, the concentration of soluble Fe was significant, reaching as

much as 1105 $\mu\text{g/g}$. In Lanzhou, the relatively high water-soluble Fe concentration was associated with lower TD_{20} values, suggesting that water-soluble Fe in Lanzhou PM_{10} was one of the major causes of DNA damage.

Meteorological Conditions and Potential Toxicity of Lanzhou PM_{10}

In the study, the causal relationship between meteorological conditions during the sampling periods and the TD_{20} values of PM_{10} samples collected at the urban site was also investigated (Fig. 5). No significant correlation was found between TD_{20} values and average temperature during the sampling periods ($r = -0.014$, $p > 0.05$, $n = 13$). In contrast, the TD_{20} values showed a relatively strong negative correlation with relative humidity ($r = -0.611$, $p < 0.05$, $n = 13$) and a relatively strong positive correlation with wind speed ($r = 0.682$, $p < 0.05$, $n = 13$), suggesting that lower

wind speed and higher relative humidity may affect the particles in some way that indirectly increases oxidative damage to DNA. Meteorological conditions have a dominant influence on aerosol particle concentrations, especially under the lower wind speed and higher relative humidity (Li *et al.*, 2011). Some results also indicated that the physical properties or mixed degree of particulate matter can be changed under different relative humidity, which could weaken and enhance secondary aerosol formation (Von Hessberg *et al.*, 2009; Cao *et al.*, 2011; Li *et al.*, 2010, 2011; Ram *et al.*, 2012). Therefore, one possible explanation is that low wind speed and high relative humidity could favor more water-soluble trace metals to be adsorbed and produced on PM. It is possible that high relative humidity contributed to secondary aerosol formation by promoting chemical reactions on the PM, resulting in more water-soluble transition metals that could impart more bioreactivity to DNA.

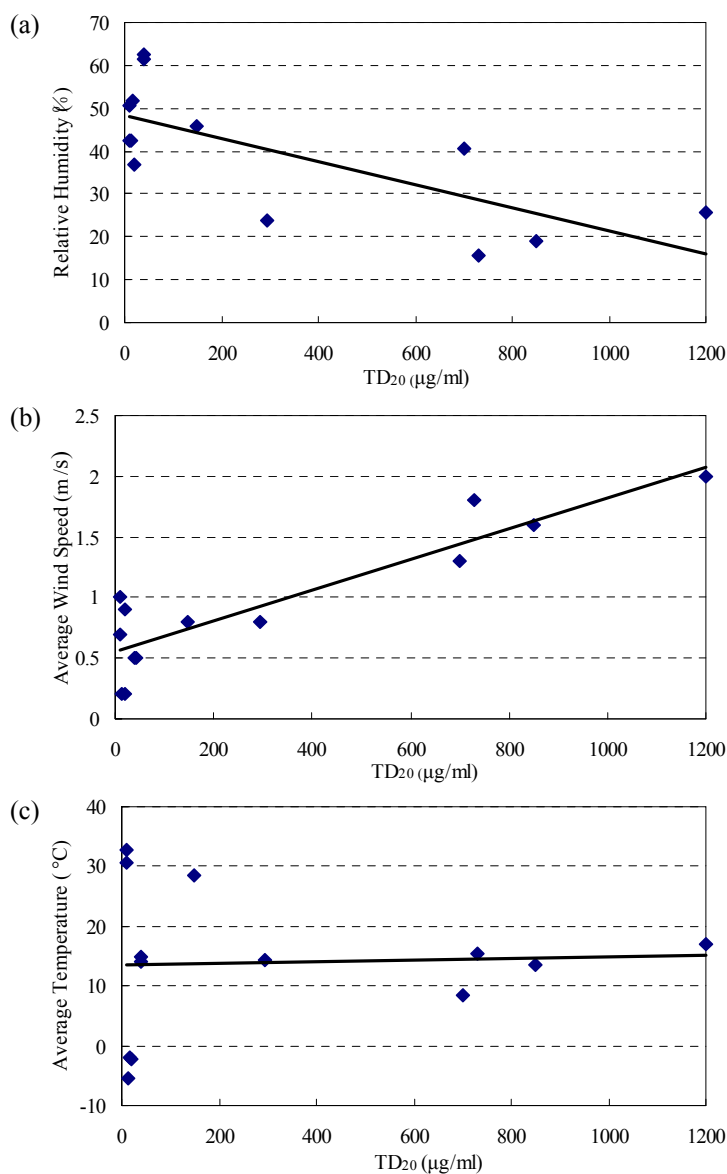


Fig. 5. The relationship between the TD_{20} values and meteorological conditions for (a) relative humidity, (b) wind speed, and (c) temperature.

In this study, there was no obvious correlation between the TD₂₀ values of PM₁₀ and the corresponding sampling temperatures. However, the PM₁₀ samples collected in summer (i.e., with the highest temperatures, more than 30°C) exhibited higher oxidative capacity (Fig. 5). Kao and Wang (2002) and Venkatachari *et al.* (2005) demonstrated previously that the concentration of reactive oxygen species in airborne particles exhibited a strong relationship with photochemical reaction strength. Furthermore, it has been reported that the photochemical smog in Lanzhou in summer is very serious (Chen *et al.*, 1986; Wang *et al.*, 1989; Jiang *et al.*, 2001). Therefore, the strong oxidative capacity of Lanzhou airborne PM₁₀ samples collected in summer appeared to be associated with photochemical reactions. One potential explanation is that extremely hot weather in summer could contribute to photochemical conversion, which might increase the solubility of transition metals (i.e., Fe, Pb, and Mn) and increase their bioreactivity with DNA.

CONCLUSIONS

The monitoring data revealed that mass concentration of Lanzhou PM₁₀ exhibited seasonal variation at both the urban and suburban sites, with higher values in winter and spring, lower values in autumn, and lowest values in summer.

ICP-MS analysis showed that Zn displayed the highest concentration in both the whole and water-soluble fractions of all the analyzed heavy metals, followed by Fe, Pb, and Mn. ICP-MS analysis also demonstrated that Zn, Cd, and As in Lanzhou PM₁₀ were present mostly in their soluble forms, unlike Fe, Pb, and V, which were present mostly in their insoluble states.

Lanzhou airborne PM₁₀ collected at both the urban and suburban sites caused damage to supercoiled DNA at various levels. The TD₂₀ values of Lanzhou PM₁₀ ranged from as low as 10 µg/mL to more than 1000 µg/mL. In addition, the oxidative damage induced by Lanzhou PM₁₀ collected at both the urban and suburban sites exhibited seasonal variation, with the highest average TD₂₀ values and thus the lowest toxicity in spring, lower values and thus higher toxicity in autumn, and the lowest values and thus highest toxicity in winter and summer.

The results presented here indicate that oxidative damage induced by Lanzhou PM₁₀ collected at both the urban and suburban sites was sourced mainly from water-soluble Zn, Fe, Pb, and Mn. Furthermore, meteorological conditions (such as lower wind speed and higher relative humidity) during sampling periods may influence particle physical and chemical characteristics, which, in turn, may increase oxidative damage to the DNA.

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REFERENCES

- Adamson, I.Y.R., Prieditis, H., Hedgecock, C. and Vincent, R. (2000). Zinc is the Toxic Factor in the Lung Response to an Atmospheric Particulate Sample. *Toxicol. Appl. Pharmacol.* 166: 111–119.
- Cao, J.J., Li, H., Chow, J.C., Watson, J.G., Lee, S.C., Rong, B., Dong, J.G. and Ho, K.F. (2011). Chemical Composition of Indoor and Outdoor Atmospheric Particles at Emperor Qin's Terra-cotta Museum, Xi'an, China. *Aerosol Air Qual. Res.* 11: 70–79.
- Charrier, J.G. and Anastasio, C. (2011). Impacts of Antioxidants on Hydroxyl Radical Production from Individual and Mixed Transition Metals in a surrogate Lung Fluid. *Atmos. Environ.* 45: 7555–7562.
- Chen, C.H., Huang, J.G., Ren, Z.H. and Peng, X.A. (1986). Meteorological Conditions of Photochemical Smog Pollution during Summer in Xigu Industrial Area, Lanzhou. *Acta Sci. Circumstantiae* 6: 334–342 [In Chinese with English abstract]
- de Kok, T.M.C.M., Drieste, H.A., Hogervorst, J.G. and Briede, J.J. (2006). Toxicological Assessment of Ambient and Traffic-related Particulate Matter: A Review of Recent Studies. *Mutat. Res.* 613: 103–122.
- Di Pietro, A., Visalli, G., Munaò, F., Baluce, B., La Maestra, S., Primerano, P., Corigliano, F. and De Flora, S. (2009). Oxidative Damage in Human Epithelial Alveolar Cells Exposed in Vitro to Oil Fly Ash Transition Metals. *Int. J. Hyg. Environ. Health* 212: 196–208.
- DiStefano, E., Eiguren-Fernandez, A., Delfino, R.J., Sioutas, C., Froines, J.R. and Cho, A.K. (2009). Determination of Metal-based Hydroxyl Radical Generating Capacity of Ambient and Diesel Exhaust Particles. *Inhal. Toxicol.* 21: 731–38.
- Dreher, K.L., Jaskot, R.H., Lehmann, J.R., Richards, J.H., McGee, J.K., Ghio, A.J. and Costa, D.L. (1997). Soluble Transition Metals Mediate Residual Oil Fly Ash Induced Acute Lung Injury. *J. Toxicol. Environ. Health* 50: 285–305.
- Gotschi, T., Heinrich, J., Sunyer, J. and Kunzli, N. (2008). Long-term Effects of Ambient Air Pollution on Lung Function: A Review. *Epidemiology* 19: 690–701.
- Greenwell, L.L., Moreno, T., Jones, T.P. and Richards, R.J. (2002). Particle-induced Oxidative Damage is Ameliorated by Pulmonary Antioxidants. *Free Radical Biol. Med.* 32: 898–905.
- Greenwell, L.L., Moreno, T. and Richards, R.J. (2003). Pulmonary Antioxidants Exert Differential Protective Effects Against Urban and Industrial Particulate Matter. *J. Biosci.* 28: 101–107.
- Han, J.Y., Takeshita, K. and Utsumi, H. (2001). Noninvasive Detection of Hydroxyl Radical Generation in Lung by Diesel Exhaust Particles. *Free Radical Biol. Med.* 30: 516–525.
- Hsieh, L.T., Wu, E.M.Y., Wang, L.C., Chien, G.P.C. and Yeh, Y.F. (2011). Reduction of Toxic Pollutants Emitted from Heavy-duty Diesel Vehicles by Deploying Diesel

- Particulate Filters. *Aerosol Air Qual. Res.* 11: 709–715.
- Hung, L.J., Tsai, S.S., Chen, P.S., Yang, Y.H., Liou, S.H., Wu, T.N. and Yang, C.Y. (2012). Traffic Air Pollution and Risk of Death from Breast Cancer in Taiwan: Fine Particulate Matter (PM_{2.5}) as a Proxy Marker. *Aerosol Air Qual. Res.* 12: 275–282.
- Jiang, Y.D., Wang, S.G., Qi, B., Shang, K.Z., Yang, D.B. and Yang, M. (2000). Temporal and Spatial Variations of Ozone Concentration and Its Relations with Meteorological Factors in Lanzhou Proper. *J. Lanzhou Univ. (Nat. Sci.)* 36: 118–125 [In Chinese with English abstract].
- Jones, T.P., Moreno, T., Bérubé, K.A. and Richards, R.J. (2006). Physicochemical Characterization of Physical Airborne Particles from South Wales: A Review of the Locations and Methodologies. *Sci. Total Environ.* 360: 43–59.
- Kao, M.C. and Wang, C.S. (2002). Reactive Oxygen Species in Incense Smoke. *Aerosol Air Qual. Res.* 2: 61–69.
- Lambert, A.L., Dong, W., Selgrade, M.K. and Gilmour, M.I. (2000). Enhanced Allergic Sensitization by Residual Oil Fly Ash Particles is Mediated by Soluble Metal Constituents. *Toxicol. Appl. Pharmacol.* 165: 84–93.
- Li, W. J., Shao, L.Y. and Buseck, P.R. (2010). Haze Types in Beijing and the Influence of Agricultural Biomass Burning. *Atmos. Chem. Phys.* 10: 8119–8130.
- Li, W.J., Zhou, S.Z., Wang, X.F., Xu, Z., Yuan, C., Yu, Y.C., Zhang, Q.Z. and Wang, W.X. (2011). Integrated Evaluation of Aerosols from Regional Brown Hazes over Northern China in winter: Concentrations, Sources, Transformation, and Mixing States. *J. Geophys. Res.* 116, doi: 10.1029/2010JD015099.
- Liu, S.M., Yang, H., Fu, Z. and Shao, Z.H. (2008). Analysis of Meteorological Condition and Related Heavy Pollution of PM₁₀ in Lanzhou City during Winter and Spring. *Environ. Sci. Technol.* 31: 80–83 [In Chinese with English abstract].
- Lu, S.L., Yao, Z.K., Chen, X.H., Wu, M.H., Sheng, G.Y., Fu, J.M. and Daly Paul. (2008). The Relationship between Physicochemical Characterization and the Potential Toxicity of Fine Particulates (PM_{2.5}) in Shanghai Atmosphere. *Atmos. Environ.* 42: 7205–7214.
- Merolla, L. and Richards, R.J. (2005). In Vitro Effects of Water-soluble Metals Present in UK Particulate Matter. *Exp. Lung Res.* 31: 671–683.
- Moreno, T., Merolla, L., Gibbons, W., Greenwell, L., Jones, T. and Richards, R. (2004). Variations in the Source, Metal Content and Bioreactivity of Technogenic Aerosols: A Case Study from Port Talbot, Wales, UK. *Sci. Total Environ.* 333: 59–73.
- Nawrot, T.S., Torfs, R., Fierens, F., De, H.S., Hoet, P.H., Van, K.G., De, B.G. and Nemery, B. (2007). Stronger Associations between Daily Mortality and Fine Particulate Air Pollution in Summer than in Winter: Evidence from a Heavily Polluted Region in Western Europe. *J. Epidemiol. Community Health* 61: 146–149.
- Nawrot, T.S., Kuenzli, N., Sunyer, J., Shi, T., Moreno, T., Viana, M., Heinrich, J., Forsberg, B., Kelly, F., Sughis, M., Nemery, B. and Borm, P. (2009). Oxidative Properties of Ambient PM_{2.5} and Elemental Composition: Heterogeneous Associations in 19 European Cities. *Atmos. Environ.* 43: 4595–4602.
- Park, S., Nam, H., Chung, N., Park, J. and Lim, Y. (2006). The Role of Iron in Reactive Oxygen Species Generation from Diesel Exhaust Particles. *Toxicol. in Vitro* 20: 851–857.
- Pop, C.A., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K. and Thurston, G.D. (2002). Lung Cancer, Cardiopulmonary Mortality, and Long-term Exposure to Fine Particulate Air Pollution. *J. Am. Med. Assoc.* 287: 1132–1141.
- Ram, K., Sarin, M.M., Sudheer, A.K. and Rengarajan, R. (2012). Carbonaceous and Secondary Inorganic Aerosols during Wintertime Fog and Haze over Urban Sites in the Indo-Gangetic Plain. *Aerosol Air Qual. Res.* 12: 59–370.
- Risom, L., Møller, P. and Loft, S. (2005). Oxidative Stress-induced DNA Damage by Particulate Air Pollution. *Mutat. Res.* 592: 119–137.
- See, S.W., Wang, Y.H. and Balasubramanian R. (2007). Contrasting Reactive Oxygen Species and Transition Metal Concentrations in Combustion Aerosols. *Environ. Res.* 103: 317–324.
- Shao, L.Y., Shi, Z.B., Jones, T., Li, J.J., Whittaker, A. and Bérubé, K. (2006). Bioreactivity of Particulate Matter in Beijing Air: Results from Plasmid DNA Assay. *Sci. Total Environ.* 367: 261–272.
- Shao, L.Y., Li, J.J., Zhao H.Y., Yang, S.S., Li, H., Li, W.J., Jones, T., Sexton, K. and Bérubé, K. (2007). Associations between Particle Physicochemical Properties and Oxidative Capacity: An Indoor PM₁₀ Study in Beijing, China. *Atmos. Environ.* 41: 5316–5326.
- Shi, T., Schins, R.P.F., Knaapen, A.M., Kuhlbusch, T., Pitz, M., Heinrich, J. and Brom, P.J.A. (2003). Hydroxy Radical Generation by Electron Paramagnetic Resonance as a New Method to Monitor Ambient Particulate Matter Composition. *J. Environ. Monit.* 5: 550–556.
- Squadrito, G.L., Cueto, R., Dellinger, B. and Pryor, W.A. (2001). Quinoid Redox Cycling as a Mechanism for Sustained Free Radical Generation by inhaled Airborne Particulate Matter. *Free Radical Biol. Med.* 31: 1132–1138.
- Ta, W.Q., Wang, T., Xiao, H.L. Zhu, X.Y. and Xiao, Z. (2004). Gaseous and Particulate Air Pollution in the Lanzhou Valley, China. *Sci. Total Environ.* 320: 163–176.
- Valavanidis, A., Salika, A. and Theodoropoulou, A. (2000). Generation of Hydroxyl Radicals by Urban Suspended Particulate Air Matter. The role of Iron Ions. *Atmos. Environ.* 34: 2379–2386.
- Venkatachari, P., Hopke, P.K., Grover, B.D. and Eatough, D.J. (2005). Measurement of Particle-bound Reactive Oxygen Species in Rubidoux Aerosols. *J. Atmos. Chem.* 50: 49–58.
- Vidrio, E., Jungb, H. and Anastasio, C. (2008). Generation of Hydroxyl Radicals from dissolved Transin Surrogate Lung Fluid Solutions. *Atmos. Environ.* 42: 4369–4379.
- Vidrio, E., Phuah, C.H., Dillner, A.M. and Anastasio, C. (2009). Generation of Hydroxyl Radicals from Ambient Fine Particles in a Surrogate Lung Fluid Solution. *Environ. Sci. Technol.* 43: 922–927.

- Von Hessberg, C., Von Hessberg, P., Pöschl, U., Bilde, M., Nielsen, O.J. and Moortgat, G.K. (2009). Temperature and Humidity Dependence of Secondary Organic Aerosol Yield from the Ozonolysis of β -pinene, *Atmos. Chem. Phys.* 9: 3583–3599.
- Wang, S.G., Zhang, L., Chen, C.H. and Yuan, J.Y. (1999). Retrospect and Prospect for the Studies of Atmospheric Environment in the Lanzhou Area. *J. Lanzhou Univ. (Nat. Sci.)* 25: 189–201 [In Chinese with English abstract].
- Wei, F.S., Teng, E.J., Wu, G.P., Hu, W., Wilson, W.E., Chapman, R.S., Pau, J.C. and Zhang, J. (1999). Ambient Concentrations and Elemental Compositions of PM₁₀ and PM_{2.5} in four Chinese Cities. *Environ. Sci. Technol.* 33: 4188–4193 [In Chinese with English abstract].
- Wei, F.S. and Hu, W. (2000). The Analysis of the Impact of Air Pollution of Four Cities in China on Children's Respiratory Health. *World Sci-Tech R & D* 22: 9–13 [In Chinese with English abstract].
- Whittaker, A., BéruBé, K., Jones, T., Maynard, R. and Richards, R. (2004). Killer Smog of London, 50 Years on: Particle Properties and Oxidative Capacity. *Sci. Total Environ.* 334–335: 435–445.
- Wu, G.P., Hu, W., Teng, E.J. and Wei, F.S. (2001). Analysis of the Effect of Air Pollution on the Adult's Respiratory Health. *Environ. Monit. Assess.* 17: 33–38 [In Chinese with English abstract].
- Zhong, C.Y., Zhou, Y.M., Smith, K.R., Kennedy, I.M., Chen, C.Y., Aust, A.E. and Pinkerton, K.E. (2010). Oxidative Injury in the Lungs of Neonatal Rats Following Short-term Exposure to Ultrafine Iron and Soot Particles. *J. Toxicol. Environ. Health* 73: 837–847.

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