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Original Research Paper

Study on the Toxicology of PM10 in Manas River Basin of China

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ABSTRACT

PM10 samples were collected in spring and summer 2010 in Manas River Basin, Xinjiang, China. The water-soluble fraction was subjected to plasmid DNA assay, and several conclusions were obtained. Damages to DNA caused by PM10 with the same mass concentration were different and related to the source of particulate matters and their complex components. The correlation between mass concentration and damage rates of the samples was not linear, indicating that the rate of particulate matter damage reached a threshold value under the synthetic action of their complex components, and then the range of damage changed with increased mass concentration. Damage to DNA caused by PM10 samples in spring was significantly lower than that in summer. Little difference in toxicity was observed between whole samples and their water-soluble fractions in both spring and summer. In other words, in the same city and under similar weather conditions, the toxicity of the whole particulate matter samples was close to the toxicity of their water-soluble fraction, indicating that the damage to DNA was usually due to the water-soluble fraction of particulate matters.

INTRODUCTION

Studies on the influence of inhalable particulate matters on health mainly focus on epidemiology and toxicology (Yin 2014). A great number of experiments on the two aspects conducted by scholars from home and abroad demonstrate that mortality rate and pathogenicity rate have a significant positive correlation with the level of particulate-matter pollution (Schwartz 1994, Chapman et al. 1997, Ostro et al. 1999, Maynard 2001, Xi et al. 1999). Dai et al. (2004)'s correlation and regression analysis between average pollution concentration and the number of daily deaths using Poisson's generalized additive models indicated that the number of deaths grows by 0.53% and 0.85%, respectively, with increased concentration of PM10 and PM2.5 in air at 10 μg/m^3. Chapman et al. (1997) experimented on the toxicology of particulate matters collected from Lan Zhou's downtown area and concluded that particulate matters damage MRC-5 cells. Ivars-Martinez et al. (2008) studied organic extracts of particulate matters with different sizes and found that the organic extracts harm the DNA of human peripheral blood lymphocytes.

No hypothesis can fully explain all damages that particulate matters inflict onto the human body. However, the transition-metal-ion hypothesis has recently attracted extensive attention. Many scholars hold the view that transition metal ions in particulate matters can generate free radicals (strong oxidizing groups, such as HO$^\bullet$, O$^\bullet$, etc.), which cause oxidative damage to object cells. Some researchers (Stohs 1995, Costa & Dreher 1997, Zhongru et al. 2005, Wendy et al. 2005, Bonzini & Tripodi 2010, Jun et al. 2013) believe that heavy metal ions on the surface of particulate matters can generate free radicals after being catalysed, and these free radicals are absorbed on the PM surface. They showed that both the samples and their water-soluble fractions can damage supercoiled DNA by oxidizing Fe^{2+} and Cr^{3+} to high-valence metal ions under the action of active oxygen (free oxygen atom O$^\bullet$) (Gilli et al. 2007, Bing et al. 2004, Donaldson et al. 1997, Geng et al. 2010). Donaldson (1997) also proved that Fe^{2+} particles under catalysis can generate HO$^\bullet$ free radical. Jing et al. (2010) showed that transition metals can break double-stranded plasmid DNA through hydrolytic cleavage and oxidative scission. Fe^{2+}, instead of directly acting on DNA, generates HO$^\bullet$ and Fe^{3+} through the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO^\bullet$, and then abstracts hydrogen from the carbon atom of deoxyribose, thereby causing the damage.

Some foreign scholars believe that the water-soluble heavy metal Zn may cause DNA oxidative damage (Bing et al. 2004), whereas Galazyn held the opinion that Zn may be the primary factor affecting PM that induces heart break (Galazyn-Sidorczuk et al. 2009).

SAMPLING AND EXPERIMENTS

Plasmid DNA assay is a method of testing DNA damage

that is highly sensitive, easy to operate, quick, and able to quantitatively evaluate the damage that PM10 inflicts to DNA. Thus, the toxicity of particle matters can be determined using a relatively small amount of sample, i.e., about 500-1000 μg.

Principle of Plasmid DNA Assay

Plasmid is a kind of DNA molecule found in many kinds of bacterial cells in large amounts. Plasmids often exist in host cells in the form of supercoiled DNA and covalent closed circular DNA, which under the effect of many *in vitro* factors can break into linear DNA (chemical bonds are broken) or open circular DNA (chemical bonds are not broken).

Plasmid DNA assay, an *in vitro* method, can be applied to evaluate the toxicity of PM10, which refers to the effect of particulate matters on DNA. This assay can also be used to test the bioreactivity of active oxygen toward plasmid DNA. The principle of this method is that free radicals absorbed on the surface of particulate matters bring oxidative damage (Bing et al. 2004) to supercoiled DNA. At first, DNA damage involves the relaxation of supercoiled DNA while chemical bonds are not yet broken, followed by the break of chemical bonds. DNA then becomes linear, which leads to the change in DNA's electrophoretic mobility. Three kinds of plasmid DNA (i.e., damaged DNA, relaxed DNA, and linear DNA) have different electrophoresis velocities (Fig. 1): Vsupercoiled DNA > Vlinear DNA > Vrelaxed DNA. Based on this principle, the three kinds of plasmids can be separated from agarose gel. After separation, we can use densitometry to measure the proportion of the three plasmids (Fig. 1). The ratio of the brightness of linear DNA and relaxed DNA to that of the three plasmids is the toxicity, with ultra pure water (electrical conductivity = 18.2 Mfl) serving as the control sample. In the calculation, the influence of ultra pure water on DNA was subtracted, and the result was PM's damage to DNA. We can use TD20 or TD50 (or TD30, TD40, etc.) to represent the toxicity of particulate matters. The value of TDX was the dosage of PM needed to damage X% DNA. A smaller TDX indicates higher toxicity, and vice versa. Given that particulate matters with different dosages have different toxicities, we can use to decide the value of TDX and evaluate PM10's damage to DNA.

Plasmid DNA Array Experiment

Equipment: The equipment used were as follows: (1) micropipettes (different sizes); (2) ultrasonic oscillator; (3) high-pressure steam sterilizer; (4) centrifuge (15 mL) and Eppendorf (1.5 and 0.2 mL) tubes; (5) Erlenmeyer flask, graduated cylinders, a microwave oven; (6) vortex oscillator and high-speed centrifuge; (7) gel-imaging system with UV light base; (8) ultra-low temperature freezer; (-28°C) and a regular freezer; (9) electrophoresis meter, including an electrophoresis cell, a gel tank, combs, an electrical power generating system; and (10) a microbalance and pincers.

Reagents: The reagents used were as follows: (1) bromophenol blue/propanetriol staining reagent; (2) HPLC water autoclaved four times; (3) ethidium bromide (EB), severely poisonous; (4) TEB butter solution (Sigma, USA); (5) X174-RF DNA (Promega, USA) stored in the -28°C ultra-low temperature freezer; and (6) agarose.

Sample preparation: The sample (a whole membrane) was cut into small pieces and placed in an asepsis tube. The tube was filled with sterilized water whose volume was calculated based on the maximum concentration. The tube was placed in the oscillator and oscillated for 20 h. The obtained solution was the sample with the highest concentration. The prepared sample was divided into two equal parts, one for whole sample and the other one to be dissolved in water.

The whole sample was placed in the freezer for later use. The supernatant obtained after oscillation was the sample to be dissolved in water. The total volume of samples with different concentrations was 50 μL, including 7 μL of colouring agent, 41 μL of solution (sterilized water plus original solution), and 1.8 μL of DNA (counted as 2 μL). For instance, if the mass concentration had five levels (200, 400, 600, 800, and 1000), the mass concentration of 1000 needed 41 μL of original solution. The equation mass concenration × 41 = X × 1000 (Table 1) to calculate the volume of the original solution that the other mass concentration levels needed.

No. 6 Eppendorf tubes were each filled with 1.8 μL of 10 μg/mL Xl74-RFDNA (Promega, London, UK). The original solution, whose volume was calculated according to Table 1, was added to each Eppendorf tube using pipettors and mixed with sterilized water, whose volume was also calculated. Five levels of mass concentration were prepared every time, placed in line from low to high level, sealed, and fix on the vortex oscillator to oscillate for 6 h.

Gel preparation: The Erlenmeyer flask, gel tank, and comb

Table 1: Example of sample-solution preparation.

PM10 mass concentration (μg/mL)	Volume of original solution (μL)	Volume of aquae sterilisata HPLC H_2O (μL)
1000	41	0
800	800×41/1000=32.8	41-X=8.2
600	600×41/1000=24.6	41-X=16.4
400	400×41/1000=16.4	41-X=24.6
200	200×41/1000=8.2	41-X=32.8
H_2O	0	41

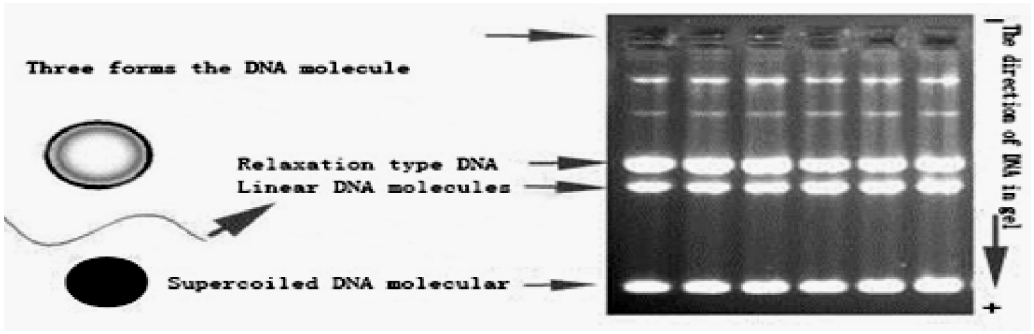

Fig. 1: Experimental principle of the bioreactivity of PM toward plasmid DNA.

were cleaned with HPLC water. About 2.6 g of agarose was placed in a 500 mL Erlenmeyer flask. A 50 mL graduated cylinder was used to measure 42 mL of TEB buffer solution, which was then poured into a 500 mL graduated cylinder, which was filled with water until the volume was 420 mL. The solution was diluted to 10% of its original concentration. The diluted TBE buffer solution was evenly blended with 2.6 g of agarose in the Erlenmeyer flask, which was left undisturbed. The flask was then placed in the microwave and heated for 3 min. The flask was gently shaken, reheated for another 1.5 min, taken out, and gently shaken again until the solution becomes clear. When the temperature of the flask decreased to around 60°C, 10 μL (0.010 g/mL) of EB was added.

Two combs were cleaned with sterilized water, dried and placed in the gel tank. Autoclaved adhesive tape was used to seal the two flanks of the gel tank. The solution was poured into the flask in the tank to remove bubbles and for natural cooling. About 1500 mL of TBE buffer solution (1/10 of the original concentration) was poured into the electrophoresis tank. The height of the liquid level was controlled to about 5 cm from the tank's top. The fully concealed gel (about 30 min later) was placed in the freezer (4°C). The adhesive tape was gently removed, and the combs were taken out. The gel tank was placed in the electrophoresis cell and 500 mL of TEB buffer solution (1/10 of the original concentration) was added to a height of 5-10 mm above the gel surface.

Injection of the mixture of DNA and samples into the gel: The six Eppendorf tubes containing the mixture of DNA and sample after oscillation for 6 h were placed in a centrifuge and centrifuged for 40s. Then, 7 μL of bromophenol blue/propanetriol staining reagent was poured into each tube so that the volume of solution in each tube becomes 50 μL. Using a pipette, 20 μL of a mixture of the sample, staining reagent, and φX174-RF DNA was injected into every hole (every sample had a parallel sample). About 20 μL of EB was added into the solution at both ends of the electrophoresis cell, whose two charges were connected to a power source. The current was set to 30 mA, and the voltage was 30 V. The current was kept on for 16 h.

Gel imaging of DNA damage and quantitative analysis: The gel was taken out of the electrophoresis cell by pouring away the redundant TEB buffer solution on the gel surface) and placed in the gel-imaging system with UV light base. The image's definition was adjusted, and the image was saved. The optical density of DNA of three forms in the gel was quantitatively analysed using Syngene Genetools. The ratio of optical density of damaged DNA (relaxed DNA + linear DNA) to that of total DNA (three forms) was the toxicity (damage rate of DNA). Damage to supercoiled DNA caused by different amounts of PM10 was quantitatively analysed by subtracting the damage of H_2O. The average value of DNA damage was calculated according to different mass concentrations, and linear regression was used to determine the TD50 or TD20 of the samples with the DNA damage rate of every concentration.

RESULTS AND DISCUSSION

Toxicity of PM10 in the atmosphere in spring, 2010 in Manas River basin: Fig. 2 is the representative gel figure (BJ3-1 and BJ3-3) showing the oxidative damage that the whole samples and water-soluble fractions of PM10 with different dosages inflicted to plasmid DNA. With increased particle mass concentration, the damage rate of DNA also slowly increased. The TD50 (the dosage needed to damage 50% of DNA) of the whole sample and water-soluble fraction was obtained by trend forecasting and goal seeking. The TD50 values of the whole samples and water-soluble fractions were 734 and 1035 μg/mL for BJ3-1, 2159 and 1961 μg/mL for BJ3-2, and 1467 and 1512 μg/mL for BJ3-3, respectively. Fig. 2 and Table 2 indicate that with increased mass concentration, the damage rate of DNA of the whole sample and water-soluble fraction of PM10 in Manas River basin only slightly varied. BJ3-2 and BJ3-4, which were much

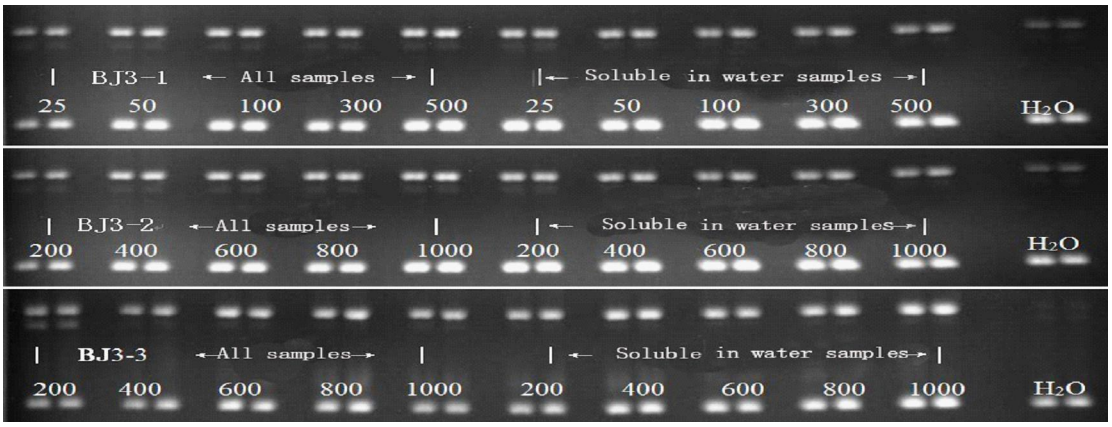

Fig. 2: Gel image showing oxidative damage to supercoiled DNA induced by PM10 samples and their water-soluble fractions collected in Manas River basin in spring, 2010.

Table 2: TD50 values of whole samples and water-soluble fractions of PM10 samples collected in Manas River basin during spring, 2010.

Mass concentration (μg/mL)	BJ3-1W/S (damage rate)	Mass concentration (μg/mL)	BJ3-2W/S (damage rate)	BJ3-3W/S (damage rate)	BJ3-4W/S (damage rate)	BJ3-5W/S (damage rate)
25	19.3/20.1	200	19.4/15.7	30.4/39.5	27.3/23.5	41.9/29.3
50	24.4/23.9	400	19.8/14.1	31.7/38.8	34.0/23.6	41.3/36.1
100	29.0/25.4	600	21.9/11.7	35.1/37.4	34.4/41.4	47.4/40.5
300	28.8/45.8	800	22.8/11.9	34.9/37.1	38.9/39.0	46.6/41.1
500	23.4/38.3	1000	25.7/14.0	40.1/42.6	43.2/42.7	49.3/41.6
TD50	734/1035		2159/1961	1467/1512	1382/1712	1068/2419
R2	0.9907/0.8032		0.9773/0.9091	0.9276/0.7729	0.9524/0.7719	0.8174/0.9969

average value of TD50 in normal weather 1090/1655; average damage rate/% with mass concentration is 500μg/mL 31.2/40.8

clearer, showed that toxicity mainly came from water-soluble components, in line with the conclusion obtained by Bing et al. (2004), who studied the oxidative damage ability of particle matters in dust weather and non-dust weather through the method of plasmid DNA assay.

Notably, 11 groups of samples in the gel image and every group had two parallel samples. The first five groups were whole samples (W), the five in the middle were water-soluble fractions (S), and the last one was ultra pure water (the control). Numbers in the figure represent the sample number and mass concentration.

Toxicity of PM10 in the atmosphere in summer, 2010 in Manas River basin: The gel image of oxidative damage to supercoiled DNA induced by PM10 samples and their water-soluble fractions collected in Manas River basin during summer of 2010 is shown in Fig. 3. The toxicity of PM10 in the atmosphere during the summer in Manas River basin substantially varies. Overall, BJ8-5 can better represent damage to the samples collected in summer. Its TD50 (W/S) was 668 μg/mL/503 μg/mL, and the average rate of DNA damage was 46.7%/48.5% when the mass concentration was 500 μg/mL. Samples BJ8-4 and BJ8-1 abruptly changed. When the mass concentrations were 50 and 100 μg/mL, the whole samples and their water-soluble fractions did more serious damage to DNA than those with higher concentrations; when the mass concentration of BJ8-4 was 500 μg/mL, the average damage rate was 59.5%/39.9%, higher than the average rate of damage in all 500 μg/mL samples, i.e., 52.8%/48.12%. The TD50 (W/S) values of BJ8-1 and BJ8-4 were 33 μg/mL/149 μg/mL and 99 μg/mL/970 μg/mL, respectively. The toxicity of whole samples was far higher than those of the water-soluble ones. This finding indicated one of the following: 1) the samples collected in the same place at different times caused different damages to DNA because of particulate matters' sources and complicated components, 2) after dissolving in water, the valence of metallic ion changed (dropped), or 3) metallic ions and water complex together became hydrated metal ions, leading to the decrease in electronic potentials (decreased oxidation).

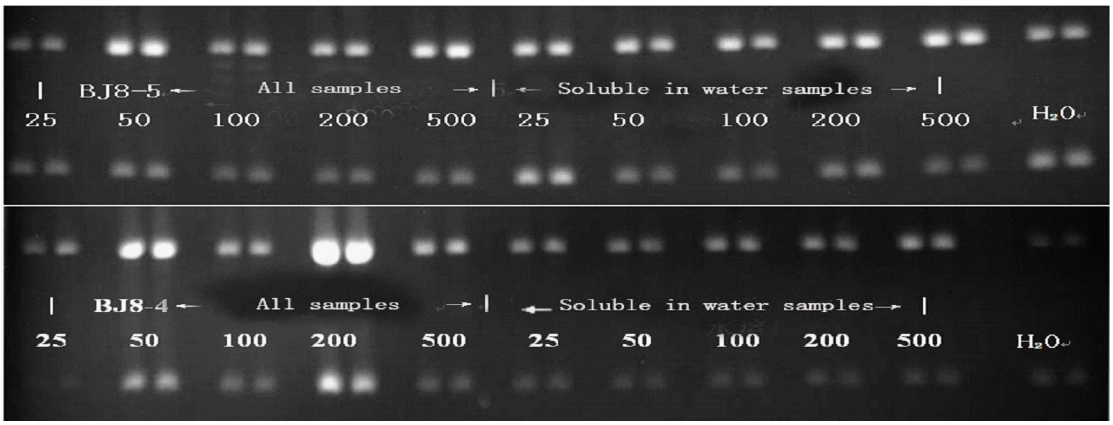

Fig. 3: Gel image showing oxidative damage to supercoiled DNA induced by PM10 samples and their water-soluble fractions collected in Manas River basin in summer, 2010.

Table 3: TD50 value of whole samples and water-soluble fractions of PM10 samples collected in Manas River basin in summer, 2010.

Mass concentration(μg/mL)	BJ8-1W/S (damage rate)	BJ8-2W/S (damage rate)	BJ8-4W/S (damage rate)	BJ8-5W/S (damage rate)	BJ8-6W/S (damage rate)
25	50.4/40.4	7.76/5.66	59.2/38.4	37.4/22.9	38.8/37.5
50	51.2/46.2	10.2/10.6	50.5/36.7	41.6/35.2	40.2/44.9
100	45.4/50.9	18.8/14.9	51.0/37.6	39.2/42.4	42.8/42.3
200	66.9/50.9	23.1/24.7	52.7/37.5	43.3/42.4	44.3/44.5
500	66.9/58.2	40.7/43.1	59.5/39.9	46.7/48.5	50.2/50.9
TD50	33/149	625/950	99/970	668/503	480/490
R2	0.957/0.8722	0.9953/0.9981	0.8225/0.7916	0.8071/0.9826	0.7603/0.9814

Average TD50 under normal weather 381/612.4; The average damage rate/% when mass concentration is 500μg/mL 52.8/48.12

The average TD50 (W/S) in summer in Manas River Basin was 381 μg/mL/612.4 μg/mL. When the mass concentration was 500 μg/mL, the average damage rate to DNA was 52.8%/48.12%.

CONCLUSIONS

Samples of the same mass concentration collected in Manas River Basin of China in spring and summer had different damage rates depending on different resources and complicated components of particulate matters. The correlation between mass concentrations and damage rates of the samples was not linear, indicating that the rate of particulate matters' damage reached a threshold value under the synthetic action of their complex components and then increased with increased mass concentration.

The samples collected in spring had significantly lower ability to damage than those collected in summer. No significant difference in toxicity existed between the samples and their water-soluble fractions in spring and summer. Thus, the toxicity of particulate matters under the same meteorological condition was similar to their water-soluble fractions, showing that the components of particulate matters that caused the damage were largely water soluble. In future, much effort and work should be done to study PM10 and its effect to social environment, public health and pollution control, so as to keep ecological environment quality.

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