

A toxicological investigation of the air quality in a moxibustion treatment room as measured through particulate concentration and oxidative capacity

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ABSTRACT

Background: Moxibustion is a traditional Chinese medicine (TCM) therapy in which mugwort (*Artemisia vulgaris*) floss is burned to warm and stimulate acupoints. The modality has been used traditionally for thousands of years. However, smoke-related safety issues have recently been of concern, and little is known about moxa smoke and air quality in the clinical moxibustion environment.

Objective: To assess the air quality in a typical moxibustion treatment room using particulate matter (PM) concentration and DNA oxidative damage at PM10.

Methods: The study was conducted in August and November to December, 2011, at a TCM clinic in Beijing, China, in a moxibustion treatment room. A moxa-free treatment room and the outdoor area adjacent to the clinic were used as controls. PM10 concentrations were monitored with a portable digital dust indicator. The oxidative capacity of whole and water-soluble fractions of PM10 were detected using plasmid DNA assay. The results were shown as TD40 values; that is, the amount of PM10 that causes plasmid DNA damage of 40%.

Results: Average PM10 concentrations in the moxibustion room were 2.56 mg m⁻³ in summer and 2.78 mg m⁻³ in winter, much higher than at control sites. For whole and water-soluble fractional PM10, the average summer TD40 values collected in the moxibustion room were 791.67 µg ml⁻¹ and 876.33 µg ml⁻¹ respectively, and the winter values were 779.86 µg ml⁻¹ and 879.57 µg ml⁻¹. These results of winter samples were significantly higher ($p < 0.001$) than the corresponding results from control sites. However, there was no statistical difference ($p = 0.06$) between the TD40 values of both the whole and water-soluble fractional PM10 from the moxibustion treatment room, while differences were significant in the general treatment room ($p = 0.025$) and at the outdoor site ($P < 0.001$).

Conclusion: Our study shows that moxa smoke increases PM10 concentration. However, the oxidative capacity of PM10 in the moxibustion room was much lower than that at control sites with the same particulate burden, and the bioactivity at that site was mainly from the water-soluble fraction, another difference from the controls. This unexpected bioactivity is assumed to relate to the low toxicity of moxa smoke or to its proven antioxidant activity. Overall, further research is needed.

Key words: Moxibustion treatment room, moxa smoke, PM10, mass concentration, oxidative capacity

1. INTRODUCTION

Moxibustion is a traditional Chinese medicine (TCM) therapy using mugwort (*Artemisia vulgaris*). The herb is usually aged, then ground into floss or formed into a cigar-shaped stick. TCM practitioners burn the floss or stick at regions of the body and acupuncture points to warm an area, stimulate circulation, and induce smoother flow of blood and *qi* in order to prevent and treat diseases. Moxibustion has been used for thousands of years, but recently smoke-caused safety issues are of concern.

Moxa smoke, the inevitable product of moxibustion, contains respirable particulates (RP). RP refers to particulate matter with an aerodynamic diameter smaller than 10 µm

(PM10), which is easily inhaled and deposited in the respiratory system. Epidemiological studies have shown that PM10 has positive correlations with morbidity and mortality in respiratory and cardiovascular diseases^[1-4]. Thus PM10 levels are significant from the perspective of environmental health.

Indoor air quality has become a great concern in recent years because more than half of the body's intake during a lifetime is air inhaled in the home^[5]. It has been reported that cigarette smoke, home heating such as wood and coalburning stoves, cooking, candle and incense burning are the main sources of indoor particulate exposure^[6-9].

Moxa smoke is a significant source of particulates in the air of moxibustion treatment rooms. But mugwort is different from other burned materials. A frequently used herb in TCM practice, it contains volatile substances such as juniper, camphor, caryophyllene oxide, and caryophyllene^[10]. Thus its smoke has a distinctive odor and special applications. For example, moxa smoke was used to cure cough, headache, toothache, and aphthous ulcers in ancient China. Even today in China, some people use it to sterilize the air. The smoke has been reported to be an antioxidant^[11–12], to have anti-inflammatory^[13] and anti-tumor effects^[12, 14], and to regulate autonomic functions^[15].

Despite its many benefits, the safety of moxa smoke is in question^[16], especially for practitioners who work for prolonged periods in moxibustion treatment rooms, and there is a lack of knowledge concerning the air quality in such rooms.

PM10 level is the main measure of air quality. As opposed to ordinary evaluations such as mass and number concentrations, PM10-induced oxidative damage to supercoiled DNA is a molecular-level index that allows us to estimate air quality. Plasmid DNA assay is a *vitro* method to study this capability. The method is based on the widely accepted hypothesis that free radicals produced by bioavailable transition metals on particle surfaces can induce oxidative damage^[17]. It has already been applied to study the bioactivity of fiber glass^[18], carbon black^[19], and urban airborne particles^[20]. Greenwell^[21, 22] used an optimized and sensitized method to evaluate the bioactivity of diesel exhaust particles and urban airborne particles in Cardiff, UK. Shao also employed the method to study PM10 and PM2.5 bioactivity in Beijing air^[23].

The aim of our study was to survey PM10 concentration and to determine the TD40 of PM10, the toxic burden of PM10 that can cause 40% plasmid DNA damage, using plasmid DNA assay to evaluate air quality in a moxibustion treatment room.

2. METHODOLOGY

2.1. Sampling sites and descriptions

This study was conducted during the August of the summer of 2011 and from November to December of that year in a moxibustion treatment room and a moxa-free treatment room which was about 15 m² in area each room, in a TCM clinic in northeastern Beijing. An open field about 10 meters to the north of the clinic was selected as the outdoor sampling site. The moxa-free room and the outdoor site were used as controls.

Both treatment rooms are occupied by patients between 8AM and 8PM every day. During the summer sampling, the moxibustion treatment room was not air conditioned; as the body is often exposed during treatment, patients could easily take cold if the temperature in the room were too low. The air conditioning was kept off in the control room as well, and a small window was opened in each room. During the winter sampling, all windows and doors were closed. Smoking is

prohibited in the treatment rooms, and the rooms are frequently cleaned in the daytime.

2.2. Sample collection

The method of sample collection is that detailed by Wang^[24]. Particulate samples were collected by the Mini VolTM PM₁₀ portable air sampler with polycarbonate filters 47mm in diameter that were numbered and dried for 24h at a constant humidity of 40–42% and a temperature of 20–22°C before and after sampling. The mass margin of each filter before and after sampling was calculated as the mass of each particulate sample. The inlets of the indoor samplers, with an air flow rate of 5L min⁻¹, were located at about 1.1m above ground level in the sampling sites to simulate the breathing zone of the patients. Outdoors, the sampler was located on the tree 1.1 meters above the ground. For three days during summer sampling and seven days during the winter period, sample collection started from 8AM every day, and 18–24 h PM10 collection a day were incessantly made at different sites in each area, because the PM10 mass concentrations are vary from different sites and seasons. The particulates collected on the filters were subjected to plasmid DNA assay.

Moxa sticks made by Nanyang Hanyi moxibustion technology development Co., Ltd were used.

2.3. Mass concentration

A P-5L2C portable digital dust indicator (Beijing BINTA Green Technology Ltd) that shows results in 3 minutes was used. Mass concentration was measured every hour between 8AM and 8PM at different sites in each room and averaged to give the PM₁₀ concentration of that room. Relative humidity and wind speed were monitored with a Kestrel NK3000 handheld aerometeorograph (USA).

2.4. Plasmid DNA assay

Plasmid DNA assay was briefly summarized here, and it was performed according to the method detailed by Lu^[25].

The damage proceeds in three stages: firstly, as the DNA is damaged by the free radical, the supercoiled form is ruptured to bulkier, relaxed coiled conformation with a lower electrophoretic mobility than the undamaged plasmid, allowing separation by electrophoresis. Further nicking breaks the plasmid ring and converts it to a linearised form with higher mobility than the relaxed form, but lower mobility than the supercoiled form. In an agarose gel, the above three forms of DNA which separate, using densitometry, different forms of DNA could be semi-quantified. (Figure 1)

(1) Preparation of samples

- 1) The whole filter was immersed in 1 ml sterile HPLC-grade water (resistivity > 17.8 MΩ). The sample was shaken gently for about 20 hours and then sonicated for 2 minutes so that the particulates could be released from the filter substrate into the solution, and the mass concentration of the whole sample fraction was calculated. The sample fraction was shaken; half of it was

centrifuged to deposit particulates on the bottom of the tube, and this was filtered. The supernatant, the water-soluble fraction, was removed carefully into another tube. Both the whole and the water-soluble fraction were stored at -80°C .

- 2) Both the whole and the soluble samples were diluted to the required concentrations with HPLC-grade water, which varied depending on the original concentration of the PM and the estimated bioactivity. Every sample in this study was diluted to five grades. For example, if the original PM₁₀ concentration of the sample was $1000\ \mu\text{g ml}^{-1}$, its experimental concentrations might be $1000\ \mu\text{g ml}^{-1}$, $800\ \mu\text{g ml}^{-1}$, $600\ \mu\text{g ml}^{-1}$, $400\ \mu\text{g ml}^{-1}$ and $200\ \mu\text{g ml}^{-1}$. Sterile HPLC-grade water was used as a solvent control for each experiment.
- 3) $1.8\ \mu\text{l}$ ($200\ \text{ng ml}^{-1}$) ϕ X174-RF DNA (Promega, London, UK) was added to an EP tube that contained a $41\ \mu\text{l}$ PM₁₀ sample (whole or soluble fraction) of each concentration. The tubes were centrifuged for 1 minute at a rate of 2000 rounds/minute and put onto a vortex (Scientific Industries, Vortex Genie2) to vibrate gently for six hours at room temperature. The frequency was adjusted to 2 in order to prevent mechanical deterioration to the plasmid.

(2) Gel preparation

- 1) Agarose gel (2.6 g) was put into a 500 ml tapered measuring flask, and 420 ml 10% Tris-Borate-EDTA (TBE) was added.
- 2) The flask was heated in a microwave oven for 6 minutes.
- 3) When the agarose was dissolved, the flask was cooled to below 60°C , $10\ \mu\text{l}$ 10mg/ml ethidium bromide was added into the solution, and the flask was shaken gently. The gel was then put into an electrophoresis apparatus filled with TBE buffer.

(3) Loading PM solution onto the gel

- 1) After vibration for six hours, the EP tubes were centrifuged for 1 minute, and $7\ \mu\text{l}$ dye (glycerol/bromophenol blue) was loaded into each tube and mixed with the solution.

- 2) From each EP tube, $20\ \mu\text{l}$ of the solution was added to a well of gel. The process was repeated one time for each grade of solution.
- 3) The gel was processed in the electrophoresis apparatus for 16 hours at 30 V.

(4) Gel imaging and plasmid DNA quantification

- 1) A Syngene Gene Tools ultraviolet imaging system was used to visualize and image the gel.
- 2) The optical density of DNA streaks was quantified, and the average of the oxidative damage rate of each sample concentration was calculated using the Syngene Gene Tools program.

2.5. Statistical analysis

A regression equation was plotted to calculate the dosage of PM (whole and soluble fraction) that would cause 40% (TD40) damage to the supercoiled form of plasmid DNA. Single-factor analysis of variance (ANOVA) was applied to identify significant differences between winter PM₁₀ TD40 in the moxibustion treatment room from that of the control sites. Pearson paired t-test was used to detect the bioactive difference between whole and the soluble fractions. Summer data was shown as (means $\pm$ SD); the sites were not compared, because of the small sample size: only three experiments were made during the summer.

3. RESULTS

3.1. Mass concentration of PM₁₀ at monitored sites

Figure 2 shows the average concentration of PM₁₀ at each site. Average PM₁₀ concentrations in the moxibustion room were $2.56\ \text{mg m}^{-3}$ in summer and $2.78\ \text{mg m}^{-3}$ in winter; in the general treatment room, $0.75\ \text{mg m}^{-3}$ and $0.91\ \text{mg m}^{-3}$ respectively; at the outdoor site, $0.83\ \text{mg m}^{-3}$ and $0.84\ \text{mg m}^{-3}$.

3.2. Oxidative DNA damage induced by PM₁₀ at monitored sites

Gel images and the average PM₁₀ TD40 of each site are shown in Figure 3 and Table 1, respectively.

The average PM₁₀ TD40 values, both whole and water-soluble fractions, from the moxibustion treatment room in summer are much higher than those of the control sites;

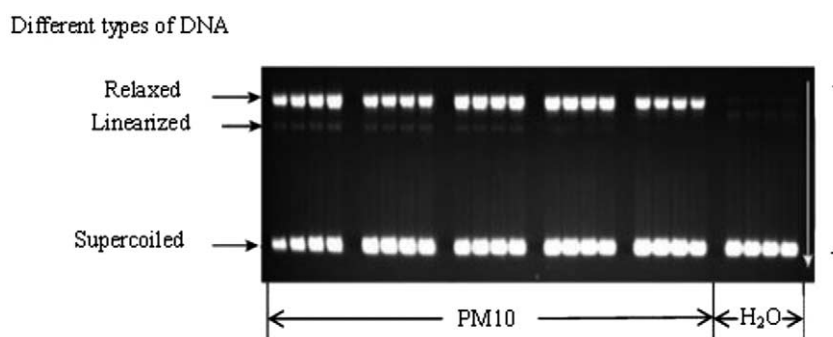


Figure 1. Experimental principle of plasmid DNA assay.

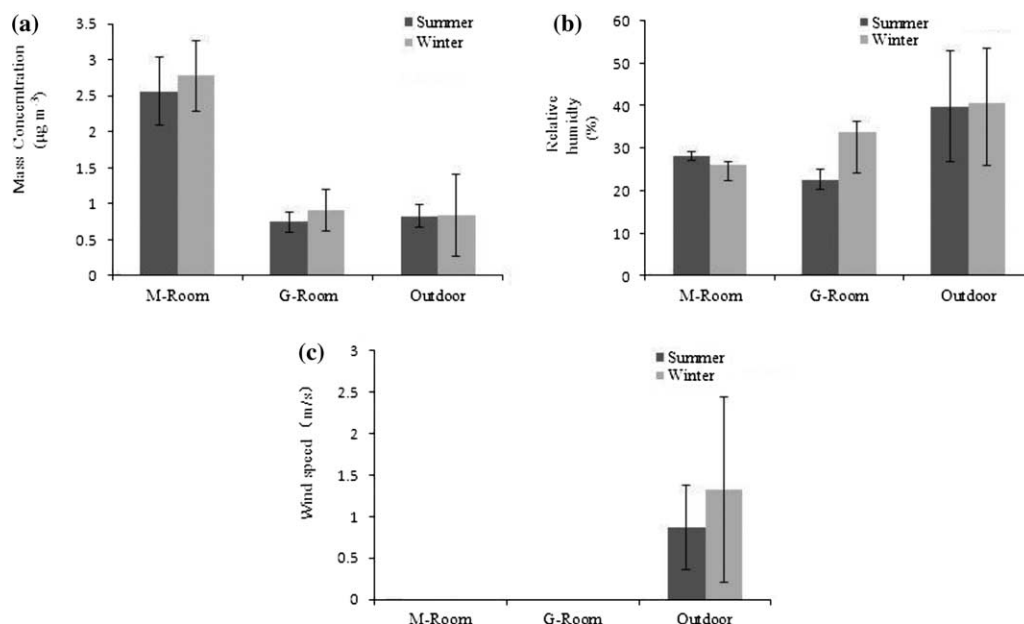


Figure 2. Sample information at monitored sites. M-Room, moxibustion treatment room; G-Room, general (moxa-free) treatment room.

winter values are significantly higher ($P < 0.001$) than the corresponding results from control sites. PM10 TD40 winter values of both fractions from the control sites are very similar to each other. ($P = 0.35$, $P = 1.00$ respectively). There was no statistical difference ($P = 0.06$) between the two winter PM10 fractions from the moxibustion treatment room, while those of the general treatment room ($P = 0.025$) and outdoor site ($P < 0.001$) were significantly different.

4. DISCUSSION

4.1. Mass concentration in the moxibustion treatment room

This is the first comprehensive air quality report in China that is based on PM10 concentration in a moxibustion treatment room. Our results show that the average mass concentrations observed in the moxibustion room in summer and winter are 2.56 mg m^{-3} and 2.78 mg m^{-3} , respectively, much higher than concentrations at the control sites. Because of a shortage of sampling equipment, sampling was not performed simultaneously in the two treatment rooms. Nevertheless, the data

shows that the mass concentrations in the general treatment room were relatively stable (Figure 2-a), making the concentrations in the rooms comparable. In addition, since only some outdoor particles penetrate the indoor environment, indoor sources often predominate in the indoor particle concentrations^[26–27, 9, 28–30]. Moxa smoke was the primary indoor source in the moxibustion room, as other factors such as wind speed (Figure 2-c) were almost the same at all sites. Thus the moxa smoke was the factor that increased PM10 concentration in the moxibustion room.

4.2. Oxidative capacity of PM10 samples from different monitored sites

Our study shows that PM10 TD40 values were much higher in the moxibustion room than at the control sites, indicating that when the PM10 burden was the same, the oxidative capacity of PM10 samples of both fractions was much lower in that room than in either control. This is an unexpected finding. However, there was no difference between the two control sites in PM10 bioactivity, which suggests that the PM10 components of the moxibustion room are different from those at the control sites.

Table 1. Summer and winter PM10 TD40 values

	Summer		Winter	
	Whole fraction ($\mu\text{g ml}^{-1}$)	Soluble fraction ($\mu\text{g ml}^{-1}$)	Whole fraction ($\mu\text{g ml}^{-1}$)	Soluble fraction ($\mu\text{g ml}^{-1}$)
General Treatment room	211.33 ± 11.02	342.00 ± 64.90	306.71 ± 120.23	$359.57 \pm 111.62^*$
Outdoor site	259.33 ± 102.77	451.67 ± 23.76	224.57 ± 50.36	$356.86 \pm 43.94^*$
Moxibustion Treatment room	791.67 ± 189.98	876.33 ± 146.87	$779.86 \pm 165.39^{***}$	$879.57 \pm 102.46^{***}$

Summer and winter data represent means $\pm$ SD from three and seven independent experiments respectively, each consisting of two duplicate samples. Due to the small sample size, the summer data was not analyzed statistically. $^{***}p < 0.001$, indicating statistically significant difference in winter PM10 values of whole fraction and water-soluble fraction between the moxibustion treatment room and each control site. $^*p < 0.05$, $^*p < 0.001$, indicating statistical differences between the two winter fractions from the general treatment room and outdoor site, respectively.

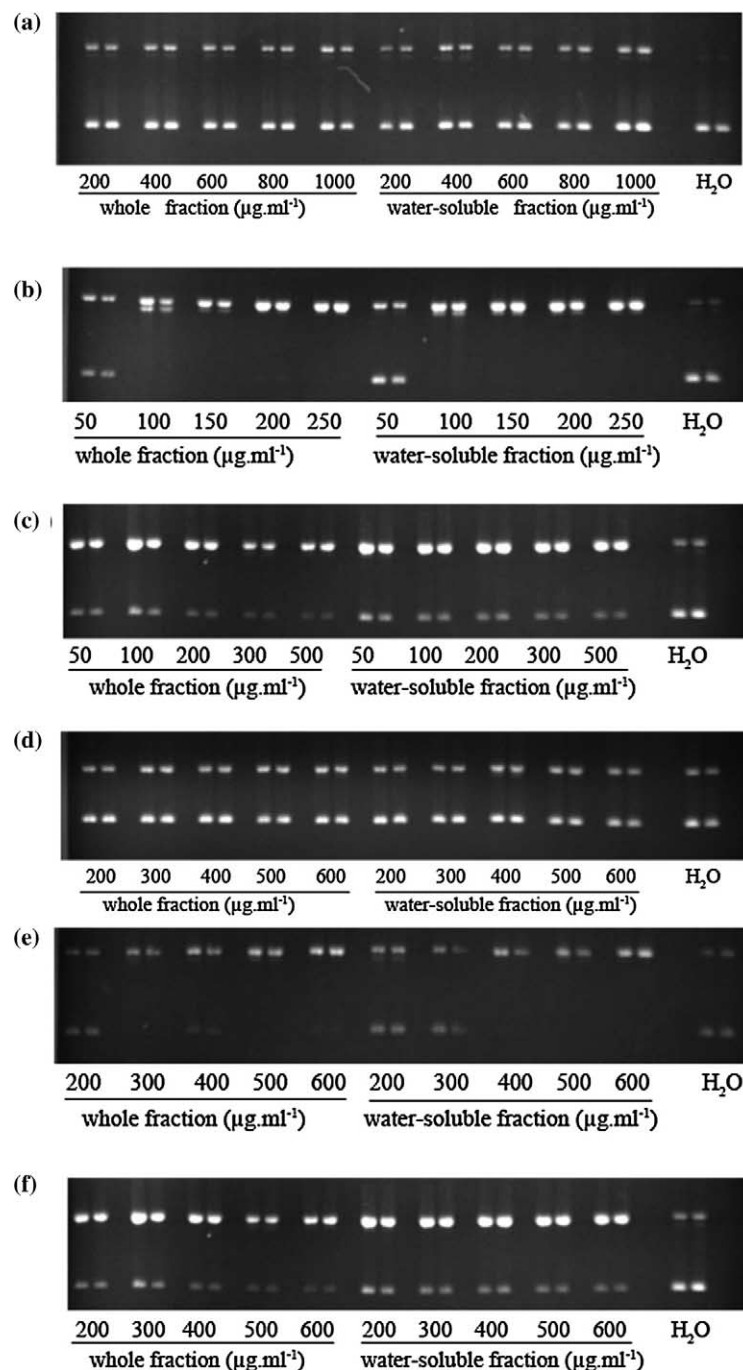


Figure 3. Gel image showing oxidative damage on supercoiled DNA induced by sample: (a) MS1; (b) GS2; (c) OS2; (d) MW2; (e) GW2; (f) OW1 MS, moxibustion treatment room summer; GS, general(moxa-free) treatment room summer; OS, out door summer; MW, moxibustion treatment room winter; GW, general (moxa-free) treatment room winter; OW, out door winter.

Furthermore, moxa smoke is the main cause of the difference between the treatment rooms, because all other indoor sources of PM10 and the relevant meteorological parameters are almost the same.

We conjecture that there may be several underlying causes of this difference in bioactivity: 1) the oxidative capacity of the components of moxa smoke is much lower than that of the air at the control sites; 2) moxa smoke is likely to reduce the capacity of PM10 to cause DNA oxidative damage by

scavenging free radicals in the air, and in fact it has been reported that moxa smoke does have that capability^[13].

4.3. Oxidative capacity of the whole and water soluble fraction of PM10 samples

In the present study, the differences in oxidative damage to supercoiled DNA between the two moxibustion room fractions collected in winter are insignificant, indicating that the oxidative capacity of the PM10 mainly derives

from the water-soluble fraction. It is widely considered that bioactive atmospheric particles predominantly belong to the water soluble fraction^[31, 32], although the insoluble fraction of atmospheric particles reportedly can trigger alveolus macrophage responses^[33–35]. We found significant differences among the three sites in the two fractions collected during winter, which clearly shows that the PM10 components in the moxibustion room are different from those at the control sites.

4.4. Oxidative capacity of PM10 samples in different seasons

Unfortunately, we could not compare the oxidative capacity of the PM10 samples from summer and winter due to the small summer sample size.

Moreover, this is only a preliminary evaluation of the air quality in a single moxibustion treatment room, and many problems remain. For example, more treatment rooms and more samples could provide more comprehensive and reliable data. Although the oxidative capacity of RP to affect plasmid DNA was much lower in the moxibustion room than at the control sites at the same concentration, the mass concentration in the moxibustion treatment room was much higher, so the safety of working and being treated in such a situation is still unknown. The safe concentration needs to be determined. We will investigate that and the antioxidant capability of moxa smoke to affect DNA damaged by PM10 in future studies.

5. CONCLUSIONS

The mass concentration of PM10 and its oxidative effects on plasmid DNA were evaluated in a moxibustion treatment room of Beijing, China. We determined that moxa smoke increases PM10 concentrations in a treatment room. However, the oxidative capacity of the PM10 in that room was much lower than that at the control sites. Moreover, PM10 bioactivity in the moxibustion room mainly derived from the water-soluble fraction. This was not the case at the control sites. We hypothesize that these differences result from low toxicity of the moxa smoke or from moxa's antioxidant activity. Clearly, further research is warranted.

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