Atmospheric Environment 122 (2015) 439–447

Contents lists available at ScienceDirect

Atmospheric Environment

journal homepage: www.elsevier.com/locate/atmosenv

Characteristics of bacterial and fungal aerosols during the autumn haze days in Xi'an, China

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HIGHLIGHTS

- On haze days, concentrations of viable bacteria and fungi were much higher than on non-haze days.
- There was different size distribution for airborne bacteria between the haze and non-haze days.
- For fungal aerosols, similar size distribution can be found between the haze and non-haze days.
- Compared to the non-haze days, some more allergic and infectious genera can be found during the haze days.
- More attention should be paid to the potential health risk related to bioaerosols during the haze days.

ARTICLE INFO

Article history:
Received 9 February 2015
Received in revised form
25 September 2015
Accepted 28 September 2015
Available online 1 October 2015

Keywords:
Bioaerosols
Concentration
Size distribution
Haze
Impactor-sampler

ABSTRACT

In recent years, haze pollution has become one of the most critical environmental issues in Xi'an, China, with particular matter (PM) being one of the top pollutants. As an important fraction of PM, bioaerosols may have adverse effects on air quality and human health. In this study, to better understand the characteristics of such biological aerosols, airborne microbial samples were collected by using an Andersen six-stage sampler in Xi'an from October 8th to 22nd, 2014. The concentration, size distribution and genera of airborne viable bacteria and fungi were comparably investigated during the haze days and non-haze days. Correlations of bioaerosol levels with meteorological parameters and PM concentrations were also examined. The results showed that the daily average concentrations of airborne viable bacteria and fungi during the haze days, 1102.4–1736.5 and 1466.2–1703.9 CFU/m^3, respectively, were not only much higher than those during the non-haze days, but also exceeded the recommended permissible limit values. Comparing to size distributions during the non-haze days, slightly different patterns for bacterial aerosols and similar single-peak distribution pattern for fungal aerosols were observed during the haze days. Moreover, more allergic and infectious genera (e.g. *Neisseria*, *Aspergillus*, and *Paecilomyces*) in bioaerosols were identified during the haze days than during non-haze days. The present results reveal that bioaerosols may have more significant effects on public health and urban air quality during the haze days than during non-haze days.

1. Introduction

With the acceleration of Chinese urbanization and industrialization recently, the urban population, the energy consumption and the number of traffic vehicles have greatly increased. These result in the increase of industrial and traffic emissions from fossil fuel combustion and biomass burning, and soil dust resuspension from intense construction operations. As a result, the air quality has become more and more deteriorated in Chinese metropolitan areas (Chan and Yao, 2008; Zhang et al., 2012; Cheng et al., 2013b). As the largest city in northwestern China with a population of 8.468 million and more than 1.60 million vehicles, Xi'an is also experiencing heavy air pollution and frequent hazy events. According to the official report (Xi'an Environmental Protection Bureau, 2014), the number of haze days in Xi'an was about 155 days in 2014, and 14 haze days only in October.

Haze is a weather phenomenon, characterized by atmospheric

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http://dx.doi.org/10.1016/j.atmosenv.2015.09.070
1352-2310/

visibility of less than 10 km and a relative humidity (RH) of lower than 90% (China Meteorological Administration, 1979). The fine particulate matters ($PM_{2.5}$) play an important role in the formation of haze (Watson, 2002). Since it affects not only air quality and public health, but also cloud formation and even global climate, the haze days have been attracting wide attentions of people and society (Okada et al., 2001; Yu et al., 2011). Numerous studies have been conducted to investigate the physical and chemical properties, source apportionment and evolution processes of atmospheric PM during the haze events in Chinese cities, such as Beijing (Quan et al., 2014), Shanghai (Yang et al., 2012), Guangzhou (Tan et al., 2009) and Xi'an (Cheng et al., 2013a). However, little is known until now about the biological properties of PM during haze episodes.

Biological particles in the atmosphere, often defined as bioaerosols, are airborne particles or large molecules carrying living organisms or released from living organisms (e.g., bacteria, fungi, viruses, pollen) (Ariyap and Amyot, 2004). Bioaerosols originate from almost any environmental reservoir for microorganisms, such as fresh and marine surface waters, soil, plants, bioreactors, wastes, animals and human. Bioaerosols can contribute as much as 25% to the atmospheric aerosols (Jaenicke, 2005). The concentration and size distribution of bioaerosols greatly vary in different environments, depending on such biotic and abiotic factors as the type of microorganism species, environmental conditions, and human activities (Jones and Harrison, 2004; Nasir and Colbeck, 2010; Haas et al., 2013; Li et al., 2013, 2015). Exposure to bioaerosols is associated with a wide array of adverse health effects, including infectious diseases, acute toxic effects, allergies and cancers (Ross et al., 2000; Douwes et al., 2003; Goldman and Huffnagle, 2009; Bolashikov and Melikov, 2009). Despite the importance of bioaerosols from the perspective of public health and atmospheric sciences, the practical measurements of bioaerosols are not yet sufficient, especially for special events (Heo et al., 2014). In particular, the quantitative measurement data for haze events are very scarce (Cao et al., 2014). Therefore, it is essential to characterize the differences in properties of bioaerosols between haze and non-haze episodes.

In this study, fieldwork was conducted to collect bioaerosol samples during both haze and non-haze days from Oct. 8th to 22nd, 2014, in Xi'an, China. The properties of bioaerosols under the two conditions, such as concentration, size distribution and genera, were characterized and compared. The objective of this study is to get knowledge of bioaerosol characteristics during haze days in Chinese urban areas. The results can provide valuable data for hazard evaluation of bioaerosols on human health and for future establishment of Chinese official standard of ambient air quality.

2. Materials and methods

2.1. Sampling sites

The field sampling was carried out on the roof of the School of Environmental Science and Engineering building of Chang'an University from Oct. 8th - Oct. 22nd, 2014 in Xi'an, China (34°26′N, 108°94′E and 424 m above sea level). The building is approximately 22 m above the ground, which is situated between the 2nd and 3rd ring roads in Xi'an. The distance of the site from nearby major roads is about 400 m. The site is surrounded by residential and school buildings. There are no nearby industrial emission sources.

Xi'an is located in the centre of the Guanzhong Plain with an area of 39,064 km^2, surrounded by the Loess Plateau and Qinling Mountain. As a typical semi-arid inland city, Xi'an has distinct seasonal variations in meteorological conditions: hot and humid in summer and cold and dry in winter. The annual average temperature is 13.0–13.4 °C and the annual precipitation is 558–750 mm. The prevailing wind direction is North-East (12%) and East–North–East (8%).

2.2. Instruments and measurement

An Andersen six-stage samplers (Westech, UK) with six glass Petri dishes of 93 mm in diameter was employed to collect bacterial and fungal aerosols with different size ranges, respectively (Andersen, 1958). The range of aerodynamic diameter at each stage was: ≥7.0 μm (stage 1), 7.0–4.7 μm (stage 2), 4.7–3.3 μm (stage 3), 3.3–2.1 μm (stage 4), 2.1–1.1 μm (stage 5) and 1.1–0.65 μm (stage 6). Fine particles were defined here as particles with aerodynamic diameter less than 2.1 μm (Yu et al., 2011; Quan et al., 2014), corresponding to the size range between stages 5–6.

The sampler was installed on the rooftop of the building at a height of about 1.0 m above the floor surface. The bioaerosol samples were collected in triplicate at a flow rate of 28.3 L/min for about 10 min each at two time intervals (8:00am and 1:00pm) during each sampling day. Before each sampling, the sampler was disinfected with 75% ethanol. After the alcohol thoroughly evaporated, agar plates were loaded to the sampler inside of a Class II biosafety cabinet (BSC-1500, Biobase, China). The sampler was then carried in a dust-free box to the sampling sites. Nutrient agar (3 g beef extract, 10 g peptone, 5 g sodium chloride, 15 g agar, 1000 ml distilled water, pH = 7.2, and 500 mg cycloheximide for inhibition of fungal growth) and Sabouraud dextrose agar (40 g glucose, 10 g peptone, 20 g agar, 1000 ml distilled water, pH = 6.2, 100 mg chloramphenicol for inhibition of bacterial growth) were used as bacterial and fungal culture medium, respectively (Fang et al., 2008). After sampling, the agar plates were immediately transported to the laboratory for further incubation. Bacterial samples were incubated at 37 °C for 48 h and fungal samples were incubated at 25 °C for 72 h. It is worth noting that the microorganism can be categorized into psychrophiles, mesophiles and thermophiles according to the optimal temperature of their growth and survival. The pathogens are all subject to mesophiles and the corresponding optimal temperature is in the range of 20–40 °C. Therefore, these temperatures and incubation time were employed in the laboratory procedure.

The concentration of $PM_{2.5}$ was concurrently determined by the portable Haze-dust EPAM-5000 particulate monitor (SKC Inc., USA). The meteorological data during the sampling period, including ambient temperature, RH, wind speed and direction, solar radiation and atmospheric visibility were simultaneously recorded by a portable automatic meteorological station (JLC-QGL, China).

2.3. Microbial identification

After incubation, the colonies were counted followed by the positive-hole correction method to correct for colony overlapping. Each concentration of bioaerosols, generally expressed as total colony-forming units (CFU/m^3) for respective particle size, was then calculated by dividing the number of colonies formed on the culture medium at each stage by the sampling air volume.

According to Kim and Kim (2007), the genera of all the cultured airborne bacteria were identified according to the classification method of Bergey's manual and, after dying the bacteria by Gram's method, additional identification was carried out by conducting biochemical test through the Biolog Microstation System (Biolog, Hayward, USA). The airborne fungal genera were identified according to the classification methods of Ainsworth and Baron by observing the form, shape and color of colony and spore through the optical microscope (Kirk et al., 2001; Murray et al., 2003). Although this identification method is susceptible to human optical and interpretation error, it is the widely used analytic method in bioaerosol research and was adopted in this study due to the advantages of simplicity, cost-effectiveness and high efficiency.

2.4. Data analysis

The Air Quality Index (AQI) is a simple and generalized way to assess the air quality in China. It derives from the concentrations of six major air pollutants: $PM_{2.5}$, PM_{10}, SO_2, NO_2, CO and O_3. AQI ($PM_{2.5}$) is assigned to the level of $PM_{2.5}$ in this study. When the value is > 100 (the limit of Chinese air quality standards Grade II), the environmental condition can be defined as a haze pollution.

The mean and standard deviation of the concentration data were calculated. The concentration differences at different days and in different size ranges were also compared by using Student's t-test, in which p values lower than 0.05 were considered to be statistically significant. In addition, number median diameter (NMD) and geometric standard deviation (GSD) based on the cumulative size distributions were calculated to describe the size distribution. Pearson's correlation analysis was performed to examine the statistical significance of correlation of viable bioaerosols with meteorological parameters and PM concentrations.

3. Results

3.1. General characteristics of haze days

Table 1 shows the variations of $PM_{2.5}$ concentrations, AQI ($PM_{2.5}$), visibility and meteorological parameters during the sampling period. As mentioned above, air quality can be considered as a pollution haze when AQI ($PM_{2.5}$) is > 100 in China. Additionally, the haze days are generally referred to days with atmospheric visibility less than 10 km and RH below 90% according to China Meteorological Administration. Evidently, the visibility was much lower from October 8th to October 10th, and from Oct. 18th to Oct. 22nd than that from Oct. 11th to Oct. 14th. Moreover, the AQI ($PM_{2.5}$) values in Xi'an were larger than 100 during these time. Therefore, it is considered that the city of Xi'an experienced two continuous haze events: Oct. 8–10 and Oct. 18–22, 2014.

During the haze days, the daily average concentrations of $PM_{2.5}$ ranged from 119.0 ± 14.4 to 214.7 ± 10.0 $\mu g/m^3$ and were significantly higher than Chinese daily average standard of 75 $\mu g/m^3$. Further, $PM_{2.5}$ concentrations during the haze days were 3–6 times higher than those during the non-haze days (31.3 ± 4.7 to 53.7 ± 10.5 $\mu g/m^3$). The RH also increased contemporaneously, indicating that both aerosol concentrations and RH had strong impact on the reduction of visibility, causing the occurrence of the haze events. Another important feature was that the wind speeds were often less than 2 m/s, implying that the horizontal transport of aerosols was very weak. It is also found that the solar radiation was lower during the haze days than that during the non-haze days. This can be explained by the fact that solar radiation was absorbed or scattered by aerosols on haze days.

Fig. 1 shows the surface weather pattern over the eastern Asia on Oct. 8th and 11th, 2014. It can be seen from Fig. 1(a) that the Guanzhong Plain region was under the control of a high-pressure system and the large scale stagnation, as indicated by the large spacing between the isobars, at 08:00 LST on Oct. 8th, 2014. The surface pattern on Oct. 8–10 and Oct. 18–22 remained similar to what is shown in Fig. 1(a). This weather system would lead to a lower surface wind speed and stably stratified atmosphere, which is favorable for the accumulation of air pollutants. A cold front passed through Xi'an On Oct. 11th. As seen in Fig. 1(b), the wind direction changed to easterly, wind speed and visibility increased, and precipitation occurred in the evening, which helped to terminate this haze event between Oct. 8–10, 2014.

3.2. Concentrations of airborne bacteria and fungi

The daily average concentrations of airborne viable bacteria and fungi during the sampling period are shown in Fig. 2. The concentrations of bacterial aerosols during the haze days, 1102.4–1736.5 CFU/m^3, were significantly higher than those during the non-haze days, 497.7–629.0 CFU/m^3 ($^*p < 0.05$). Similar to the bacterial aerosol concentrations, the concentration of fungal aerosols during the haze days, 1466.2–1703.9 CFU/m^3, were significantly higher than those during the non-haze days, 247.6–398.4 CFU/m^3 ($^{**}p < 0.01$). These results exhibited the significant increase of the fungal and bacterial concentrations during the haze events.

3.3. Size distributions of airborne bacteria and fungi

Fig. 3 shows the size distribution of airborne viable bacteria detected in this trial. On non-haze days, the dominant stages for airborne viable bacteria were stage 4, stage 3, and stage 2 with the fractions of 25.6 ± 6.7%, 23.8 ± 8.3%, and 20.4 ± 3.9%, respectively, while the lower proportions of airborne bacteria were detected on stage 5 and stage 6 with each fraction of less than 10% ($^*p < 0.05$). Statistically, no significant differences in airborne bacteria were found among the three stages 4, 3, and 2 ($p > 0.05$), and also no difference between stages 5 and 6 ($p > 0.05$). In contrast, the highest proportion of airborne bacteria during the haze days (25.0 ± 6.8%) was in the size range of 1.1–2.1 μm (stage 5) ($^*p < 0.05$), while the lowest proportion (12.1 ± 4.7%) was detected on stage 6 (0.65–1.1 μm). The results demonstrated a somewhat difference in distribution patterns of airborne bacteria during the haze and non-haze days.

Fig. 4 illustrates the size distribution of airborne viable fungi detected in this trial. Similar size distribution can be found between

Table 1
Variations of $PM_{2.5}$ concentration, AQI ($PM_{2.5}$), visibility and meteorological parameters during sampling period.

Date	WS (m/s)	T (°C)	RH (%)	SR (W/m^2)	Visibility (km)	$PM_{2.5}$ ($\mu g/m^3$)	AQI	Weather
2014/10/8	0.52 ± 0.35	18.40 ± 3.62	82.68 ± 11.24	3.5755 ± 5.3074	1.7	210.0 ± 7.8	264	Haze
2014/10/9	1.83 ± 0.89	20.60 ± 3.96	71.43 ± 15.55	5.4298 ± 7.6000	2.0	214.7 ± 10.0	267	Haze
2014/10/10	0.57 ± 0.37	20.31 ± 3.62	63.57 ± 9.23	5.5550 ± 7.8851	2.7	119.0 ± 14.4	156	Haze
2014/10/11	2.50 ± 0.49	15.74 ± 1.84	76.98 ± 16.21	2.9771 ± 2.6010	9.3	53.7 ± 10.5	94	Non-haze (rainy)
2014/10/12	2.26 ± 1.00	11.10 ± 0.70	74.69 ± 5.96	3.0541 ± 1.7324	10.6	31.3 ± 4.7	45	Non-haze (rainy)
2014/10/13	1.95 ± 0.48	12.96 ± 3.51	71.38 ± 11.24	12.8797 ± 8.2392	10.4	35.7 ± 11.7	51	Non-haze
2014/10/14	1.14 ± 0.44	12.50 ± 6.88	72.53 ± 17.98	16.7346 ± 9.7718	10.1	37.3 ± 7.0	68	Non-haze
2014/10/18	1.18 ± 0.67	16.58 ± 3.92	77.79 ± 13.13	4.2821 ± 6.9321	2.8	125.3 ± 11.7	165	Haze
2014/10/19	0.83 ± 0.61	17.77 ± 3.30	84.43 ± 13.40	5.9105 ± 8.5088	2.5	136.7 ± 4.2	181	Haze
2014/10/22	0.80 ± 0.42	13.73 ± 2.35	83.45 ± 15.68	7.6808 ± 10.6721	1.3	162.3 ± 12.3	112	Haze

WS: wind speed, T: temperature, RH: relative humidity, SR: solar radiation.

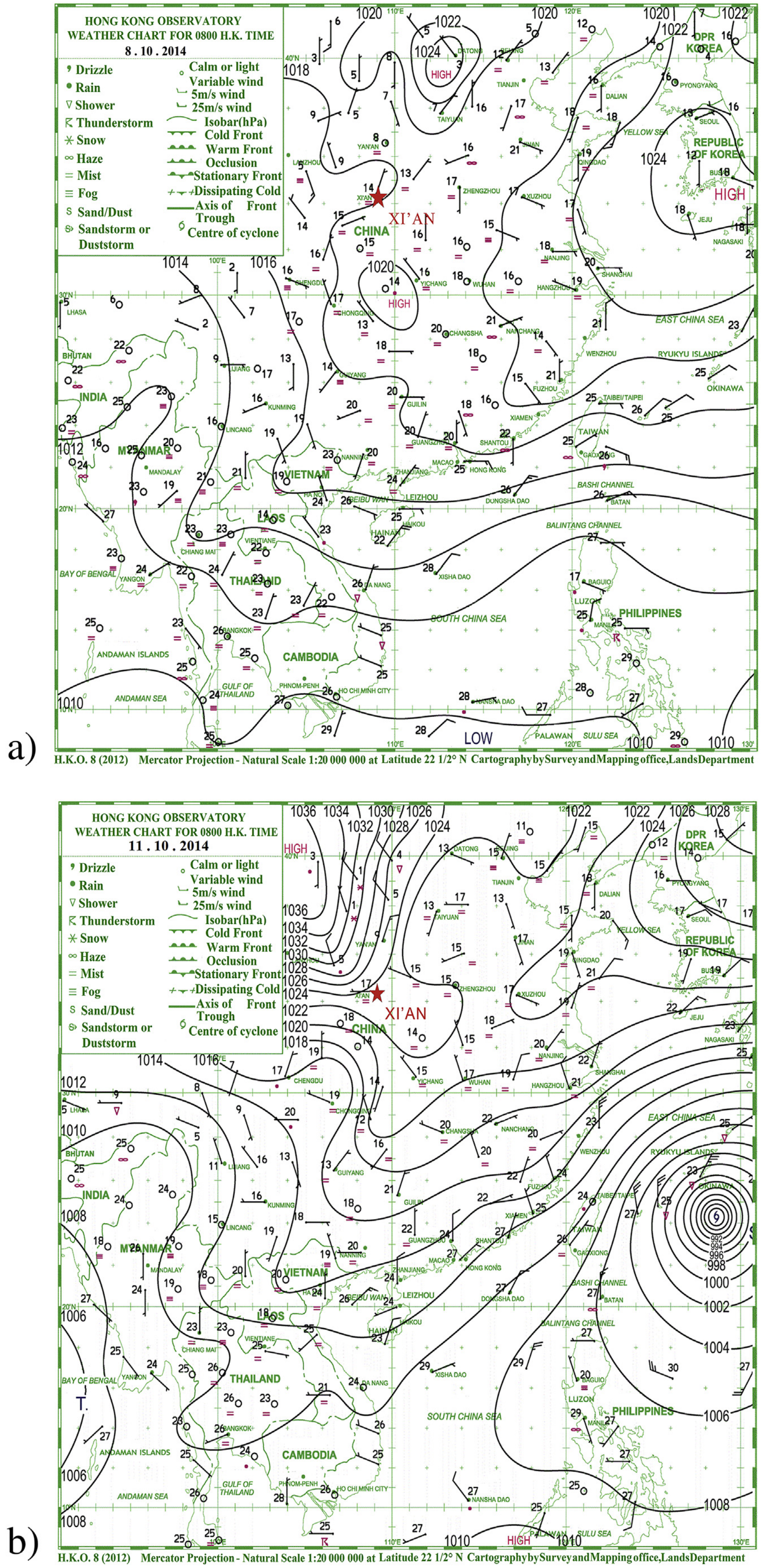


Fig. 1. Surface weather patterns over the eastern Asia at 0800 LST on a) October 8th, 2014 and b) October 11th, 2014.

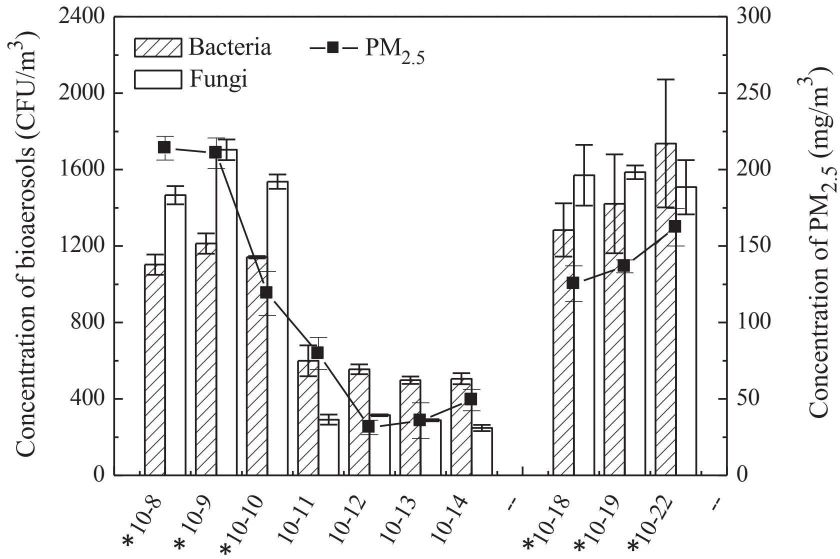
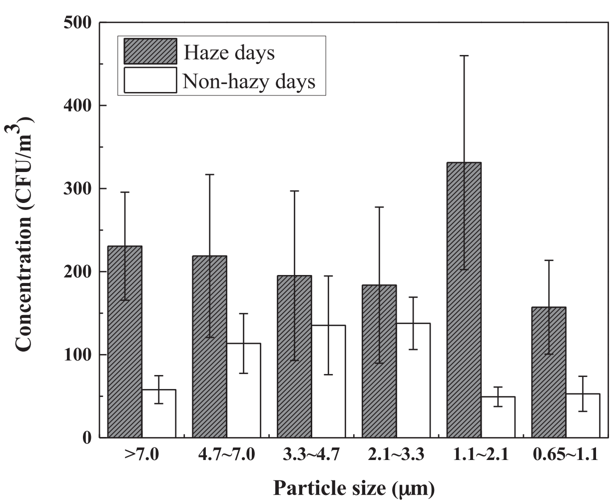
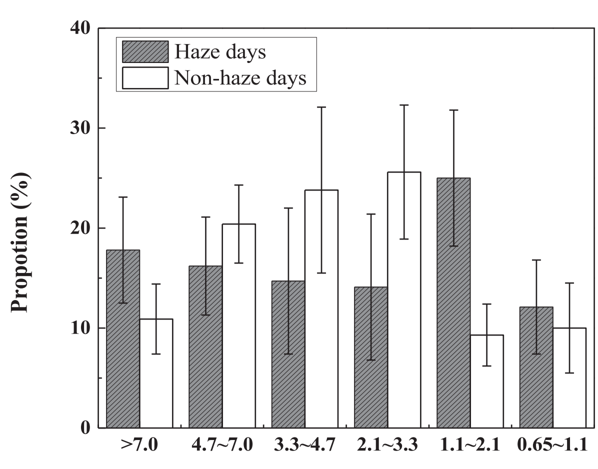


Fig. 2. Daily average concentrations of bacterial and fungal aerosols and concentrations of $PM_{2.5}$ in Xi'an during October 8–22, 2014 (* represents the days with haze).



(a) Size distribution by number



(b) Size distribution by number frequency

Fig. 3. Size distributions of airborne bacteria detected in Xi'an during October 8–22, 2014.

the haze and non-haze days. On haze days, the highest proportion was found in the size range of 4.7–3.3 μm with fraction of 29.4 ± 4.1%, while the lowest proportion was on stage 5 with fraction of 8.2 ± 3.2% (*$p < 0.05$). On non-haze days, the highest collection rates were on stage 2 and stage 3, while the lowest proportions were on stage 5 and stage 6 (*$p < 0.05$). There were no statistically significant differences between stages 2 and 3 ($p > 0.05$), and between stages 5 and 6 ($p > 0.05$).

3.4. Composition of airborne bacteria and fungi

Table 2 represents the relative abundance of airborne viable bacteria and fungi genera in the samplers collected during the haze and non-haze days. *Staphylococcus* and *Micrococcus* were detected on both the haze and non-haze days, and their percentage of the total airborne bacteria were 50.0% and 16.7% on haze days, 60.9% and 34.8% on non-haze days, respectively. Another bacterial genus, *Neisseria*, was detected at 25.0% of the total airborne bacteria only on haze days.

For airborne fungi, *Aspergillus* and *Aureobasidium* were the dominant genera during non-haze days, accounting for 84.4% of the total airborne fungi, followed by *Penicillium* with 12.5% of the total. In contrast, *Aspergillus* showed the highest count, constituting 65.1% of the total fungal genera during the haze days. The second most common fungal aerosol was *Paecilomyces*, constituting 24.4% of the total fungi. Others like *Penicillium* and *Cephalosporium*, had a lower relative abundance of less than 5%.

4. Discussion

Airborne particles are recognized to have significantly important impact on air quality and public health. Numerous studies have revealed the elevated concentration of most airborne particles, including $PM_{2.5}$ and PM_{10}, during the haze days (Watson, 2002; Tan et al., 2009; Yang et al., 2012; Cheng et al., 2013a; Quan et al., 2014). However, it is still not clear whether the concentrations of bioaerosols increases during the haze days. In this trial, we first examined airborne microbe levels during the haze days and non-haze days. Our measurements clearly showed that the airborne viable bacterial and fungal concentrations during the haze days were approximately 2–4 times and 4–7 times higher than those

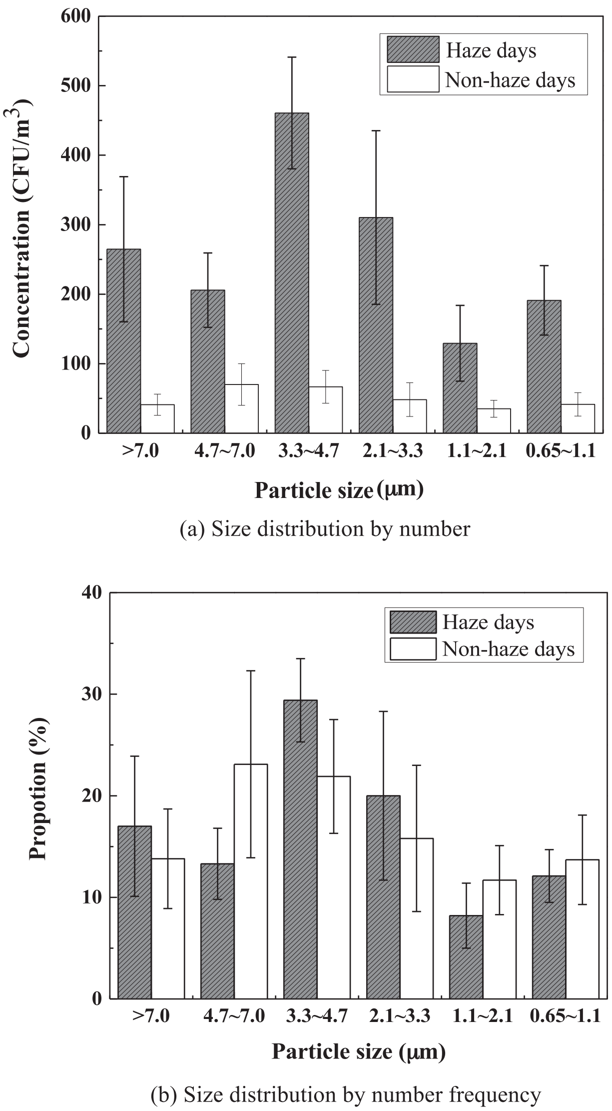


Fig. 4. Size distributions of airborne fungi detected in Xi'an during October 8–22, 2014.

during the non-haze days, respectively, suggesting that the concentrations of fungal and bacterial aerosols increased significantly during haze events.

Table 3
Spearman's correlation coefficients between meteorological parameters, visibility and AQI ($PM_{2.5}$) and the concentration of airborne bacteria and fungi from total concentration, coarse particle and fine particle concentration.

	T	RH	Solar radiation	Visibility	AQI ($PM_{2.5}$)
Bacteria					
B_{coarse}	0.016	0.478	−0.489	−0.657*	0.808**
B_{fine}	−0.055	0.418	−0.335	−0.724**	0.615*
B_{total}	0.088	0.500	−0.467	−0.635*	0.786**
Fungi					
F_{coarse}	−0.027	0.330	-0.291	−0.578*	0.571*
F_{fine}	0.151	0.801**	-0.316	−0.658*	0.902**
F_{total}	0.176	0.533	-0.423	−0.567*	0.902**

* represents $p < 0.05$ (2-tailed), ** represents $p < 0.01$ (2-tailed).

Although there are no similar studies on airborne viable microorganisms during the haze events, a comparison with previous studies on sandstorm events will be helpful to uncover the reason of high concentration of bioaerosols during the haze days. Li et al. (2011) observed that the concentrations of airborne viable microbes during a sandstorm and a non-sandstorm day were 830 and 372.5 CFU/m^3 in the Qingdao coastal region in Mar. 2010, respectively. Griffin et al. (2003) founded 19 bacteria and 28 fungi in a total of 3652 L air during the non-dust conditions, while during the dust conditions 171 bacteria and 76 fungi were recovered in a total of 2369 L air in the northern Caribbean. The atmosphere does not constitute an environment where microbes can grow and develop, but is a means for rapid dispersion of many types of microbes. Dust particles or fine particles could act as carriers for those microbes and can provide them nutrition, leading to elevated concentrations of airborne microorganisms. Therefore, bioaerosol concentrations increased greatly during the sandstorm. During the haze events, much more number of fine particles, relatively higher humidity and lower solar radiation were observed comparing to sandstorm events, which would provide a more favorable condition for microbial proliferation and growth. As a result, it is not surprising that an increase of viable microbe concentrations in ambient air was found during the haze days.

The above explanation can be also supported by correlations of meteorological parameters, visibility and AQI ($PM_{2.5}$) with the concentrations of airborne bacteria and fungi, shown in Table 3. The concentrations of viable microbes are positively correlated with the RH and AQI ($PM_{2.5}$), while negatively correlated with visibility and solar radiation. The significant positive correlation between the bioaerosols and $PM_{2.5}$ concentrations is consistent with

Table 2
Viable bacterial and fungal genera identified on haze and non-haze days.

	Non-haze days		Haze days	
	% Of total bacterial or fungal genera	% Occurrence	% Of total bacterial or fungal genera	% Occurrence
Bacteria				
Staphylococcus	60.9	100	50.0	100
Micrococcus	34.8	100	16.7	100
Neisseria	0.0	0.0	25.0	100
Unidentified	4.3	50	8.3	50
Fungi				
Aspergillus flavus	28.1	100	29.1	100
Aspergillus Japonicus	6.3	100	0	0
Aspergillus niger	3.1	50	36.0	66.7
Aureobasidium pullulans	46.9	100	0	0
Penicillium Chrysogenum	12.5	50	0	0
Penicillium patulum	0.0	0.0	4.7	33.3
Cephalosporium acremonium	0.0	0.0	3.5	33.3
Paecilomyces variotii	0.0	0.0	24.4	33.3
Unidentified	3.1	50	2.3	33.3

observations reported by Boreson et al. (2004) and Adhikari et al. (2006), suggesting that the level of airborne viable microbes increases with an increase of $PM_{2.5}$ concentrations. The correlation analysis results also indicate that a higher RH is associated with a higher airborne bacterial and fungal concentration. According to Heo et al. (2014), moisture in ambient air may alter integrity of cell walls or viral coats, and thereby humid condition may facilitate the growth and survival of airborne microorganism during the haze events. Solar radiation is an effective sterilization method for bioaerosols (Hwang et al., 2010), and thus reduced die-off rates due to reduction in solar radiation may cause the accumulation of microbes during the haze events.

It is well known that aerosols containing microorganisms can undergo long-range transport from their sources (Brown and Hovmøller, 2002; Prospero et al., 2005). Therefore, input of microorganisms from transportation paths may be another factor leading to the high concentration of bioaerosols during the haze days. In order to characterize the transport pathway of the air parcels that passed through Xi'an, backward trajectory analysis was carried out by HYSPLIT-4 in the present study (HYSPLIT Model; Draxler and Rolph, 2011; Rolph, 2011). Fig. 5 presents the result of 72-h back trajectory during the haze and non-haze days. It clearly shows that air mass arriving at Xi'an on October 8th came from the two main pathways: the southwest and southeast direction, instead of Guanzhong Plain (east-west direction). Similarly, air mass arriving at Xi'an on October 18th did not come from the Guanzhong Plain either. These results imply that except for local sources, bioaerosols detected in the present campaign may not come from the Guanzhong Plain during the haze periods of Oct. 8–10 and Oct. 18–22, 2014.

There are currently no official reference concentrations for ambient fungal and bacterial aerosols in outdoor air in China, even in Europe and the US. Therefore, it is not easy to evaluate the present level of air contamination with microorganisms in Xi'an. Nevertheless, China Scientific Ecology Center (CSEC) recommended that the guideline values should be less than 1000 CFU/m^3 for total airborne bacteria and less than 500 CFU/m^3 for airborne fungi, respectively. It is clear that the concentrations of bacterial and fungal aerosols during the non-haze days do not exceed the CSEC guidelines. In contrast, all of those bioaerosol concentrations detected during the haze days exceed the CSEC guidelines. Such a comparison suggests that the high concentrations of bioaerosols during the haze days may have great potential to threaten the health of the residents in Xi'an.

Effects of bioaerosols on human health depend not only on their concentration, but also on their size distribution because bioaerosols with different aerodynamic diameters may be deposited in different positions of the respiratory system and result in various respiratory illnesses (Thomas et al., 2008). The present study shows that the majority of airborne bacteria and fungi (about 79.7% and 74.6%, respectively) during the non-haze days exists in coarse size range (>2.1 μm). This distribution feature is comparable to previous results reported in normal outdoor environments. For example, the majority of airborne bacteria in coarse size range (>2.1 μm) were also reported by Fang et al. (2008), Xu and Yao (2013) and Li et al. (2015). In addition, Zuraimi et al. (2009) concluded that the highest distribution of outdoor airborne fungi in Singapore ranged from 2.1 to 3.3 μm; whereas Fang et al. (2005) found that the highest proportion of culturable fungi in Beijing was detected in stage 4 (2.0–3.5 μm). The maximum proportion of airborne terrestrial and marine fungi in Qingdao, China lied in the range of 2.1–3.3 μm, whereas the minimum proportion was in the range of 0.65–1.1 μm (Li et al., 2011). The above data, together with our results, imply that the abundance of bacterial and fungal aerosols in the coarse size fraction (>2.1 μm) seems independent on geographical factors. The size distribution feature is most likely because bioaerosols may appear as agglomerates or attached to other non-biological matrices rather than single cells (Fang et al., 2008).

It is interesting that the distribution pattern of bacterial aerosols during the haze days is slightly different from that during the non-haze days. The difference is that there exists a trend of concentration peak shifting to fine size range during the haze days. However, there is little difference in the distribution pattern of fungal aerosols. This can be also reflected by the number median diameter of airborne bacteria and fungi. The NMD and GSD of bacterial and fungal concentrations were 1.96 ± 0.29 μm and 2.44 ± 0.23 μm during the haze days, respectively, while both values were 2.32 ± 0.12 μm and 2.48 ± 0.24 μm during non-haze days, respectively. The relatively smaller NMD of airborne viable bacteria during the haze days may be due to more free-floating individual cells and a lower number of larger agglomerates. Another reason may be due to new formation of atmospheric fine particles. As discussed earlier, $PM_{2.5}$ concentrations during the haze days are 3–6 times higher than those during the non-haze days. The higher $PM_{2.5}$ concentrations indicate more carriers for airborne microorganisms and nutrients for their growth, resulting in the elevated percentage of bacterial particles presented in the fine size range.

Humidity of ambient air is another factor that can influence the size of bioaerosols (Kim and Kim, 2007; Nasir and Colbeck, 2010). The high RH on haze days can promote hygroscopic growth of bioaerosols by condensation or water absorption. The size

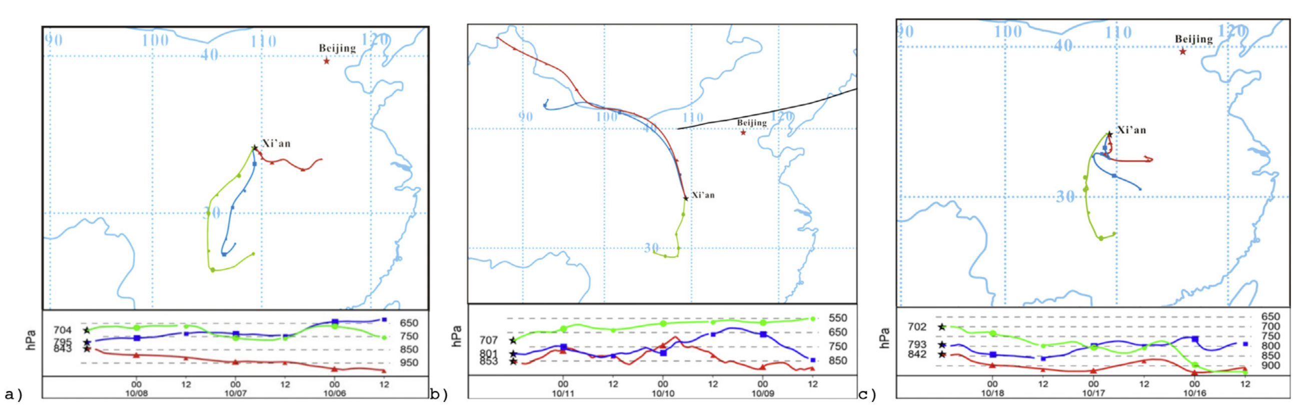


Fig. 5. Cluster analysis of 72 h backward trajectory of air mass arriving at Xi'an at 20:00 on (a) Oct. 8th, (b) Oct. 11th and (c) Oct. 18th, 2014 (red, blue and green represent air track at height of 500 m, 1000 m and 2000 m, respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

distributions of bioaerosols also depend on genus and the spores age. Reponen (1995) indicated that the aerodynamic sizes of the freshly released spores were larger than size of spores which have been airborne for a longer time. Unfortunately, the present study can not provide evidences for the above viewpoint due to the lack of information on the age and nutrient sources. We will focus on this phenomenon in our future studies.

Besides the concentration and size distribution, genera or species composition also play important role in assessment of human exposure to airborne microorganisms. The abundance of airborne bacteria and fungi during the non-haze days has a little difference with previous reports. Pastuszka et al. (2000) found that *Micrococcus* spp. and *Staphylococcus epidermidis* were dominant bacteria in indoor and outdoor environments in Upper Silesia, Poland, both of which contributed 50% of the total bacteria concentration. Fang et al. (2005) carried out a systematical survey on the culturable airborne fungi for 1 year in Beijing urban area and found that the prevalent fungal groups in the outdoor environments of Beijing were *Cladosporium*, *Alternaria*, *Pencillium* and *Asperigillus*. The dominant genera identified inside and outside the public buildings of Korea were *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., and *Bacillus* spp., for airborne bacteria and *Penicillium* spp., *Cladosporium* spp., and *Aspergillus* spp., for airborne fungi, respectively (Kim and Kim, 2007). Although *Corynebacterium, Bacillus, Cladosporium* and *Alternaria* were not detected in the atmosphere in Xi'an, our result is consistent with other studies in China (Zhai et al., 2000; Ju et al., 2003). It may be due to differences in genera sources, sampling methods and sampling environments. Singh et al. (1990) indicated that some of airborne microbes showed seasonality and in particular, *Cladosporium* was predominant during winter months, *Alternaria* during summers, while *Penicillium* species dominated in autumn. Furthermore, *Aspergillus*, *Penicillium,* and *Cladosporium* were considered to be closely related to local microenvironments and urbanization (Awad, 2005). It was worthy to note that the present results were obtained based on a short sampling period (less than 20 days only in October) and using a sampling method for only viable microbes. Therefore, the present distribution pattern for airborne bacterial and fungal genera seems to be reasonable.

It is important to point out that the residents in Xi'an were exposed to more allergic and infectious bioaerosols during the haze days in comparison to the non-haze days. Two Gram positive bacteria (*Staphylococcus* and *Micrococcus*) were identified during the non-haze days, while a Gram negative bacteria, *Neisseria*, was identified during the haze days. Some species of *Neisseria* are known to be pathogens and may cause meningitis and gonorrhea (Tsai, 2011). Moreover, exposure to the most prevalent fungi species identified during the haze days (i.e. *Aspergillus* and *Paecilomyces*) has been strongly associated with allergic and infectious respiratory diseases, such as asthma and pneumonia (Douwes et al., 2003). Accordingly, a further study should examine the relationship between exposure to airborne microbes and occurrence of respiratory diseases on haze days.

It is worth noting that the number of microorganisms in bioaerosols reported in this study is only for culturable ones on selective media, probably accounting for only ~1% of the total microbes. Moreover, culture-dependent techniques do not allow for the characterization of damaged, stressed, or nonviable microbes and their byproducts (Chi and Li, 2007; Lighthart, 2000). As a result, the number and type of microorganisms presented in ambient air may be underestimated in this study. Nonetheless, it is still expected from the present results that exposure to bioaerosols during haze days may have significantly adverse health effects on human beings. Therefore, more attention should be paid to the potential health risks related to bioaerosols accompanied by the haze days, which is an often neglected issue of aerosols during the haze days in previous studies. To reduce exposure to potentially harmful bioaerosols during the haze days, we should take some possible measures, including the use of enclosures around sources to restrain bioaerosol production, spraying water on the road, and using efficient personal protective equipment (e.g. masks and gloves).

5. Conclusions

A study was conducted to compare the difference in characteristics of bioaerosols during the hazy and non-haze days from October 8th to 22nd, 2014, in Xi'an. The results are highlighted as follows:

(1) During the haze days, the daily average concentrations of airborne viable bacteria and fungi were 1102.4–1736.5 and 1466.2–1703.9 CFU/m^3, respectively. They were not only much higher than those detected during the non-haze days, but also exceeded the reference concentrations suggested by a Chinese non-governmental organization, indicating that the level of air contamination with microbes significantly increases and the exposure to airborne microbes may threaten the health of population in the megacity during haze events.

(2) During the non-haze days, the majority of airborne viable bacteria and fungi was in coarse size range (>2.1 μm). During the haze days, more airborne viable bacteria were in the size range of less than 2.1 μm. Unlike bacterial aerosols, there was little difference in the size distribution of airborne fungi between the haze and non-haze conditions. These findings suggest that during the haze days bioaerosols are more easily to reach bronchi and alveolus, and thus cause greater adverse health effects on the residents in Xi'an.

(3) The genera identified during the haze days included *Staphylococcus*, *Micrococcus* and *Neisseria* for airborne viable bacteria and *Aspergillus*, *Paecilomyces*, *Penicillium* and *Cephalosporium* for airborne viable fungi, respectively, suggesting that there are more exposures to allergic and infectious bioaerosols during the haze days than during non-haze days.

Acknowledgments

The authors would like to thank the National Natural Science Foundation of China (41230314, 51208059) and the Special Fund for basic Scientific Research of Central Colleges, Chang'an University (2013G2291013).

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