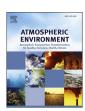
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Characteristics of bacterial and fungal aerosols during the autumn haze days in Xi'an, China



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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.

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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/m³, 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.

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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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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, $108^{\circ}94'E$ 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 km², 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: $\geq 7.0~\mu m$ (stage 1), $7.0-4.7~\mu m$ (stage 2), $4.7-3.3~\mu m$ (stage 3), $3.3-2.1~\mu m$ (stage 4), $2.1-1.1~\mu m$ (stage 5) and $1.1-0.65~\mu m$ (stage 6). Fine particles were defined here as particles with aerodynamic diameter less than $2.1~\mu m$ (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/m³) 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

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