High Time-Resolved Measurements of Microbial Activity Levels in Bioaerosols During Typical Heavy Pollution Processes

Shengli Du¹, Yanpeng Li¹, ², *
¹School of Water and Environment, Chang'an University, Xi'an, 710054, PR China
²Key Laboratory of Subsurface Hydrology and Ecology in Arid Areas, Ministry of Education, Xi'an, 710054, PR China

*Corresponding author: liyanp01@chd.edu.cn

Abstract. All manuscripts must be in English, also the table and figure texts, otherwise we cannot publish your paper. Please keep a second copy of your manuscript in your office. When receiving the paper, we assume that the corresponding authors grant us the copyright to use the paper for the book or journal in question. Should authors use tables or figures from other Publications, they must ask the corresponding publishers to grant them the right to publish this material in their paper.

Keywords: Bioaerosol, microbial activity, high time-resolved measurements.

1. Introduction

The complex composition of PM₂.₅ include inorganic compounds such as water-soluble ions (Na⁺, NH₄⁺, K⁺, Mg²⁺, Ca²⁺), carbon substances such as elemental carbon and organic matter, and microbial components such as fungi and bacteria [1-3]. Bioaerosols generally refer to aerosols and particulate matter of biological origin, including fungal spores, bacteria, and viruses, as well as pollen, animal and plant decomposition products [4-6]. The living microorganisms in bioaerosols participated in the physical and chemical processes of the atmosphere, and reacted with the reactive oxidants in the atmosphere to form secondary aerosols, which contributed to the formation of haze [7, 8]. At the same time, microorganisms can constitute cloud condensation nuclei and ice nuclei to affect the atmospheric circulation, and most of these nucleating microorganisms were still active [9-11]. Molds, thermophilic actinomycetes, gram-negative bacteria and viruses in bioaerosols were infectious and allergic pathogens [12-14]. The metabolism and other physiological activities of the active microorganisms in the bioaerosols will have serious impact on human health and the atmospheric environment. Therefore, the knowledge of the activity of microorganisms in bioaerosols is important to better understand the role of bioaerosols in various meteorological activities.

Microbial activity refers to the intensity of microbial metabolic activity, which is often instructed by the activity of microbial proteases, lipases, esterizes, and dehydrogenases [15]. There are many standards for measuring total microbial activity: for example, the measurement of microbial respiration, the fluorescein diacetate hydrolysis method (FDA), and the microcalorimetry focuses [16]. Fluorescein diacetate (FDA) can be hydrolyzed by a variety of hydrolytic enzymes to produce fluorescein, which can be determined by spectrophotometry. FDA hydrolysis was an efficient and
accurate method that can be used to determine microbial activity in soil and multiple environmental samples [17, 18]. Qi et al. improved the method to establish the relationship between luciferin produced by FDA hydrolysis and enzymes [17], and then used it to determine the enzyme activity of microorganisms in bioaerosol samples. Qi et al.’s subsequent studies have shown that the activity of microorganisms in bioaerosols has a significant correlation with meteorological factors, and the activity of microorganisms in Qingdao has obvious seasonal changes [19].

Not only physical, chemical, and biological factors can affect the activity of microorganisms [5], but also regional, seasonal and meteorological factors (temperature, humidity, wind speed, etc.) also affect the activity of microorganisms [19, 20]. Air quality also has an important impact on the activity of microorganisms in bioaerosols [20], and there are many organic and inorganic ions required for the metabolism of microorganisms in particulate matter. Among them, Na+, Mg2+ and Ca2+ were significantly positively correlated with the activity of microorganisms, but as air quality deteriorates, the activity of microorganisms will decrease [20, 21]. The complex relationship between microorganisms, meteorological factors, and pollutants needs more in-depth research.

In this study, high time-resolved sampling was performed on a winter high-pollution day in Xi'an, an important city in northwestern China. The sampling time was December 20, 2019, December 28, and January 14, 2020. This paper collected high time-resolved samples on highly polluted days (PM$_{2.5}$ concentration higher than 150μg/m$^3$), high time-resolved samples from haze generation to dissipation, and high time-resolved samples on highly polluted snow days. This research aimed to study the continuous changes of microbial activity and its influencing factors under highly polluted weather, and to explore the role of bioaerosols in the haze generation process.

2. Materials and methods

2.1. Sampling location
The sampling site was located at the Yanta Campus of Chang'an University in Xi'an, China. The bioaerosol sampler was placed on the building roof of the College of Water Conservancy and Environment, about 27 meters above the ground (34°13′N,108°57′E). The sampling site is surrounded by urban roads, green belts, school buildings, residential areas and commercial areas, and there are no potential pollution sources.

2.2. Sample Collection
The air sampler was placed on a tripod, 1.5 meters above the building ground. Two air samplers (ZR-3930, Qingdao China) were used to collect two samples (PM$_{2.5}$ and PM$_{10}$ particles) simultaneously, and the samples were collected on a sterilized 47mm polycarbonate membrane (Whatman, UK). Each sample was collected at a flow rate of 16.7 L / min for 30 minutes, and the samples were refrigerated at low temperature(-80°C) immediately after the collection.

The first sampling started at 8:00 on December 20th, 2019 and ended at 24:00 on December 22nd. Three days of high-contaminated bioaerosol samples were collected. The second sampling was from 8:00 on December 28, 2019 to 24:00 on December 30, 2019. The processes of PM$_{2.5}$ concentration rising sharply and disappearing were collected. The last sampling started at 16:00 on January 14, 2020 and ended at 24:00 on January 15, 2020. The sampling interval was 4 hours. During the sampling process, we recorded the meteorological data of the weather station, including PM$_{2.5}$ concentration, temperature (T), relative humidity (RH), wind speed (WS) and total radiation (TR).

2.3. Microbial activity testing
The microorganisms were eluted from the polycarbonate membrane into physiological saline. Fluorescein diacetate solution was added to react for a period of time in the dark, and then a terminator was added. The supernatant is extracted and placed in a multifunctional microplate reader to measure the fluorescence intensity of the reaction at a specific wavelength. For detailed experimental procedures, refer to our other study [22].
Before calculating the microbial activity in PM$_{2.5}$, subtract the fluorescence intensity of the blank sample, and calculate the microbial activity level in the bioaerosol of ng/m$^3$. The formula is:

$$M_{a(PM)} = 1000 \times C_{(solution)} \times \frac{V}{Q \times t}$$

(1)

where $M_{a(PM)}$ is the activity of microorganisms in PM$_{2.5}$ (ng/m$^3$), $C_{(solution)}$ is the concentration of sodium fluorescein in the solution after the reaction was terminated, $V$ is the volume of the solution after the reaction was terminated, $Q$ is the flow rate of each sample when sampling, and $t$ is the length of the sampling.

2.4. Data analysis

Origin2019 and The R (64 3.6.3) statistical computing environment were used for statistical analysis of data. The spss (version25) software was used to calculate the spearman correlation coefficient. Spearman correlation analysis was used to determine the relationship between meteorological factors and microbial activity in bioaerosols and establish a correlation matrix. If the $p \leq 0.05$, the correlation between the two can be considered significant.

3. Results and discussion

3.1. Temporal variations of microbial activity

Figure 1. The PM2.5 concentrations and microbial activities in PM2.5 during the present sampling periods

Figure 1 compares the relationship between the microbial activities and PM$_{2.5}$ concentrations during the three haze pollution processes. During the entire sampling period from December 20 to December 22, the PM2.5 concentration was above 150μg/m$^3$, which was a severely polluted day. It can be observed that the activity of microorganisms was maintained at a higher level when the PM$_{2.5}$ concentration increased during this sampling period, and the activity of microorganisms reached the lowest in the morning during this period. The morning was the lowest temperature of the day during the sampling period. This was because temperature was significantly positively correlated with the activity of microorganisms. During the sampling period from December 28 to December 30, the microbial activity showed a downward trend as the entire pollution process progressed, indicating that the increase in the concentration of pollutants has a toxic effect on microorganisms and reduced the activity of microorganisms [23]. At the same time, there have been studies it showed that the viability
of microorganisms in dusty weather was lower than that in non-dust weather [24], so as the concentration of pollutants increased, the activity of microorganisms tended to decrease, but the concentration of PM$_{2.5}$ reached 100μg/m$^3$ (high pollution weather), the activity of microorganisms began to decline, which may indicate that the influence of pollutant concentration on the activity of microorganisms has a certain lag. However, during the rapid increase of PM$_{2.5}$ concentration, the activity of microorganisms reached the highest level during the sampling period, which showed that the secondary aerosol produced by the activities of microorganisms promoted the increase of PM2.5 concentration. During the sampling period, snowfall began at 5 am on January 15, 2020, and the snowfall continued until 8 pm of the same day. During this period, the concentration of pollutants was also high, but the microbial activity was the lowest among the three samples. The influence of inorganic ions will be discussed in detail later. In summary, although no clear relationship between the activity of microorganisms and the concentration of PM$_{2.5}$ has been found, and the activity of microorganisms may aggravate the production of PM$_{2.5}$. There was a lag in the influence of high pollutant concentration on the activity of microorganisms. On the whole, high pollution concentration will reduced the activity of microorganisms.

3.2. Comparison with other regional studies
The microbial activity in the sample was in the range of 6.47 to 13.44 ng/m$^3$ fluorescein sodium, with an average value of 9.90 ng/m$^3$ fluorescein sodium. In a previous study by Wang [22], bioaerosol samples were collected in Xi'an from March to September 2019, and the study found that the microbial activity in PM$_{2.5}$ was within the range of 5.0 ng/m$^3$ to 11.0 ng/m$^3$ fluorescein sodium, the average microbial activity level in PM$_{2.5}$was 8.2 ng/m$^3$ fluorescein sodium. It can be observed that the activity of microorganisms in winter haze in Xi'an was significantly higher than that in other periods. In the Qingdao's study, Qi found that the average microbial activity showed significant seasonal differences. The microbial activity ranged from 5.59–102 ng/m$^3$ (average 34.4 ng/m$^3$), but according to weather conditions, hazy days (37.8 ng/m$^3$) followed by foggy days (42.1 ng/m$^3$) [19]. Compared with this research, the activity of microorganisms in bioaerosols in Qingdao area in winter was much higher than that in Xi'an area, and the similarity between the two regions is that the microbial activity was the highest in haze days. But in another study, it showed completely different results. Zhang et al. [20] collected PM$_{2.5}$ particles in Beijing and found that in winter the average activity of microorganisms in bioaerosols is 0.91 ng/m$^3$. In the same season in China, the microbial activities of Xi'an, Beijing and Qingdao show great differences. There are many possible reasons for this phenomenon. First of all, this study is the same as the study in Beijing area to collect PM2.5 particle samples to study the activity of microorganisms in the bioaerosol. The FA-1 six-stage microbial sampler was used in the study of Zhong et al. and they collected more than 0.65μm bioaerosol particles [19]. Previous studies have shown that as the particle size of bioaerosols changes, the concentration and community structure of microorganisms in them have significant changes [25-27], the concentration of microorganisms in particles larger than 0.65μm in diameter was much higher than that in PM$_{2.5}$, and the community structure was also significantly different, so the particle size of the collected samples will have a certain impact on the experimental results. Secondly, compared to the research of Zhong [19], the sources of bioaerosols in different regions were different, and the sources of marine bioaerosols in Qingdao and inland areas were also significant. All in all, all in all, there were significant geographic differences in the activity of microorganisms in bioaerosols.
3.3. Day and night comparison of microbial activity

Figure 2 compares the difference in microbial activity between daytime samples and nighttime samples during the sampling period. The average value of microbial activity in the daytime was 9.86 ng/m$^3$, compared to the average value of 10.16 ng/m$^3$ at night. The maximum temperature during the sample 11.9 °C, the lowest temperature of 0.5 °C, and the maximum temperature difference between day and night was 8.5°C. Overall, the day microbial activity was low, compared to high microbial activity at night, many observations have shown that the concentration of biological aerosols at night is higher than that during the day, indicating that the night was more conducive to the reproduction of microorganisms [28, 29], at the same time, the conditions of low radiation at night were also conducive to the reproduction of microorganisms. It can also be found that the increase in PM$_{2.5}$ concentration occurs during the day, due to a large number of gaseous pollutants produced by human activities, and some studies have shown that the concentration of microorganisms is significantly positively correlated with the concentration of SO$_2$, NO$_2$ and CO in the air [23, 27]. It shows that human activities also had a certain impact on the microbial activity.

3.4. The relationship between microbial activity and meteorological conditions

Earlier studies on airborne fungal spores and climate in the Stockholm area found that temperature and precipitation are very important factors, but the concentration of fungal spores varied with wind speed and total cloud cover [30]. In the United Kingdom, in addition to exploring temperature and other factors, the influence of the North Atlantic Ocean current and the number of summer days on the survival rate of microorganisms was also studied [31]. Microorganisms in the air are in different regions, and due to different climates, the influence of various meteorological factors is different, and even the main factors in different seasons were different [32]. The microorganisms living in the air are not only affected by meteorological factors, but also by pollutants. A large number of existing studies have shown that meteorological factors are important factors affecting microorganisms in the air.
Table 1. Meteorological data during the sampling period.

| Time          | T (℃) Range | RH (%) Average | PM2.5(μg/m³) Range | TR(W/m²) Average | WS(m/s) Range | Averages  |
|---------------|-------------|----------------|---------------------|------------------|--------------|-----------|
| Decembe 20, 2019 | 2.6-7.1    | 5.2            | 77.6-82.4 80.2      | 0-694.7          | 156.7        | 0.2-1.0   |
| Decembe 21, 2019 | 3.5-7.7    | 5.4            | 77.3-83.6 79.9      | 0-382.3          | 102.8        | 0.1-0.2   |
| Decembe 22, 2019 | 1.7-6.6    | 4.3            | 79.1-91.2 84.7      | 0-681.5          | 158.1        | 0-1.0     |
| Decembe 28, 2019 | 3.7-11.3   | 6.2            | 57.3-75.9 69.2      | 0-797.3          | 181.3        | 0-2.5     |
| Decembe 29, 2019 | 3.4-11.9   | 7.4            | 64.0-77.7 72        | 0-776.5          | 180.5        | 0-1.8     |
| Decembe 30, 2019 | 4.1-8      | 5.8            | 47.2-74.8 62.4      | 0-888.7          | 207.2        | 0-5.4     |
| January 14, 2020 | 0.5-1.7    | 1.2            | 78.5-81.2 79.8      | 0-407.5          | 98.6         | 0.9-1.4   |
| January 15, 2020 | 0.5-1.5    | 0.9            | 79.0-59.7 87.4      | 0-58.4           | 12.4         | 0.7-1.9   |

Table 2. Spearman correlation coefficient

|          | T     | RH    | PM2.5 | TR    | WS    |
|----------|-------|-------|-------|-------|-------|
| MA       | 0.49**| -0.35*| 0.04  | 0.02  | -0.33*|

By studying the effect of temperature on the stability of aerosols, it was found that as the temperature increased to a certain extent, the activity of microorganisms decreased [33]. When the temperature decreased [34], the fluidity of the cell membrane and enzyme activity decreased, thereby reducing the activity of microorganisms. As shown in Table 1, during the sampling period, the lowest temperature was 0.5°C, the highest temperature was 11.9°C, and the average temperature was 4.9°C. The Spearman correlation coefficient of PM2.5 microbial activity and temperature was 0.49 (n=10, p<0.01), and they have a significant positive correlation. The activity of microorganisms was directly proportional to temperature and increased with increasing temperature, which was consistent with Zhong [19]. The results of the study show that even in winter with relatively low temperatures, temperature is still a significant factor affecting the microbial activity in bioaerosols.

During the sampling period, the humidity range was 47.2%-91.2%, and the average humidity was 76.1%. In haze days, the activity of microorganisms was significantly negatively correlated with relative humidity (r=40, p<0.05). High humidity may cause microorganisms to combine with non-biological particles, and the formed large particles were easier to settle, therefore, humidity affects the microbial activity by affecting the number of microorganisms in the air [35]. At the same time, studies have shown that when the RH of the air is high, a layer of NH₃-, NOX and volatile organic compounds (VOCs) in the air will be formed on the surface of the bacteria [8], and those had toxic effects [23], so when the relative humidity was high, the activity of microorganisms was relatively low. Similarly, during the sampling period, the activity of microorganisms in bioaerosols was also significantly
negatively correlated with wind speed (Spearman =-0.33*, p<0.05), which may be due to the dilution effect of wind on microorganisms [36]. In haze days, the factors affecting microbial activity are complex, and more research is needed to obtain more complete conclusions.

4. Conclusions
There are significant seasonal differences in the activity of microorganisms. The activity of microorganisms in the winter haze in Xi'an area reaches the highest level, with an average value of 9.90ng/m³ fluorescein sodium. Under high-resolution sampling, it was found that during the rapid increase of PM₂.₅ concentration, the activity of microorganisms also increased and maintained a high level. The secondary aerosol produced by the activities of microorganisms may aggravate the haze. Meteorological factors such as temperature, humidity and wind speed are significantly correlated with the activity of microorganisms. Among them, temperature has a significant positive correlation with the activity of microorganisms, and humidity, wind speed and the activity of microorganisms have a significant negative correlation. Through multiple linear regression simulation, meteorological factors account for 44.8% of the interpretation of microbial activity. Research on the effects of pollutants in the atmosphere on microbial activity is still insufficient, and further research is needed.

References
[1] ROGULA-KOZŁOWSKA W, KLEJNOWSKI K, ROGULA-KOPIEC P, et al. Spatial and seasonal variability of the mass concentration and chemical composition of PM 2.5 in Poland [J]. Air Quality, Atmosphere & Health, 2014, 7(1): 41-58.
[2] STEENHOF M, GOSENS I, STRAK M, et al. In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential-the RAPTES project [J]. Particle and fibre toxicology, 2011, 8(1): 26.
[3] TAO J, CHENG T, ZHANG R, et al. Chemical composition of PM 2.5 at an urban site of Chengdu in southwestern China [J]. Advances in Atmospheric Sciences, 2013, 30(4): 1070-84.
[4] CAO C, JIANG W, WANG B, et al. Inhalable microorganisms in Beijing’s PM2.5 and PM10 pollutants during a severe smog event [J]. Environmental science & technology, 2014, 48(3): 1499-507.
[5] JONES A M, HARRISON R M. The effects of meteorological factors on atmospheric bioaerosol concentrations—a review [J]. Science of the total environment, 2004, 326(1-3): 151-80.
[6] DOUWES J, THORNE P, PEARCE N, et al. Bioaerosol health effects and exposure assessment: progress and prospects [J]. The Annals of occupational hygiene, 2003, 47(3): 187-200.
[7] GUO S, HU M, ZAMORA M L, et al. Elucidating severe urban haze formation in China [J]. Proceedings of the National Academy of Sciences, 2014, 111(49): 17373-8.
[8] ESTILLORE A D, TRUEBLOOD J V, GRASSIAN V H. Atmospheric chemistry of bioaerosols: heterogeneous and multiphase reactions with atmospheric oxidants and other trace gases [J]. Chemical science, 2016, 7(11): 6604-16.
[9] DELORT A-M, VAITILINGOM M, AMATO P, et al. A short overview of the microbial population in clouds: potential roles in atmospheric chemistry and nucleation processes [J]. Atmospheric Research, 2010, 98(2-4): 249-60.
[10] SUN J, ARIYA P A. Atmospheric organic and bio-aerosols as cloud condensation nuclei (CCN): A review [J]. Atmospheric Environment, 2006, 40(5): 795-820.
[11] MöHLER O, DEMOTT P, VALI G, et al. Microbiology and atmospheric processes: the role of biological particles in cloud physics [J]. 2007.
[12] FUNG F, HUGHSON W G. Health effects of indoor fungal bioaerosol exposure [J]. Applied occupational and environmental hygiene, 2003, 18(7): 535-44.
[13] HERR C, ZUR NIEDEN A, JANKOFSKY M, et al. Effects of bioaerosol polluted outdoor air on airways of residents: a cross sectional study [J]. Occupational and Environmental Medicine, 2003, 60(5): 336-42.

[14] BURGER H. Bioaerosols: prevalence and health effects in the indoor environment [J]. Journal of Allergy and Clinical Immunology, 1990, 86(5): 687-701.

[15] SCHNüRER J, ROSSWALL T. Fluorescein Diacetate Hydrolysis as a Measure of Total Microbial Activity in Soil and Litter [J]. Applied and Environmental Microbiology, 1982, 43(6): 1256-61.

[16] CALVET E, PRAT H. Recent progress in microcalorimetry [M]. Elsevier, 2016.

[17] Qi J, ZHONG X, SHAO Q, et al. Microbial activity levels in atmospheric bioaerosols in Qingdao [J]. Aerobiologia, 2015, 31(3): 353-65.

[18] ADAM G, DUNCAN H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils [J]. Soil Biology and Biochemistry, 2001, 33(7-8): 943-51.

[19] ZHONG X, Qi J, LI H, et al. Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region [J]. Atmospheric Environment, 2016, 140(506-13.

[20] ZHANG S, DU R, CHEN H, et al. Seasonal variation of microbial activity and pathogenic bacteria under non-serious pollution levels in Beijing [J]. Aerosol and Air Quality Research, 2019, 19(8): 1798-807.

[21] MENG X-B, LI M-Z, LI H-T, et al. Microbial activity in bioaerosols in winter at the coastal region of Qingdao [J]. Huan jing ke xue= Huanjing kexue, 2016, 37(11): 4147-55.

[22] WANG B, LI Y, XIE Z, et al. Characteristics of microbial activity in atmospheric aerosols and its relationship to chemical composition of PM2. 5 in Xi'an, China [J]. Journal of Aerosol Science, 2020, 105572.

[23] XIE Z, LI Y, LU R, et al. Characteristics of total airborne microbes at various air quality levels [J]. Journal of Aerosol Science, 2018, 116(57-65.

[24] HARA K, ZHANG D. Bacterial abundance and viability in long-range transported dust [J]. Atmospheric Environment, 2012, 47(20-5.

[25] GAO M, QIU T, JIA R, et al. Concentration and size distribution of viable bioaerosols during non-haze and haze days in Beijing [J]. Environmental Science and Pollution Research, 2015, 22(6): 4359-68.

[26] HAAS D, GALLER H, LUXNER J, et al. The concentrations of culturable microorganisms in relation to particulate matter in urban air [J]. Atmospheric Environment, 2013, 63(215-22.

[27] DONG L, QI J, SHAO C, et al. Concentration and size distribution of total airborne microbes in hazy and foggy weather [J]. Science of the Total Environment, 2016, 541(1011-8.

[28] ZENG X, KONG S, ZHENG S, et al. Variation of airborne DNA mass ratio and fungal diversity in fine particles with day-night difference during an entire winter haze evolutoin process of Central China [J]. Science of The Total Environment, 2019, 694(133802.

[29] WEI K, ZOU Z, ZHENG Y, et al. Ambient bioaerosol particle dynamics observed during haze and sunny days in Beijing [J]. Science of the Total Environment, 2016, 550(751-9.

[30] HJELMROOS M. Relationship between airborne fungal spore presence and weather variables: Cladosporium and Alternaria [J]. Grana, 1993, 32(1): 40-7.

[31] HOLLINS P, KETTLEWELL P, ATKINSON M, et al. Relationships between airborne fungal spore concentration of Cladosporium and the summer climate at two sites in Britain [J]. International Journal of Biometeorology, 2004, 48(3): 137-41.

[32] ZHEN Q, DENG Y, WANG Y, et al. Meteorological factors had more impact on airborne bacterial communities than air pollutants [J]. Science of the Total Environment, 2017, 601(703-12.

[33] MOHR A J. Fate and transport of microorganisms in air [M]. Manual of Environmental Microbiology, Third Edition. American Society of Microbiology. 2007: 961-71.
[34] SLONCZEWSKI J L, FOSTER J W. Microbiology: An evolving science: Third international student edition [M]. WW Norton & Company, 2013.

[35] ALGHAMDI M A, SHAMY M, REDAL M A, et al. Microorganisms associated particulate matter: a preliminary study [J]. Science of the Total Environment, 2014, 479(109-16).

[36] KURKELA T. The number of Cladosporium conidia in the air in different weather conditions [J]. Grana, 1997, 36(1): 54-61.