Aerosol and Air Quality Research

The Microbial Activity in $PM_{2.5}$ in Indoor Air: As an Index of Air Quality Level

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ABSTRACT

Bioaerosols are a major source of pollution in indoor environments, where people spend approximately 90% of their time, and the microorganisms adhered to $PM_{2.5}$ adversely affect human health. However, most research has focused on the concentration of these aerosols and the factors that influence it rather than the correlation between microbial activity and air quality. Thus, this study used a modified technique of fluorescein diacetate (FDA) hydrolysis to evaluate the activity of microorganisms in the $PM_{2.5}$ during three seasons (summer, autumn and winter) in Beijing. 0.155–5.388 ng m^{-3} and 0.091–5.740 ng m^{-3} of sodium fluorescein, a marker of microbial activity, were measured indoors and outdoors, respectively; thus, no significant difference in concentration between the two environments was detected, but the indoor activity was affected by outdoor conditions to an extent. The most active season was autumn, followed by winter and summer. Furthermore, the highest activity in summer and autumn was observed during conditions of excellent air quality, and in summer, the activity during conditions of good air quality also obviously exceeded that during conditions of slight pollution. Additionally, the microbial activity in a room varied according to the room's ventilation (or lack thereof), suggesting a strong association between these parameters. In general, when the air quality was excellent, 20 minutes of ventilation achieved the optimal air exchange, but this duration should be reduced during polluted conditions. Our results provide new insights into evaluating the indoor air quality based on the microbial activity.

Keywords: Fluorescein diacetate, Season, Pollution level, Ventilation

1 INTRODUCTION

Indoor air quality is closely related to human health and work efficiency. Studies have estimated that people spend approximately 90% of their time in private and public indoor environments (Cincinelli *et al.*, 2017; Lakey *et al.*, 2017), and some elderly people and infants who are less mobile may spend more time indoors. Large-scale COVID-19 outbreaks occurred all over world this year, and residents were isolated indoors for a long time, increasing the importance of indoor air quality. Furthermore, the health risks associated with exposure to poor quality indoor air may far exceed those associated with outdoor pollution, and are more likely to cause harm to vulnerable groups (Mendes *et al.*, 2013).

Indoor air pollution refers to the accumulation and diffusion of various chemical, biological and physical pollutants, resulting in a decline in indoor air quality and risks to daily life activities, work and health (Cincinelli *et al.*, 2017). Bioaerosols accounts for 5–34% of indoor air pollution (Srikanth *et al.*, 2008), and mainly include bacteria, fungi, viruses and spores (Ariya *et al.*, 2004). Microbial aerosols are an important fraction of bioaerosols, and high levels of microbial aerosols directly decrease indoor air quality; some harmful microorganisms can directly or indirectly threaten human health depending on the environmental conditions. To date, the association between indoor air quality and disease has been widely studied. For example, people who live and work indoors long-term might show many symptoms, such as headache, nausea, dizziness and inattention, and this has been termed *sick building syndrome* (SBS) (Hedge *et al.*, 1996;
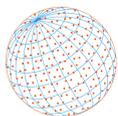
Jones, 1999). Likewise, related research has suggested that indoor microbial pollution can cause respiratory diseases, such as asthma, rhinitis and lung diseases (Douwes *et al.*, 2003; Fung *et al.*, 2003; Meng *et al.*, 2012; Liu *et al.*, 2014); increase the incidence of infectious and allergic diseases; and even cause mutual infections of human and livestock diseases (Husman, 1996; Barker *et al.*, 2001).

Indoor microorganisms include outdoor pollution sources and indoor pollution sources, two types of sources. Regarding outdoor pollution sources, contaminants in the atmosphere enter the indoor environments through indoor and outdoor air exchanges, which are common sources of indoor pollution (Happo *et al.*, 2013). Epidemiological evidence has shown a strong correlation between outdoor pollution and human health, and numerous outdoor pollutants can penetrate into the indoor environment (Chen *et al.*, 2012). Microorganisms attached to $PM_{2.5}$ mainly come from vegetation, soil, industrial activities and daily life activities (Smets *et al.*, 2016). Several researchers have reported the human health threat posed by $PM_{2.5}$ in the atmosphere. For example, $PM_{2.5}$ can lead to an increase in cardiopulmonary damage and mortality (Hu, 2009; Lu *et al.*, 2019) and promote the occurrence and development of diabetes mellitus (Chen *et al.*, 2013). Furthermore, recent research has shown that even very low levels of $PM_{2.5}$ are a public health hazard (Franklin *et al.*, 2007; Feng *et al.*, 2016).

Regarding indoor pollution sources, the main pollution includes humans, animals, plants, building decoration materials and air conditioning systems (Weschler, 2009). Previous studies have reported the detection of microorganisms in indoor environments with and without humans, and strong signals from human-associated microorganisms have been found in indoor environments where human activities occur (Qian *et al.*, 2012). Moreover, several studies have documented the significant impacts on microorganisms of changes in the number of people, the ratio of males to females, and the presence of animals or plants (Berg *et al.*, 2014; Barberan *et al.*, 2015; Kozdroj *et al.*, 2019). The indoor ventilation system will affect the indoor air quality. Ventilation systems mainly include natural ventilation and mechanical ventilation. Natural ventilation achieves indoor and outdoor air exchange through the opening and closing of doors or windows. The common methods of mechanical ventilation are air conditioning and ventilation systems; ventilation systems have entered into public use only in recent years, and air conditioning is still the most widely used mechanical ventilation system. However, using air conditioning as a daily ventilation method has great disadvantages. When an air conditioning system is running, it will affect the indoor ventilation frequency, and thereby the concentration of indoor microorganisms (Yu *et al.*, 2009). Research on SBS and the type of ventilation system in office buildings has revealed that the prevalence of SBS symptoms was observably higher, by approximately 30–200%, in air-conditioned spaces than in naturally ventilated spaces (Seppanen *et al.*, 2002). Furthermore, one of the possible causes of the large-scale infection by COVID-19 on the Japanese Diamond Princess cruise ship was the air conditioning system spreading aerosols containing the virus, but this has yet to be confirmed. Notably, indoor pollutant concentrations may be increased by either too much or too little ventilation (Hoisington *et al.*, 2019).

In recent decades, research on indoor microbial aerosols has mainly focused on the concentration, particle size distribution, and relationship between environmental factors and indoor microorganisms. To reduce the impact of indoor air microbes on human health, many countries have formulated relevant indoor microbial limit standards, but no unified standard exists yet. Pastuszka *et al.* (2000) used an Anderson 6-stage sampler to measure the concentrations of bacteria and fungi and compare their levels between a residence and an office and between a healthy and moldy residence in the Upper Silesia Industrial Zone. The typical levels of bacteria in the residence and office were 10^3 CFU m^{-3} and 10^2 CFU m^{-3}, respectively. In winter, the concentration of fungi in the moldy residence (10–10^3 CFU m^{-3}) was higher than that in the healthy residence (10–10^2 CFU m^{-3}), and the data measured in summer were significantly higher than those measured in winter. *Penicillium* constituted up to 90% of the total fungi in the moldy residence but only 3–50% in healthy residences. Happo *et al.* (2013) collected particulate matter samples with different particle size ranges ($PM_{10-2.5}$, $PM_{2.5-0.2}$ and $PM_{0.2}$) using a high-volume sampler, and then performed toxicological experiments: The $PM_{10-2.5}$ samples triggered the highest inflammatory and cytotoxic responses, and the $PM_{2.5-0.2}$ samples had the greatest effect on the apoptotic activity of macrophages.

Many of the current quantitative methods for indoor microorganisms still utilize the culturable
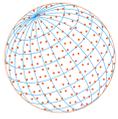
method. Sedimentation or the Anderson sampling method has been used to collect microorganisms on a culture medium, and then classify and cultivate different microorganisms; however, this traditional method has great limitations: Only a small fraction of the microorganisms (< 1%) in the atmosphere can be cultured (Heidelberg *et al.*, 1997; Bridge *et al.*, 2001; Despres *et al.*, 2012), and the cultivation method is time- and energy-intensive. Thus, some researchers have leveraged gene analysis and quantitative real-time polymerase chain reaction (PCR) to analyze the changes in microbial aerosols in indoor environment. A method based on SYBR Green real-time quantitative PCR was developed to detect and identify allergenic *Aspergillus versicolor*, which can often be found in indoor environments, and it has been applied in practice (Libert *et al.*, 2015). High-throughput sequencing was applied to analyze the dominant and pathogenic microorganisms in the air of five hospitals in eastern and southern China, and the abundance of bla_{CTX-M} and $mecA$ resistance genes was measured by real-time quantitative PCR. Based on these findings, hospitals were recommended to reduce air emissions during daily operation to protect the health of the surrounding communities (Gao *et al.*, 2018). Unfortunately, none of the above methods can directly reflect the ability of microorganisms to perform their functions, because only active microorganisms can grow and propagate in an appropriate environment, and have an impact on human health. Microbial activity can accurately reflect the potentially harmful effect of atmospheric microorganisms on human health, but it is a largely underexplored domain.

Therefore, in this study, microbial activity was determined to evaluate air quality. The microbial activity in $PM_{2.5}$ collected at different indoor and outdoor sampling points was measured by the fluorescein diacetate (FDA) hydrolysis method (Qi *et al.*, 2015; Zhang *et al.*, 2019). The results will make a crucial contribution to reducing indoor microbial pollution and protecting human health and provide basic data for the establishment of microbial limits. Specifically, this work aims to address the following three questions: (1) How do indoor and outdoor air quality levels change in different seasons and under different pollution levels with microbial activity as an indicator? (2) Does the outdoor environment have an impact on indoor microbial activity? (3) How can a scientific ventilation method be adopted in winter to reduce the impact of indoor microorganisms on health?

2 METHODS

2.1 Sample Site and Groups

The indoor samples were collected in the Yard Four 345 office of Huairou East Campus, University of Chinese Academy of Sciences (40°24′12″N, 116°40′47″E, 8 m above the ground). The office area is approximately 24 m^2, with two sets of office desks, chairs and computers; a three-person sofa; two sets of metal bookcases; and a small number of green plants. The office was equipped with an air conditioning system. Usually, indoor staff activities involved no more than 2 people day^{-1}. The outdoor samples were collected from the roof of the first teaching building on the Huairou West Campus of the University of Chinese Academy of Sciences (40°24′29″N, 116°40′28″E, 30 m above the ground). This site is in northeast Huairou, Beijing. Southwest of the sampling site is a lake (230 km^2), to the west and north are densely forested mountains, and to the east is a national highway. In particular, no direct source of industrial pollution exists near the sampling site. Sample collection at the two sampling points was performed simultaneously.

Sampling was conducted on 54 days at the indoor and outdoor sampling sites during a 14-day period in summer (from May 11 to July 18, 2017), a 19-day period in autumn (from September 23 to October 22, 2017), and a 21-day period in winter (from December 4 to December 28, 2017). We classified the collected $PM_{2.5}$ samples based on the ambient air quality index (AQI), which is an index for reporting levels of air pollution: Pollution Level 1 ("excellent air quality," AQI: 0–50), Pollution Level 2 ("good air quality," AQI: 51–100) and Pollution Level 3 ("slight pollution," AQI: 101–150) (Table 1).

2.2 Collection of Indoor and Outdoor $PM_{2.5}$ Samples

$PM_{2.5}$ samples were collected on sterilized quartz membranes (Whatman, UK) using a small-volume particulate matter air sampler (Omni™ FT; BGI, USA) 1.5 m above the ground. The quartz
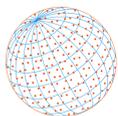
Table 1. Information of experimental treatment groups.

Season	Pollution level	Range of AQI	Air quality level	Number of samples	Percentage	Sampling date
Summer	Level 1	0–50	Excellent	3	5.56%	11-May-2017; 11, 14-Jul-2017
	Level 2	51–100	Good	9	16.67%	13-May-2017; 26, 27-Jun-2017; 9, 10, 13, 16, 17, 18-Jul-2017
	Level 3	101–150	Slight pollution	2	3.70%	18-May-2017; 12-Jul-2017
Autumn	Level 1	0–50	Excellent	9	16.67%	27, 28-Sep-2017; 11, 12, 13, 14, 15, 16, 19-Oct-2017
	Level 2	51–100	Good	10	18.52%	23, 24, 25, 26, 29, 30-Sep-2017; 17, 20, 21, 22, -Oct-2017
	Level 3	101–150	Slight pollution	0	0.00%	–
Winter	Level 1	0–50	Excellent	16	29.63%	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 19, 21, 23, 24, 25, 26-Dec-2017
	Level 2	51–100	Good	3	5.56%	20, 22, 27-Dec-2017
	Level 3	101–150	Slight pollution	2	3.70%	15, 28-Dec-2017

membranes were wrapped independently with aluminum foil and placed in a muffle furnace at 550°C for 5 hours. The sampling flow rate was 5 L min^{-1}, and the sampling duration was 40 minutes (8:00–8:40 a.m.).

To investigate the effect of indoor ventilation on microbial activity in winter, indoor samples were collected in a closed environment. Then, the window was ventilated for a predetermined length of time (30 minutes, 20 minutes or 10 minutes), and new samples were collected. Each ventilation duration was collected separately for 1 week. At the same time, outdoor samples were collected using the same experimental method. Two blank membranes were placed at each sampling point and processed simultaneously.

2.3 Analytical Method

A modified fluorescein diacetate hydrolysis method was used to determine the microbial activity in PM$_{2.5}$. After sampling, the membranes were immediately returned to the laboratory for processing. First, the sample was taken out on the clean bench, and the sampling membrane was cut into pieces with scissors in disposable culture dishes. 5 mL of sterile physiological saline (0.85–0.90% NaCl solution) was added to sterilized 10-mL centrifuge tubes, in which the membrane pieces were placed. The centrifuge tubes were shaken at 30°C for 30 minutes and 150 r min^{-1} to separate the adherent microbes from the membranes. Next, 50 μL of 200 μg mL^{-1} FDA solution was added to the pretreated samples, and the samples were hydrolyzed at 30°C for 90 minutes in the dark. After the reaction was completed, 5 mL of acetone was added to terminate the reaction for 15 minutes. After centrifugation at 10,000 r min^{-1} for 1 minute, the fluorescence intensities of samples and blanks were measured by a SpectraMax Paradigm Multi-Mode Detection Platform (λex = 490 nm, λem = 530 nm). The microbial activity in PM$_{2.5}$ was calculated using the following formula (Ren *et al.*, 2018):

$$Ma_{(air)} = \frac{1000VMa_{(solution)}}{Qt} \tag{1}$$

where $Ma_{(air)}$ and $Ma_{(solution)}$ denote the microbial activity levels in atmospheric aerosols per unit volume of air (ng m^{-3}) and solution (ng mL^{-1}), respectively, V is the volume of solution (mL), Q is the air sampling flow rate (L min^{-1}), and t is the sampling duration (min).

3 RESULTS AND DISCUSSION

3.1 Comparison of the Microbial Activity in Indoor and Outdoor Air

During the sampling period, the microbial activity in indoor air ranged from 0.155 to
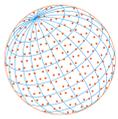
5.388 ng m^{-3} sodium fluorescein, with an average of 1.388 ng m^{-3} sodium fluorescein, and the microbial activity in outdoor air ranged from 0.091 to 5.740 ng m^{-3} sodium fluorescein, with an average of 1.102 ng m^{-3} sodium fluorescein. The daily analysis of microbial activity revealed that the indoor microbial activity was greater than the outdoor microbial activity for 32 days, but the opposite was true on the other sampling days. In addition, the highest indoor and outdoor microbial activity was recorded in autumn (outdoor = 1.211 ng m^{-3} sodium fluorescein; indoor = 2.011 ng m^{-3} sodium fluorescein), followed by summer and winter. Considering the traffic congestion at the test site, the indoor and outdoor samples were collected at the same time, and the indoor microbial content was always higher than the outdoor microbial content, with a seasonal trend (Radwan *et al.*, 2019). The concentration of fungi indoors was higher than that outdoors in winter, but in the other seasons, the concentration of fungal aerosol outdoors was higher than that indoors (Lee *et al.*, 2006). However, no significant difference between the indoor and outdoor microbial activities was found in the 95% confidence interval (P = 0.104, P > 0.05).

The comparison of the variations in the indoor and outdoor microbial activity revealed no constant increasing or decreasing trend either indoors or outdoors, but the variations in the microbial activity indoors and outdoors were similar. For example, in summer, during the continuous observation from July 9 to 18, the microbial activity both indoors and outdoors increased and decreased similarly. In autumn, the microbial activity both indoors and outdoors decreased from October 11 to 12, increased gradually from October 14 to 17, and finally increased again from October 20. In winter, similar trends were observed from December 4 to 28 (Fig. 1).

According to these results, the variation in microbial activity was relatively consistent. Although the indoor environment was enclosed and the air circulation was poor, the variation in microbial activity was still affected by the outdoor environment to some extent. A study of eight classrooms at a university in the United States revealed that indoor air microbial communities are strongly associated with outdoor air microbial communities, and human-related bacterial genera are more than twice as abundant in indoor air as in outdoor air (Meadow *et al.*, 2014). 1 year of research on the indoor environment of five Danish families showed that the indoor fungi mainly come from the outdoors, and when indoor and outdoor air flow is not balanced during ventilation, a higher number of ventilation points corresponds to a higher indoor concentration of fungi (Frankel *et al.*, 2012), which was consistent with findings in Cincinnati (Lee *et al.*, 2006). Similarly, researchers in a zero-carbon building found that bacteria associated with the outdoor environment dominate indoor airborne bacteria (Leung *et al.*, 2018).
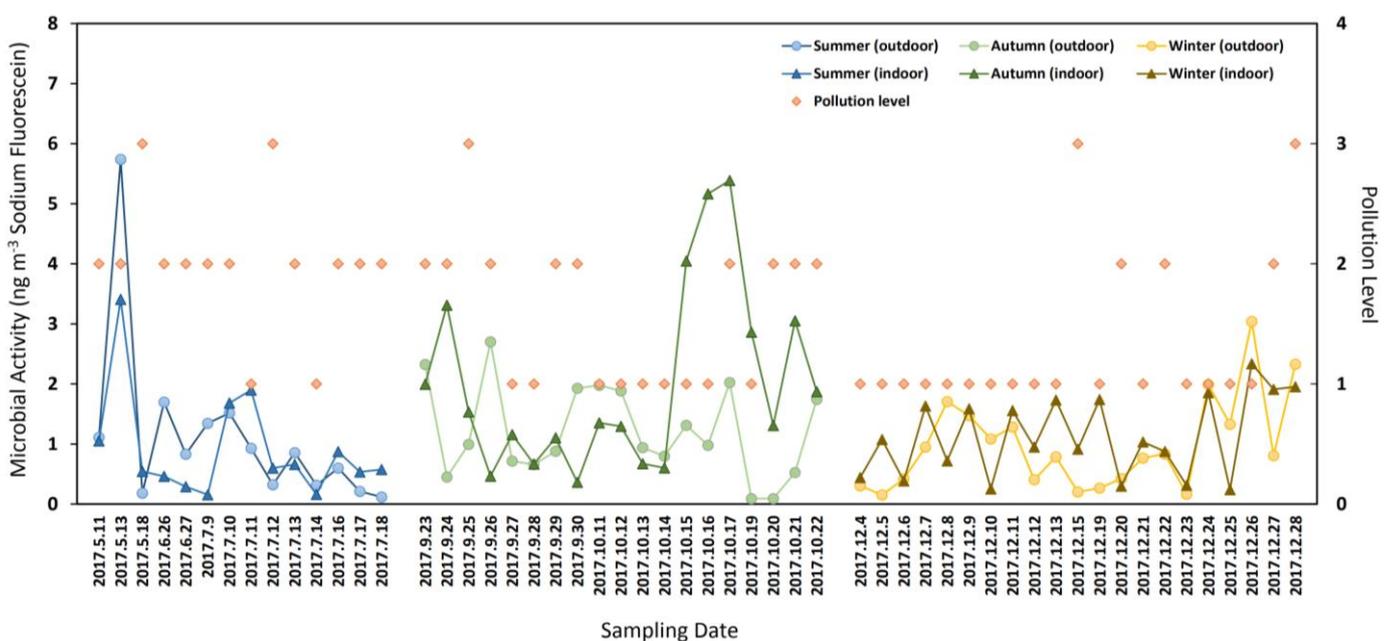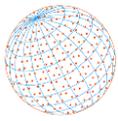

Fig. 1. Comparison of the microbial activity in indoor and outdoor PM$_{2.5}$.

In the present study, without microbial DNA sequencing, how the indoor microbial community structure, composition, and concentration changed was unclear. In particular, we could not analyze the related pathogens' information due to a lack of relevant data, and this will be the focus of future research.

3.2 Characteristics of the Indoor Microbial Activity during Different Seasons

As shown in Fig. 2, during the sampling period, 28 samples with excellent air quality (3 days in summer, 9 days in autumn and 16 days in winter), 22 samples with good air quality (9 days in summer, 10 days in autumn and 3 days in winter), and 4 samples with light pollution (2 days in summer and 2 days in winter) were collected. The statistical analysis revealed that weather with excellent air quality, good air quality and slight pollution accounted for 42%, 38% and 17%, respectively, of the 231 days from May 11, 2017, to December 28, 2017. The proportions of the collected samples are similar, indicating that the samples are representative.

The comparison of the levels of microbial activity in indoor airborne $PM_{2.5}$ in summer, autumn, and winter showed that regardless of the air quality, the indoor microbial activity was highest in autumn, followed by winter, and lowest in summer. This result is consistent with Li et al. (2011), who found a seasonal variation in the concentration of culturable terrestrial bacteria in Qingdao. Additionally, in Xi'an, researchers reported a similar trend based on measurements of the total microbial concentration in air, which was higher in autumn and lower in summer and winter (Li et al., 2017; Xie et al., 2018). For the indoor environment, Asif et al. (2018) demonstrated a significant seasonal difference in the microbial concentration between the outpatient department and the emergency department in winter and spring.

Hurtado et al. (2014) studied culturable microorganisms in the atmosphere of Tijuana, Mexico, and showed that the highest microorganism concentration occurred in summer while the lowest occurred in winter, unlike our results. In addition, different conclusions have been drawn for the same area of Qingdao. Dong et al. (2016) detected the highest concentration in winter and the lowest concentration in summer during their sampling period and noted that this may be due to the multiple effects of a low wind speed, a high particle concentration and the occurrence of heating activities during the sampling period, resulting in results different from ours. Although most of the above studies were in outdoor environments, the results of our study show that the indoor environment is affected by the outdoor environment and that no significant difference exists between the indoor and outdoor microbial activity. Hence, the studies above have reference value.

To summarize, obtaining different conclusions at the same sampling point is possible, whether to measure the concentration of culturable microorganisms or the total microorganisms in the atmosphere. Moreover, the results above directly reflect the microbial concentration changes only during the sampling period and in the context of the surrounding environment but indirectly reflect the local air quality. Meanwhile, microbial activity can be a direct indicator of the potential public health risk posed by microorganisms.
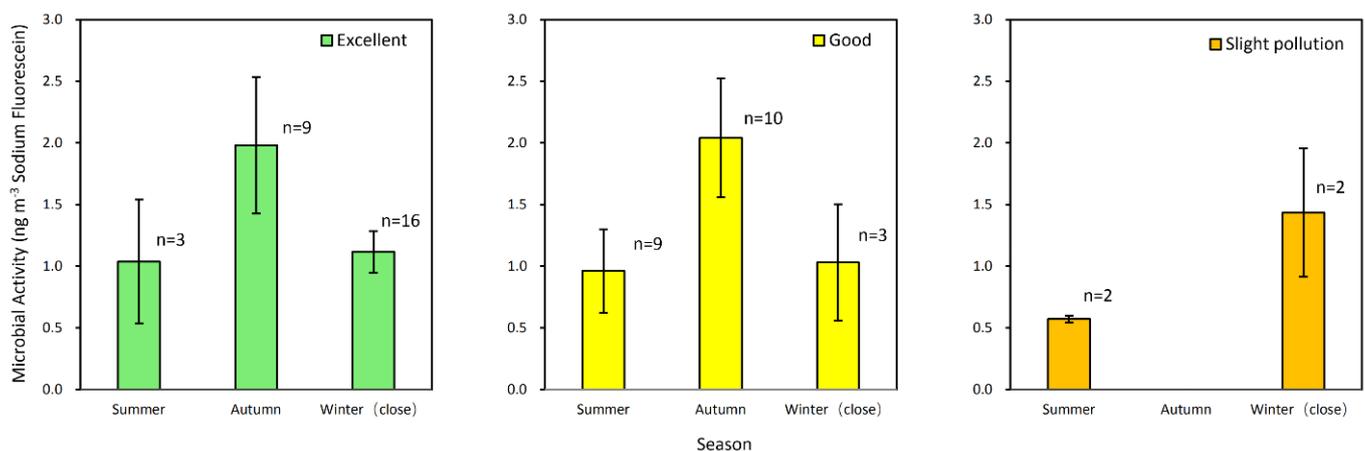
Fig. 2. Activity of microorganisms in $PM_{2.5}$ in indoor air in different seasons under the same pollution level. Error bars represent the standard error of the mean.
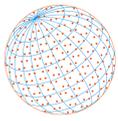
For this study, leaves fall from trees in autumn and increase the source of microorganisms, further increasing the microbial activity when the impact of the outdoor environment is higher than that of the indoor environment. In addition, the suitable temperature and relative humidity and sufficient nutrients in autumn provide favorable conditions for the growth and propagation of microorganisms, leading to the indoor microbial activity in autumn being the highest. In winter, because of heating, the indoor temperature is suitable for microorganism growth, but the outdoor temperature is too low. Zhong *et al.* (2016) explained that low temperature can inhibit the activity of microorganisms. Moreover, heating can dry the indoor air environment via the influence of both indoor and outdoor elements, and the activity of microorganism is low in winter. Finally, the lowest microbial activity occurs in summer because of the intense ultraviolet rays and frequent rainfall, which can greatly affect microorganisms.

3.3 Characteristics of the Indoor Microbial Activity under Different Pollution Levels

The level of microbial activity in summer gradually decreased with increasing pollution. In autumn, the variation in microbial activity was different from that in summer, and the level of microbial activity was very high when the air quality was excellent or good; however, the difference in activity between these two air quality conditions was very small. The relationship between the microbial activity and pollution level in autumn could not be determined due to the improving atmospheric environment, and no samples were collected on slightly polluted days. In winter, the change in the microbial activity of the samples collected when a room was closed was different from that in samples collected when a room was ventilated. In both the closed and ventilated conditions, the microbial activity was highest when air was slightly polluted or when the air quality was excellent, and lowest when the air quality was good (Fig. 3). Therefore, in the same season, the variety of indoor microbial activity was closely related to the indoor environment and ventilation conditions. The outdoor environment impacted the indoor microbial activity when a room was not closed, and this impact may be related to the source and transport of airborne microorganisms.

The microbial activity was the lowest under the slightly polluted conditions in summer. Moreover, after ventilation in winter, the microbial activity was 46.7% lower under slightly polluted conditions than under conditions with excellent air quality. Although the microbial activity was not the lowest under the slightly polluted conditions, it was significantly decreased compared with that under conditions of better air quality. Therefore, during the observation period, we can speculate that the microbial activity appeared to decrease with increasing
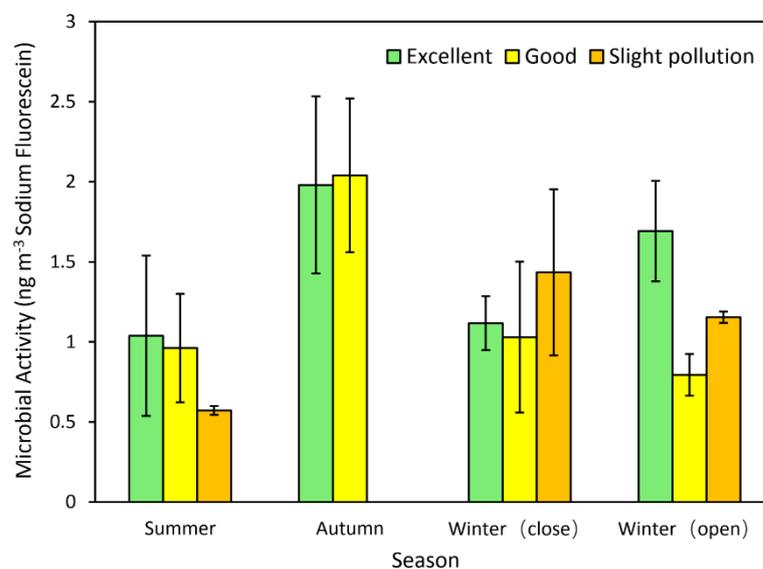
Fig. 3. Activity of microorganisms in $PM_{2.5}$ in indoor air at different pollution levels during the same season. Error bars represent the standard error of the mean.
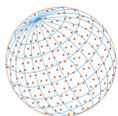
pollution. 1 year of observations in Beijing showed that the average microbial activity under conditions with excellent and good air quality was significantly higher than that under slightly and moderately polluted conditions (Zhang *et al.*, 2019).

During the observation period of this study, the sampling point was located in the suburb with a positive ecological environment, and the air quality in Beijing was dramatically improved. Therefore, few samples were collected under slight pollution. Air pollution mitigation measures have achieved obvious success, producing long periods of excellent and good air quality, but few studies have focused on the variation in atmospheric microbial activity and the possible impacts on human health. The results of this study can fill a gap in this field and provide a theoretical basis for further evaluation of air quality. In addition, to fully analyze the relationship between air quality and microbial activity, more data are needed for further analysis.

3.4 Influence of Ventilation on the Indoor Microbial Activity in $PM_{2.5}$

During long-term indoor activities, the air quality directly affects people's sensory experience and health status. An appropriate ventilation design can greatly improve the air quality, whereas unsuitable ventilation will increase the health risk of pollutants in the air. Li *et al.* (2007) reported that ventilation was associated with the spread of infectious diseases such as measles, tuberculosis, influenza and SARS. In most areas of China and under most conditions, people generally prefer ventilated indoor environments. In winter, because of the low temperatures, people must close windows for insulation. Nonetheless, long-term exposure to closed indoor environments will have negative health impacts. Therefore, this study investigated the effects of window ventilation and different ventilation times on microbial activity in winter. From December 4 to December 28, 2017, indoor ventilation was evaluated over 21 days. Three different ventilation durations—30 minutes, 20 minutes and 10 minutes—were assessed for 1 week each. Table 1 shows that during the observation period, excellent air quality occurred on 16 days, good air quality occurred on 3 days and slight pollution occurred on 2 days. In the experimental group with a duration of 20 minutes, samples with good air quality or slight pollution were collected on only 1 day each. In the experimental group with a duration of 10 minutes, excellent air quality, good air quality and slight pollution accounted for 4 days, 2 days and 1 day, respectively. The details are shown in Table 2. To ensure the representativeness of the microbial activity in $PM_{2.5}$ for each sampling group, only experimental groups with 2 or more samples were analyzed to eliminate the variations due to a small sample size.

Because of the limited number of samples, we mainly analyzed the microbial activity when the air quality was excellent, and the results are shown in Fig. 4(a). When the air quality was excellent, after a window was open for 30 minutes, 20 minutes, or 10 minutes, the microbial activity in the indoor $PM_{2.5}$ increased, decreased, or increased compared with that before the window was open, respectively. When the window was open for 10 minutes, the indoor microbial activity increased significantly. When a window is open for a short period of time, a large number of outdoor microorganisms may enter the room, but the air exchange is incomplete and does not result in good ventilation, ultimately resulting in increased indoor microbial activity. When the window was open for 20 minutes, the microbial activity decreased, and the variation was different from that during 10 minutes of ventilation, probably because the indoor and outdoor air circulation and exchange become stable as the ventilation time increases, finally achieving good ventilation effects and reducing the indoor microbial activity. When the window was open

Table 2. Grouping of experiments with different ventilation times.

| Ventilation time	Pollution level	Range of AQI	Air quality level	Number of samples	Sampling date
30 minutes	Level 1	0–50	Excellent	7	4, 5, 6, 7, 8, 9, 10-Dec-2017
20 minutes	Level 1	0–50	Excellent	5	11, 12, 13, 19, 21-Dec-2017
	Level 2	51–100	Good	1	20-Dec-17
	Level 3	101–150	Slight pollution	1	15-Dec-17
10 minutes	Level 1	0–50	Excellent	4	23, 24, 25, 26-Dec-2017
	Level 2	51–100	Good	2	22, 27-Dec-2017
	Level 3	101–150	Slight pollution	1	18-Dec-17
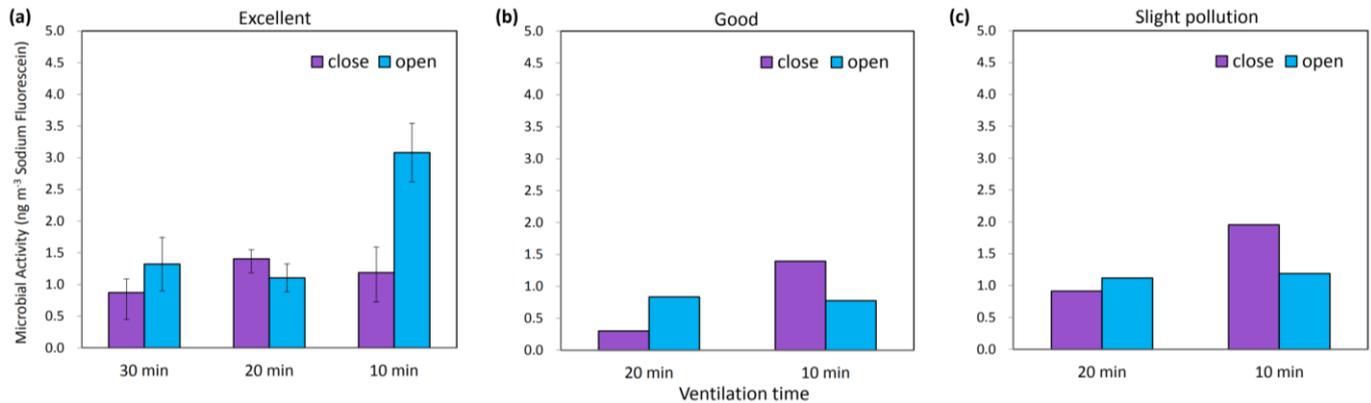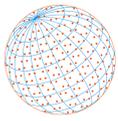
Fig. 4. Microbial activity under different ventilation time when the air quality was (a) excellent or (b) good or when (c) slight pollution was present. Error bars represent standard error of the mean.

for 30 minutes, the indoor microbial activity increased. In this case, the indoor and outdoor air exchange was imbalanced, accelerating the rate of impact of the outdoor environment and eventually triggering an increase in indoor microbial activity. Moreover, the microbial activity was very similar after the window was opened 20 minutes and 30 minutes, demonstrating that the air exchange was nearly complete after 20 minutes. In summary, when the air quality was excellent, a good ventilation effect was achieved in terms of microbial activity after 20 minutes of ventilation. Further increasing the ventilation duration had little influence on the indoor microbial activity and may decrease the indoor temperature excessively in winter; thus, the ventilation time for excellent air quality was 20 minutes.

Under good air quality and slight pollution, only 3-day and 2-day samples were collected, respectively. Because the number of samples collected was small, only a simple analysis was conducted. As seen from Fig. 4(b) and 4(c), when the air quality was good, the indoor microbial activity decreased after 10 minutes of window ventilation. However, when the indoor ventilation time increased to 20 minutes, the indoor microbial activity increased. Therefore, under polluted atmospheric conditions, 10 minutes of window ventilation achieves the purpose of indoor and outdoor air circulation and significantly reduces the indoor microbial activity. With the extension of the ventilation duration, the impact of the outdoor environment on the indoor environment increased, which led to an increase in the indoor microbial activity. Similarly, under slight pollution, the variation in microbial activity was identical after 10 minutes and 20 minutes of window ventilation. Hence, we can infer that under polluted conditions, a short ventilation duration achieves the best ventilation effect and can prevent excessive amounts of outdoor air pollutants from entering a room and causing adverse health effects. In summary, in terms of microbial activity as an indicator of air quality, this study suggests that when air quality is excellent, 20 minutes of window ventilation can achieve the ideal effect. Under polluted atmospheric conditions, the indoor and outdoor ventilation duration should be appropriately reduced.

At present, the world is severely impacted by COVID-19. Hundreds of millions of residents are required to isolate in their homes for a long period of time. Moreover, the transmission of the virus cannot rule out the existence of aerosol transmission at the current stage. Therefore, the evaluation of indoor air quality is particularly important. Using the convenient and quick measurement of microbial activity as an evaluation index and establishing a set of corresponding air quality standards may be more instructive to guiding democratic domestic life in the future. In this study, we determined a reasonable window ventilation time by measuring the indoor microbial activity. However, because of the remarkable effect of air pollution mitigation measures implemented in Beijing in 2017, few days had poor air quality. Hence, we need to further observe and verify the changes in microbial activity at different pollution levels. Furthermore, the study was performed in a sparsely staffed office, leading to limitations of the study. The community structure of indoor microorganisms was not analyzed, and the relevant information of pathogenic microorganisms was not further determined. Most importantly, the synergistic toxicity of microorganisms and other compounds adhering to $PM_{2.5}$ was not evaluated.
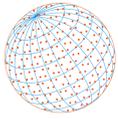
4 CONCLUSIONS

Using fluorescein diacetate hydrolysis, we measured microbial activity as an indicator of air quality and characterized the indoor and outdoor concentrations of microorganisms in Huairou, Beijing. Our results provide a theoretical basis for future evaluations of indoor air quality. During the sampling period, the indoor microbial activity produced 0.155–5.388 ng m^{-3}, with an average of 1.388 ng m^{-3}, of fluorescein sodium, which exceeded the range found outdoors; however, the increase was insignificant, and the indoor environment was also affected by the outdoor environment to an extent. The indoor microbial activity was highest during autumn and lowest during summer for all levels of air quality ("excellent air quality," "good air quality" and "slight pollution"). Additionally, the days with excellent air quality and those with good air quality exhibited minimal difference in activity during either season, and during summer, these days showed obviously higher activity than the slightly polluted ones. We discovered that variations in the indoor microbial activity were closely related to the ventilation of the room. When the air quality was excellent, 20 minutes of ventilation were ideal, but when the air quality was only good or slight pollution was present, this duration needed to be reduced.

This study has identified a novel indicator of indoor air quality, but much work remains. First, only a few samples were collected during light pollution, and no samples at all were collected during moderate, heavy or severe pollution. Second, no samples were collected during spring, precluding the comprehensive evaluation of the relationship between microbial activity and pollution and that between activity and season. Third, the sampling sites were not representative, and the community structures of the microorganisms and the pathogenic bacteria were not examined. Finally, we did not address the synergistic toxicity of microorganism and their products with other components. Nevertheless, this study offers a new method for evaluating indoor air quality by measuring microbial activity, as microorganisms are closely linked to human health.

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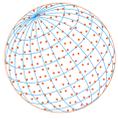
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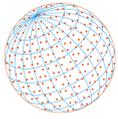
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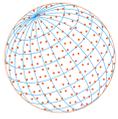
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