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Time-Dependent Size-Resolved Bacterial and Fungal Aerosols in Beijing Subway

Hanqing Fan[1+†], Xinyue Li[1,2+], Jiahao Deng[1], Guillaume Da[3], Evelyne Gehin[3], Maosheng Yao[1*]

[1] *State Key Joint Laboratory of Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China*
[2] *School of Water Resources and Environment, China University of Geosciences, Beijing 100083, China*
[3] *CERTES, Université Paris-Est Créteil, Laboratoire de Physique des Aérosols, Créteil 94000, France*

ABSTRACT

Despite of an important concern, human bioaerosol emission into subway is not well and directly characterized. Here, we used bioaerosol detector and next generation sequencing methods to investigate time-dependent bioaerosol size distributions in Beijing subway system between March and April, 2015. In contrast to weekends, weekday microbial aerosol results exhibited strong time dependence with higher bacterial and fungal aerosol levels up to 2083 CFU m^{-3} and 483 CFU m^{-3} observed, respectively, for the peak hours. During the peak hour (17:30–18:30), bioaerosol emissions at 0.8–3 μm was detected, while about three times higher concentration levels were observed compared to those during the off-peak hour (22:00–23:00). Similar bioaerosol size distributions were observed between ventilation outlets and subway platform air. During off-peak hours, subway bioaerosols had similar size distributions with the outside air. Sequence results revealed a vast array of airborne microbial species which varied from one station to another, but with *Aspergillus* spp. as dominant fungal species, and *Staphylococcus*, *Pseudomonas* as primary bacterial genera including human opportunistic ones. Our results provide direct online observations of human contributions to subway size-resolved bioaerosols, and enhancing ventilation system might help for controlling the exposure especially during the peak-hours.

Keywords: Bacteria; Fungi; Beijing subway; Fluorescent bioaerosol particle; Ventilation.

INTRODUCTION

It is now well accepted that exposures to airborne biological agents including microbial derivatives could cause numerous respiratory problems. Specifically, inhalation of infectious or opportunistic agents could disrupt microbial community in the respiratory system and damage the epithelium barrier, possibly leading to dysfunction of immune system (Bosch *et al.*, 2013). In addition, exposure to carcinogenic mycotoxins, e.g., aflatoxin and ochratoxin, can increase the risk of cancer (Douwes *et al.*, 2003). Under some situations, airborne transmission of infectious agents among people could cause pandemics such as severe acute respiratory syndrome (SARS) and H1N1 flu (Yu *et al.*, 2004; Thompson *et al.*, 2013).

Apart from many other bioaerosol investigations (Lee *et al.*, 2011; Xu *et al.*, 2011; Lee *et al.*, 2012), there has been a boom of interest in its exposures for underground transportation. As an indispensable means of public transportation for urban metropolises, subway system around the globe is carrying the largest commuter traffic daily. Humans themselves are shown to be an important source of bioaerosol emission, especially for indoor environment via coughing, breathing and shedding of human skin (Xu *et al.*, 2011; Hospodsky *et al.*, 2012; Noti *et al.*, 2012; Ghosh *et al.*, 2013; Adams *et al.*, 2015). In addition, human activities such as walking could also resuspend the particles (some of them could be the settled biological materials) into the air from the floor (Qian *et al.*, 2014). Due to the underground nature, subway stations are typically built in relatively confined spaces featuring a low degree of airflow, which can harbor still air and accumulate high concentrations of biological aerosols. Importantly, its microclimatic conditions and high volume of passengers also make underground stations and trains vulnerable to bioterrorism attacks. It is thus important to develop information about subway background bioaerosols in order to analyze bioaerosol exposure risks and differentiate them from those pathogenic ones for bio-alert.

Airborne bacteria and fungi in subway systems have been investigated in several cities in the world (Dong and

[+] These authors contributed equally.
[†] now at Department of Civil and Environmental Engineering, Stanford University, Stanford, California 94305, USA.

[*] Corresponding author.
Tel.: +86 01062767282
E-mail address: Yao@PKU.edu.cn

Yao, 2010; Hwang *et al.*, 2011; Kembel *et al.*, 2012; Robertson *et al.*, 2013; Dybwad *et al.*, 2014; Leung *et al.*, 2014; Afshinnekoo *et al.*, 2015; Hwang *et al.*, 2015). Previous studies showed a wide range of concentration levels of subway bioaerosols due to different places, seasons and different methods of sampling. Typically, the concentrations of bacterial aerosols in the subway system varied from 50 CFU m^{-3} to 12000 CFU m^{-3}, while those of fungal aerosols varied from 45 CFU m^{-3} to 1600 CFU m^{-3} (Gilleberg *et al.*, 1998; Picco *et al.*, 2000; Cho *et al.*, 2002; An *et al.*, 2004; Seino *et al.*, 2005; Yao and Mainelis, 2006; Bogomolova and Kirtsideli, 2009; Dong and Yao, 2010; Hwang *et al.*, 2010; Kim *et al.*, 2011; Dybwad *et al.*, 2012; Robertson *et al.*, 2013; Dybwad *et al.*, 2014; Hwang and Park, 2014). Through both culture and culture-independent methods, airborne bacterial and fungal community structures were investigated in subways (Dybwad *et al.*, 2012; Robertson *et al.*, 2013; Leung *et al.*, 2014). At the same time, influencing factors such as the subway structure design, humidity level and ventilation on bioaerosol levels and compositions were also subject to investigations (Kembel *et al.*, 2012). In most of these studies, the bioaerosol contribution from humans has been studied. However, all of these analyses were offline, failing to directly observe time-dependent (e.g., peak or off-peak hours) bioaerosol emissions from humans or decays in a real-time manner. Besides, size-resolved bioaerosol contribution from humans is also lacking. A comprehensive analysis combining the information of time-dependent culturable concentration (e.g., peak-hour and off-peak), size distribution and biological diversity are still lacking for most subways in the world.

Beijing underground transportation system (with ongoing constructions of several new lines for Greater Beijing Collective Development Plan), one of the largest subway systems in the world, transports more than 10 million people daily. However, the huge emission of bioaerosols from humans during the peak hour is not well understood and characterized. Here, the objectives of this work were to investigate: 1) What are the differences in time-dependent size-resolved bioaerosol concentrations, particularly between peak and off-peak hours for a typical Beijing subway system? 2) Are there any bioaerosol differences between different types of Beijing subways, e.g., year of construction and ventilation? and 3) What microbial agents are people exposed to when riding Beijing subway system? To address these questions, we employed a bioaerosol sensor-Ultraviolet Aerodynamic Particle Sizer (UV-APS) and a high throughput gene sequence method to analyze the air samples inside different subway systems during different time periods including peak and off-peak hours. The information obtained in this study can assist in managing bioaerosol exposure risk for underground transportation and providing recommendations for future and ongoing subway constructions.

MATERIALS AND METHODS

Sampling Site and Sampling Time

In this research, we selected four underground railway platforms as sites for monitoring: Beida (Line 4), Xizhi-a (Line 2), Xizhi-b (Line 4) and Xidan (Line 1) of Beijing subway system as shown in Fig. 1. The four platforms selected in this work were different in the commuter volume and the year of construction. The four stations Beida, Xizhi-a, Xizhi-b and Xidan were constructed in 2009, 1984, 2009 and 1992, respectively. Those newly constructed stations (Beida, Xizhi-b) were built with new ventilation systems. The Xizhi-a, Xizhi-b and Xidan stations were close to the urban center as illustrated in Fig. 1 with high traffic volume of passengers, while Beida station was relatively far away from urban center (close to the 4th Ring) with much smaller volume of passengers. Further, Xizhi-1 (Line 2) and Xizhi-b (Line 4) also serve as transfer stations, which share a similar volume of peak-hour passengers. The monitorings were conducted during the time period of from March to

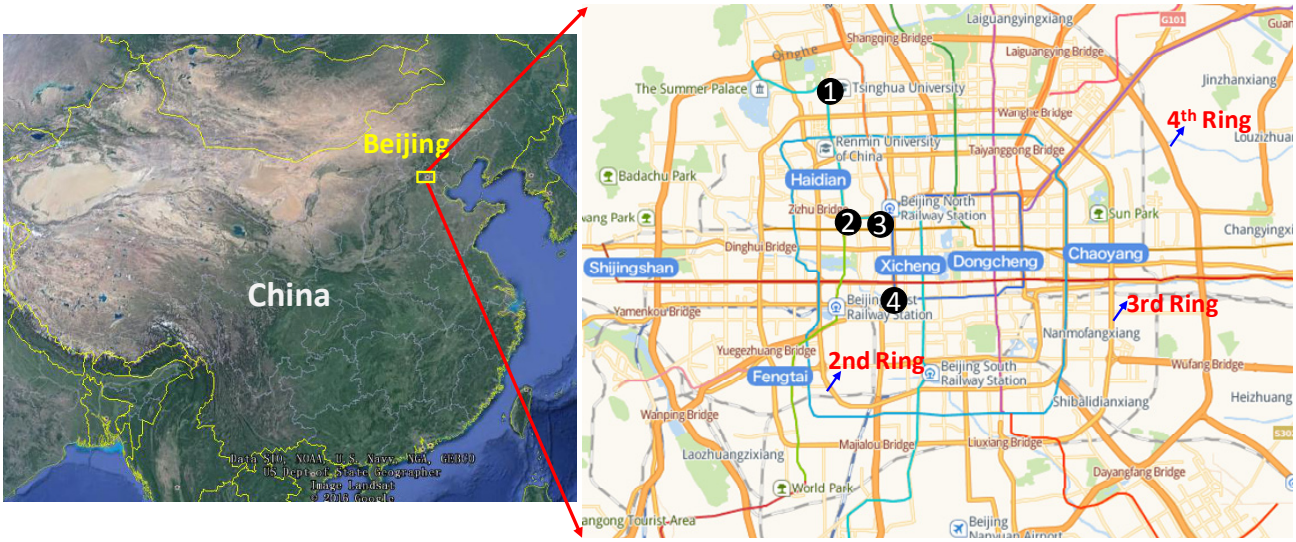

Fig. 1. Map of sampling sites of four different subway systems in Beijing with information of construction years: (1) Beida (2009), (2) Xizhi-a (1984), (3) Xizhi-b (2009), and (4) Xidan (1992).

April, 2015. The temperature and humidity data are listed in Table S1. According to the statistics on passengers of the Beijing subway system (Fig. S1), we selected five different time periods (time, # of passengers：5:30–6:30, 40000; 7:30–8:30, 460000; 11:00–12:00, 130000; 17:30–18:30, 370000; 22:00–23:00, 50000) during a typical work day for comparing the results of peak hours and off-peak hours and analyzing the bioaerosol contributions from passengers. These five periods included two peak-hour periods (7:30–8:30, 17:30–18:30) and three off-peak periods (5:30–6:30, 11:00–12:00, 22:00–23:00). We collected air samples every one hour from the subway stations and an outdoor environment adjacent to the Beida subway station. The indoor sampling sites were selected on the subway waiting platforms. The outdoor sampling sites were selected 50 m away from the subway stations. In addition, for every site air samples were collected both on weekdays and weekends. The sampling height was about 1.5 m above the floor.

Concentration of Culturable Bacterial and Fungal Aerosols

To study the concentrations of bacterial and fungal aerosols in the subway stations, we used a Reuter Centrifugal Sampler (RCS) High Flow (Biotest Diagnostics Corp., Denville, NJ) to sample the airborne bacteria and fungi in selected sampling sites. The RCS High Flow with a cutoff size of 1.2 μm (Yao and Mainelis, 2006) is a portable sampler utilizing both centrifugal and impaction forces for the biological particle collection and performed well when collecting bioaerosols (An *et al.*, 2004; Yao *et al.*, 2009; Zhen *et al.*, 2009). Agar strips were prepared using trypticase soy agar (Becton, Dickson and Company, Sparks, MD) for bacteria and Sabouraud dextrose Agar (Becton, Dickson) for fungi. We sampled bacterial and fungal aerosols six times in every one-hour period from each of the selected subway waiting platforms. Each air sample was collected at a sampling flow rate of 100 L of air for 1 min using the RCS device. Additionally, outside air was also sampled twice every hour the same as subway monitoring. After sampling, air samples were incubated at 30°C for 48 hours for bacteria and 72 hours for fungi. The colony forming units (CFUs) were manually counted, and the airborne culturable bacteria and fungi concentrations were calculated as the CFUs per unit of volume of air sampled by the sampler (CFU m^{-3}).

Characterization of Time-Dependent Size-Resolved Fluorescent Bioaerosol Particles

The time-dependent size-resolved fluorescent aerosol particles, which often refer to viable biological aerosol particles, in the subway stations were monitored by an Ultraviolet Aerodynamic Particle Sizer (UV-APS) (TSI, Inc.). The UV-APS detects the size distributions of viable bioaerosols by measuring the intrinsic fluorescence level in viable biological particles. We monitored both inside and outside the stations to analyze the differences. In addition, we also monitored the particles at the outlet of Beida subway station to investigate the effects of air ventilation system on the subway bioaerosols during the peak and off-peak hours. Because of security reasons, the UV-APS monitorings were only conducted at Beida subway station.

Taxonomical Classification

We used high-throughput gene sequence technology in taxonomical classification. The peak-hour samples collected by the RCS High Flow after culturing were further sequenced by the 454 GS-FLX pyrosequencing platform (Majorbio, Inc., Shanghai, China). The bacterial taxa were identified by 16S rRNA gene sequencing. The fungal taxa were identified by Internal Transcribed Spacer (ITS) sequencing. The entire procedure similar to the protocol previously reported was followed and detailed description can be found in the reference (Li *et al.*, 2013). In order to analyze the source of airborne microbes, the results of taxonomical classification were compared with Global Catalogue of Microorganisms (GCM) database. The source of a species was certificated by the isolation source records in the database.

Statistical Analysis

The results were subjected to statistical analyses using SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). We conducted normality testing using the Shapiro-Wilk test. Depending on whether the normality was fulfilled or not, the significance testing was further performed with the Student t test or the Mann-Whitney rank-sum test, respectively. Principal component analysis (PCA) was also used to study the difference among airborne bacterial communities from different subway stations. A p-value of 0.05 indicated a statistically significant difference.

RESULTS AND DISCUSSION

Concentration Levels of Culturable Bioaerosols

The time-dependent concentrations of culturable bacterial and fungal aerosols obtained by the RCS High Flow device at different sampling locations are shown in Figs. 2 and 3, respectively. As shown in the figure, the concentrations of airborne bacteria and fungi varied greatly with different time periods and environments, but higher levels of bacterial aerosols were observed in general. The culturable concentration of airborne bacteria ranged from 145 ± 51 CFU m^{-3} (5:30–6:30, Beida, off-peak) to 2083 ± 266 CFU m^{-3} (7:30–8:30, Xizhi-a, peak hour). In contrast, the culturable concentration of airborne fungi were observed to be lower, ranging from 38 ± 22 CFU m^{-3} (5:30–6:30, Beida) to 493 ± 222 CFU m^{-3} (7:30–8:30, Xidan, peak hour). Regardless of stations, morning (7:30–8:30) and afternoon (17:30–18:30) peak hours were shown to exhibit higher bioaerosol levels compared to those off-peak hours (5:30–6:30, 12:00–13:00; 22:00–23:00). This bioaerosol trend clearly followed the same one for time-resolved commuter traffic as shown in Fig. S1. In general, the average peak-hour culturable bacterial aerosol concentration (1174 CFU m^{-3}) was detected to be about three times as high as the off-peak hour (460 CFU m^{-3}). And the average concentration of peak-hour fungal aerosol concentration (291 CFU m^{-3}) was shown to be about two times as high as that of the off-peak hour one (142 CFU m^{-3}). Compared with bioaerosols outside the subway stations (380 CFU m^{-3} for bacteria, 121 CFU m^{-3}

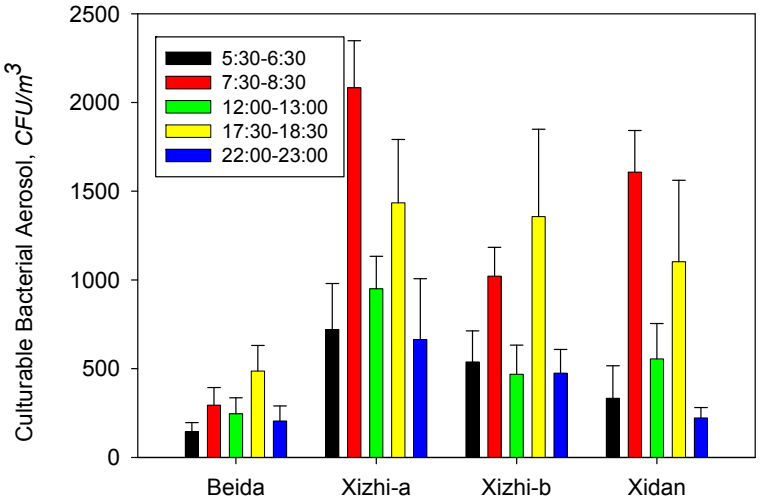

Fig. 2. Concentrations of culturable bacterial aerosols detected during different time periods (morning and afternoon rush hours, lunch time and also late night time) on different workdays (Beida on April 17, 2015, Thursday, Xizhi-a on May 16, 2015, Friday; Xizhi-b on May 23, 2015 Friday; Xidan on April 15, 2015 Tuesday).

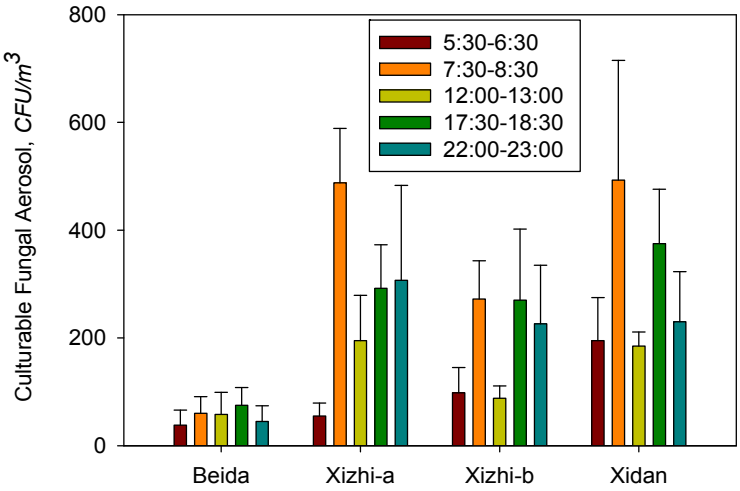

Fig. 3. Concentrations of culturable fungal aerosols detected during different time periods (morning and afternoon rush hours, lunch time and also late night time) on different workdays (Beida on April 17, 2015, Thursday, Xizhi-a on May 16, 2015, Friday; Xizhi-b on May 23, 2015 Friday; Xidan on April 15, 2015 Tuesday).

for fungi), the average subway platform exposure levels of Xizhi-a, Xizhi-b and Xidan were significantly higher, especially for bacterial aerosols as shown in Figs. 2 and 3. However, the concentration level of bioaerosols at Beida station (newly built and less crowded) was at the same level with those obtained from the outside adjacent (50 m away) to the station (Bacteria p-value = 0.236, Fungi p-value = 0.391, Mann-Whitney U Test). In contrast to the weekday concentrations, the concentrations of culturable bioaerosols on weekends demonstrated different characteristics on temporal variations (The trends were found different and time-resolved bioaerosol distributions were observed not to strongly depend on time as the weekday observation did) (Figs. S2 and S3). The diurnal pattern of subway bioaerosols observed here followed the same one reported in a Norwegian subway station study (Dybwad *et al.*, 2014). The results both from weekdays and weekends suggest that humans are important contributors to the subway bioaerosols, especially during the peak-hours.

Our results more or less resemble results reported from other subway systems around the globe. For example, in a recent Norwegian subway station study, the airborne bacterial concentration was detected up to 10^3 CFU m^{-3} with a daytime temporal variation up to 270-fold (Dybwad *et al.*, 2014). In a New York subway study, 2×10^4 microbial cells m^{-3} was detected in average from air (Robertson *et al.*, 2013). In Tehran subway, more than 1000 CFU m^{-3} airborne fungi were detected, significantly higher than those found in adjacent outdoor air (Hoseini *et al.*, 2013). With respect to bioaerosol health effects, the recommended threshold values for culturable bacteria and fungi are still disputed and vary greatly with countries. Although some guidelines are suggested on dampness and fungal growth by the world health organization (WHO, 2009), as of this

writing, no specific exposure limits are set yet for airborne biological agents. Some studies suggested the concentration of bioaerosols should be controlled under 5000 CFU m^{-3} in Denmark and Finland (Sigsgaard *et al.*, 1990; Tolvanen and Hänninen, 2005). The concentrations of both bacterial and fungal aerosols here were detected to be below this recommended threshold. However, a survey in Korea showed only 16% of its 25 stations exceeds the suggested bacterial concentration level of 800 CFU m^{-3} (Hwang *et al.*, 2010). Some authors from Finland also suggested that further measures should be implemented if the bacteria levels exceed 600 CFU m^{-3} or the fungal spore levels exceed 50 CFU m^{-3} in the indoor air during the winter (Salonen *et al.*, 2007). In a previous report, it was suggested that 150 CFU m^{-3} for the mixtures of fungal species rather than pathogens, and 300 CFU m^{-3} if the species is *Cladosporium* (Miller, 1992). In Beijing subway system, the peak-hour bacterial aerosol exposure level of some busy stations such as Xizhi-a station could exceed 2000 CFU m^{-3} which is certainly not acceptable according to these standards. Besides, fungal aerosol levels seemed to also exceed the proposed threshold of 150 CFU m^{-3}. Given that passengers have regular subway bioaerosol exposure during the peak hours, the commuters' potential health risk should be evaluated especially during the rush hours. Unfortunately, currently it is difficult to quantitatively assess the collective health effects of biological aerosol particles encountered in a typical indoor environment due to the lack of long-sought bioaerosol dose-response relationship.

The results from this work implied that the concentration level of bioaerosols of a subway station strongly depends on the two factors: air ventilation (older subway has poor ventilation) and the passenger volume. The inside subway exposure level in general was significantly higher than the outside for both bacteria and fungi especially during the peak-hours. However, the bioaerosol concentration of a newly built with a good air ventilation system and less crowded station can be reduced to a low level for both bacterial and fungal aerosols. For example, the Beida subway station here exhibited very low concentration of bioaerosols, especially with respect of fungal aerosols. No matter it was during weekday or weekend, the concentration of bioaerosols at Beida subway station was found to be at a similar level with the outside air (Bacteria p-value = 0.236, Fungi p-value = 0.391, Mann-Whitney U Test). The PCA analysis (see below) also confirmed Beida subway station and the outside air had similar bacterial community structure. The microbial community of Beida station showed a great similarity to the outside as a result of the air ventilation. Previously, the ventilation system was also shown to play a statistically significant role in reducing culturable bacterial aerosol concentrations in Seoul subway systems (Hwang *et al.*, 2015) and in a university laboratory (Hwang *et al.*, 2011). Although for Xizhi-a and Xizhi-b stations, they also serve as transfer platforms with similar passenger volume, they showed significant differences in bioaerosol concentration levels. Xizhi-a, the earlier built platform, demonstrated significantly higher level of bioaerosol exposure than Xizhi-b (Bacteria p-value = 0.001, Fungi p-value = 0.016, Mann-Whitney U Test). This observed difference is largely due to the installation of good ventilation system with the newly built station (Xizhi-b). On the other hand, the volume of commuters also strongly influenced the microbial aerosol concentration in the subway system as discussed above. It was indicated that in average for humans, bacterial cells outnumber human cells by a 10:1 ratio (Qin *et al.*, 2010). Therefore, it is no surprise that humans are an important bioaerosol contributor in an indoor environment such as subway system. Our work here provides direct evidence about human bioaerosol emission to subway air and role of ventilation (discussed below) by real-time monitoring bioaerosol levels using a fluorescent particle sensor.

Size Distribution of Time-Resolved Fluorescent Aerosol Particles

The distributions of fluorescent biological aerosol particles provide further information to understand the variation and fate of the bioaerosols in the subway system. Figs. 4 and 5 show the time-dependent size-resolved distribution patterns of fluorescent bioaerosol particles (viable biological particles) in waiting platforms, outdoor environments and outlets of subway ventilation system on a typical weekday at the Beida subway station. Fig. 4 shows clearly that the aerodynamic diameter of peak-hour fluorescent biological particles (2.5×10^6 particles m^{-3}) was mainly ranging from 0.8 to 2.5 μm (accounting for about 65% of the total) with concentration levels substantially higher (up to 2.5 times) than the outside. The fluorescent biological aerosol particles at the Beida station outlet also demonstrated a peak value between 0.8 to 2.5 μm (63% of the total) with similar levels (larger than 1 μm) compared to the subway platform during the peak hour. Given airborne bacteria may be attached to other particles, or found as agglomerates of many bacterial cells, the median aerodynamic diameter of particles containing culturable bacteria can be relatively larger. However, the fungal aerosols are usually larger than bacterial aerosols. The fungal spores are commonly found on the order of from 2 μm to 10 μm (Despres *et al.*, 2012). Therefore, the variations of size distribution from peak hours to off-peak hours actually dictate the variations of human-borne bacterial aerosols. While bioaerosol particles in subway platforms and outlets showed similar size-distribution patterns during the peak hours, the outdoor ones did not demonstrate such a trend as observed in Fig. 4. For the night time period of 22:00–23:00, the fluorescent bioaerosol particles were observed to decrease substantially especially for the peak size that was observed during the day both in subway platform and the outlet (Fig. 4). The concentration was even observed to be lower than that of the outdoor environment. However, the size distribution patterns for both Beida station subway platform and the outlet were detected to resemble each other closely. These results suggest that the ventilation can achieve a good air exchange between the outside and subway. In terms with subway air quality, this on the other hand implies that during a hazy day in Beijing outside air pollution could be easily introduced into the subway system if the subway ventilation operates regularly. Here, for the first time the UV-APS was employed to

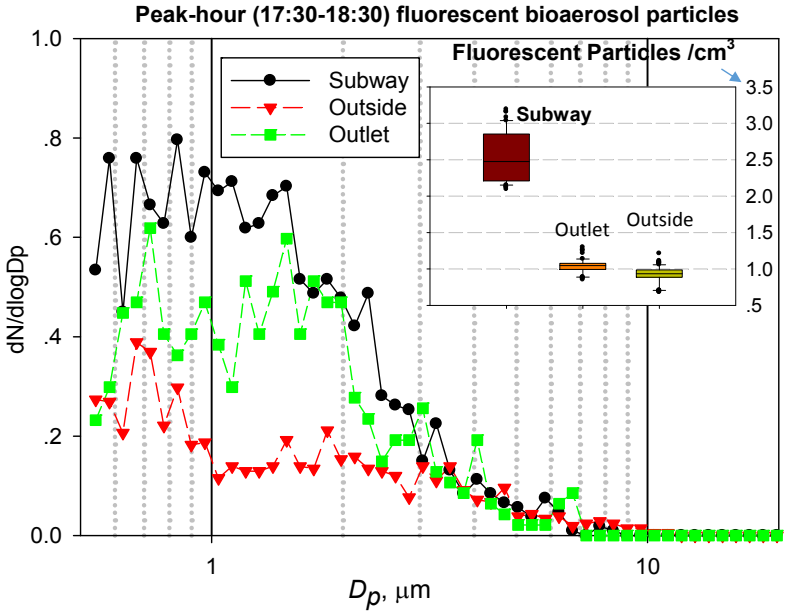

Fig. 4. Size-resolved fluorescent biological aerosol particles detected for subway, outlet of the subway Beida station and also the outside air during the peak-hour (17:30–18:30) on April 17, 2015.

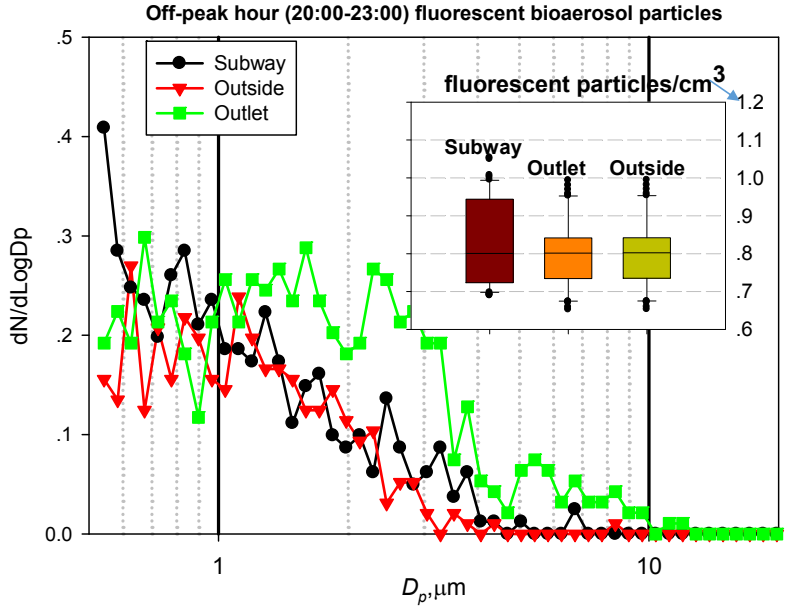

Fig. 5. Size-resolved fluorescent biological aerosol particles detected for inside, outlet of the subway Beida station and also the adjacent outside during the off-hour (22:00–23:00) on April 17, 2015.

investigate the time-dependent size-resolved bioaerosols in subway system and to see the impacts of commuters (i.e., their bioaerosol emissions) in a real-time manner. Comparing the peak-hour size distributions to off-peak hour ones, we can see that the influences from passengers were clearly reflected by the data recorded from the UV-APS.

Based on the characteristic of time-dependent data, two possible mechanisms may explain the results here. One possible explanation is that humans as direct emission source via breathing and skin are major contributors to airborne bacteria at subway stations. The rising of passenger numbers brings more emission sources for subway bioaerosols. The other possible explanation is that the bioaerosol concentrations were elevated by the re-suspension of settled dusts that contain microbes through walking (this might be the most possible case for fungi). The results from 454 GS-FLX pyrosequencing further proved that the subway bacterial aerosols and fungal aerosols originated from different emission sources as shown in Fig. 6. The subway bacterial aerosols were mainly from human emission sources, such as human skin, human lung and body fluids. On the contrary, the fungal aerosols were found to mainly

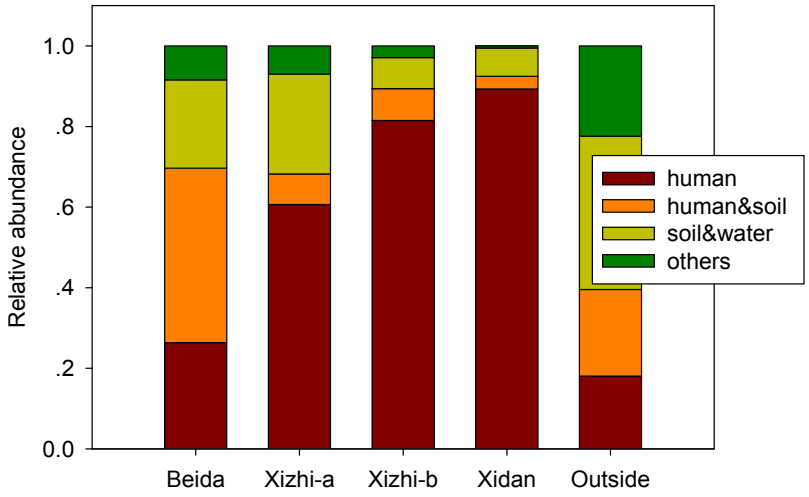

Fig. 6. Source contributions to the bacterial aerosol community structures for each of four monitoring stations. The source of a species was certificated by the isolation source records in the database of Global Catalogue of Microorganisms (GCM).

originate from soils. Therefore, the bacterial aerosols emitted from passengers accumulated at peak hours, which led to the high bacterial aerosol exposure level. In contrast, the fungal aerosols were found to mainly originate from the settled dust/soils (carried by passengers via shoes and clothes) in the subway system, or from the outdoor environment via the ventilation system. It was shown that the concentration of airborne bacteria in the subway was directly correlated with the number of commuters (Seino *et al.*, 2005). Nonetheless, the subway bioaerosol levels and species are suggested to be influenced by a complex interplay among architectural, meteorological, and anthropogenic factors (Leung *et al.*, 2014). In another work, the building attributes, e.g., the source of ventilation air, airflow rates, relative humidity and temperature, were found to be correlated with the diversity and composition of indoor bacterial communities (Kembel *et al.*, 2012). It was also found that poor ventilation and lower humidity level were associated with higher abundance of bacterial genera closely related to human pathogens in indoor environments (Kembel *et al.*, 2012). For peak-hours, human emissions including walking-induced resuspension are major sources of bioaerosols in the subway air according to the results obtained here.

The Sources of Microbial Aerosol Species

Here, we used GCM database to determine the source of microbial species. As for bacterial aerosols, we classified our results into four major categories in terms with their source. As shown in Fig. 7, some species were specifically human-originated, e.g., human skin, human lung and body fluid; some species were reported to be isolated from both humans and soil samples; some species were isolated from soil and water, while not reported from humans; some species might be from other sources such as grass and tainted food. Overall, subway airborne bacteria are most human originated. The source of bacterial diversity was different when comparing the subway bacterial aerosols to those above the ground. At Xizhi-a, Xizhi-b and Xidan stations, more than 60% relative abundance of bacteria was contributed specifically from human origin. And Beida, the station with smallest passenger volume and bioaerosol concentration, also attributed 60% of bacteria to human emissions. Unlike those subway aerosols, the outdoor bacterial aerosols were found to largely originate from soils (38%). Those human-related bacterial aerosols contributed 39% relative abundance to the bacterial aerosols above the ground. As for fungal aerosols, no species was specifically found to be human-generated. No matter in subway or outdoor environment, the relative abundance of fugal species from soil origin was more than 85%. Previously, in a New York subway study it was found that the microbial aerosol assemblage was composed of a mixture of genera and species emitted from soil, environmental water, and human skin (Robertson *et al.*, 2013). Among others, *Staphylococcus epidermidis*, *S. hominis*, *S. cohnii, S. caprae*, and *S. haemolyticus* from human skin were all detected in the subway air (Robertson *et al.*, 2013). However, one study in New City subway indicated that about half of the DNA recovered did not match any known organisms (Afshinnekoo *et al.*, 2015). Our results from the UV-APS here suggest that outdoor air during the off-peak hours is the dominant source of subway (with good mechanical ventilation) bioaerosols. Nonetheless, the results from the UV-APS might be negatively impacted by its inability of differentiating between certain fluorescent chemicals such as PAH and living bioaerosol particles at similar wavelengths.

Microbial Aerosol Community from Different Subway Stations

Top 20 bacterial genera in four subway stations and outdoor environment identified by 16S rRNA gene sequencing are listed in Table S2 and all fungal genera identified by ITS identification are shown in Table 1. As for bacterial aerosols, we identified 50 genera (87 species) of bacteria in the subway platform air samples using 16S rRNA gene sequencing. Compared with the outdoor air samples, which consisted of27 genera (43 species), the subway air samples showed a greater diversity in bacterial type during the peak-hour.

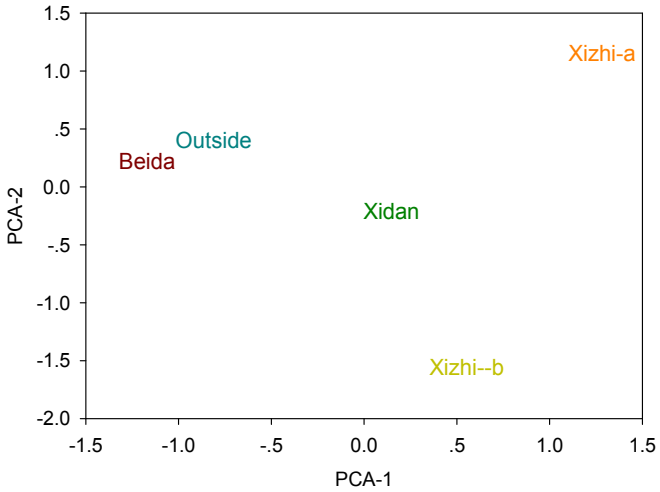

Fig. 7. Similarity analysis of airborne bacterial community on the genus level for the monitoring stations using principal component analysis (PCA). PCA-1 explained 30.7% of the variance. PCA-2 explained 28.2% of the variance.

However, the results were different for fungal aerosols. The ITS identification detected 7 genera (15 species) of fungi in the subway air samples and in contrast 9 genera (14 species) of fungi in the outdoor samples.

As observed from Table S2, subway and outdoor environment showed different dominant bacteria. The genera *Staphylococcus*, *Acinetobacter*, *Pseudomonas*, *Micrococcus*, and *Vagococcus* were found to account for a major fraction (> 80%) of the subway airborne bacterial aerosol community. Among the dominant bacteria in subway bioaerosols, *Staphylococcus* was the most abundant species. The outdoor air samples demonstrated different characteristics in bacteria composition compared to subway air. For example, the genera *Bacillus*, *Staphylococcus*, *Pantoea*, *Lactococcus* and *Acinetobacter* were detected to be dominant in the outdoor samples. *Bacillus* was found to mostly dominate in the outdoor air, while the proportion of *Staphylococcus* was relatively small when compared to that of subway air results. On the contrary, subway air and outdoor environments exhibited similarity in fungal composition (Table 1). In the subway air samples, *Aspergillus*, *Emericella*, *Rhizopus*, *Penicillium*, *Rhizomucor* were found to be the dominant species. Similarly, in the outdoor samples, more than 99% of fungi were *Aspergillus*, *Emericella*, *Rhizopus* and *Penicillium*. All of these genera of fungi detected are commonly found from the ambient air. Regardless of sampling site, *Aspergillus* had the largest relative abundance of more than 80%. Here, we also used principal component analysis (PCA) to analyze the differences in microbial diversity from different air samples (different subway systems and also outside air adjacent to the Beida subway station). We used the relative abundance of 50 bacterial genera for PCA analysis and reduced these lists with 50 parameters into a two dimensional vector (two independent linear combinations of 50 parameters). The factor-reduced analysis demonstrated that the bacterial aerosol community in the Beida station (newly built, less crowded with good air ventilation system) was very similar to the outside airborne bacterial community. For different stations, it seems their bacterial aerosol community structures were significantly different from one another as observed in Fig. 7. The same conclusions can be obtained from results listed in Table S3 by comparing OTU, Ace, Chao, Shannon and Simpson statistics together.

In addition to its plume emission, aerosol pathogenicity is also our major concern in the subway air. In general, we did not detect (with detection limits allowed) bacterial or fungal species with high pathogenicity in our subway air samples. Nonetheless, many opportunistic pathogens were isolated from the subway air. For example, *Staphylococcus haemolyticus* was found to be the top species in Beida, Xizhi-a and Xidan stations. *S. haemolyticus* is considered to be an important nosocomial pathogen. Human infections that can be caused by *S. haemolyticus* include: native valve endocarditis, septicemia, peritonitis, and urinary tract, wound, bone, and joint infection (Takeuchi *et al.*, 2005). The dominant species in the Xidan station was *Vagococcus fluvialis*, which is also reported to be connected with human infection (Teixeira *et al.*, 1997). In another work, airborne antibiotics resistant bacteria *Staphylococcus* spp. were also detected in Shanghai subway station (Zhou and Wang, 2013). In New York subway study, it was shown that majority (57%) of the bacteria recovered from the surfaces of the subway are not related to any human disease, however about 31% are potentially opportunistic bacteria, and 12% of them are human pathogens including detection of *Yersinia pestis* (Bubonic plague) and *Bacillus anthracis* (anthrax), and also *MRSA* (Afshinnekoo *et al.*, 2015). As for fungal aerosols, *Aspergillus fumigatus* and *Rhizomucor pusillus* detected from subway air were two species that can cause respiratory infection (Del Palacio Hernanz *et al.*, 1983; Latge, 1999). In a previous subway study, 50 fungal species were found, among which *Acremonium, Aspergillus, Cladosporium and Penicillium* were the most prevailing genera in St. Petersburg , Russia (Bogomolova and Kirtsideli, 2009). Another study found that majority of the fungal species detected was *Aspergillus versicolor* and the structure design of the subway station played a significant role in distribution and composition of fungal species in

Table 1. Relative abundances of all fungal genera identified in each monitoring station.

Beida		Xizhi-a		Xizhi-b		Xidan		Outside	
Genera	RA (%)	Genera	RA (%)	Genera	RA (%)	Genera	RA (%)	Genera	RA (%)
Aspergillus	95.35	Aspergillus	81.06	Aspergillus	97.16	Aspergillus	97.65	Aspergillus	93.91
Emericella	2.95	Rhizomucor	15.42	Rhizopus	2.27	Penicillium	1.76	Emericella	2.40
Rhizopus	1.05	Rhizopus	1.09	Trichocomaceae	0.57	Trichocomaceae	0.35	Rhizopus	1.80
Trichocomaceae	0.31	Penicillium	0.94			Emericella	0.23	Penicillium	1.29
Rhizomucor	0.26	Emericella	0.80			Rhizopus	0.01	Trichocomaceae	0.36
Penicillium	0.07	Trichocomaceae	0.68					Alternaria	0.13
		Guehomyces	0.01					Candida	0.04
								Pleosporaceae	0.04
								Guehomyces	0.02

Tokyo, Japan (Kawasaki *et al.*, 2010). Different from other subway stations, *Penicillium spp.* (34.88% of total airborne fungi) and *Alternaria* spp. (29.33% of total airborne fungi) were detected in subway and outdoor air, respectively, in Tehran, Iran (Hoseini *et al.*, 2013). In addition to moisture, human activities, and ventilation, these differences might in part due to different climatic conditions for different subways as studies showed different fluorescent bioaerosol size distributions for different climate zones (Wei *et al.*, 2015) and seasons (Heo and Lee, 2015). These results provide valuable information about the airborne microbiota in the subway system with respect to exposure risk management and discerning pathogenic species out from subway air.

In this work, the detection of high bioaerosol concentration level and presence of opportunistic species in the subway air underscores the potential health risks for passengers and the need of public health management. Among others, human occupants were shown to play a dominant role in contributing to bioaerosol exposure level in the subway system, especially during the peak hours. As a subway commuter, one might be exposed to a vast array of new species other than from their own and could be even quite different when riding through different subway stations which were constructed with different ventilation systems and operating conditions. Unfortunately, the relevant potential inhalation health risk except those known pathogens (currently most of the studies are only limited to environmental monitoring and health speculation) has not been analyzed, which is however greatly needed presently. From the results of our study, enhancing ventilation system seems to be one of the effective measures to control the subway bioaerosol exposure level. When balancing the cost, enhanced or improved ventilation during peak-hours particularly is highly recommended for some stations with high volume of passengers. This effort might also simultaneously help reduce the potential health impacts during a possible bio-terrorism attack or infectious disease pandemics because the ventilation can rapidly ventilate out pathogenic aerosols. Our work provides direct online observations of human contributions to subway size-resolved bioaerosols, and the results obtained here can also lend information to developing bio-hazard alert system for discerning specific pathogenic species out of microbial clouds in subway indoor environments.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

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