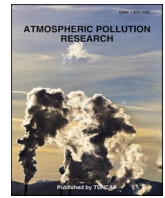




Contents lists available at ScienceDirect

Atmospheric Pollution Research

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

Assessment of seasonal variations in concentration, particle-size distribution, and taxonomic composition of airborne fungi in a courtyard space

Dingmeng Wu^{a,b}, Ying Zhang^b, Wenwen Qin^b, Yu Tian^b, Angui Li^{b,*}, Li'an Hou^b, Yicun Hou^b, Bingyang Han^b, Tianqi Wang^b, Jing Xiong^{a,b}, Ran Gao^b

^a School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an, Shaanxi, 710055, PR China

^b School of Building Services Science and Engineering, Xi'an University of Architecture and Technology, Xi'an, Shaanxi, 710055, PR China

ARTICLE INFO

Keywords:

Courtyard space
Fungal air quality
Seasonal variation
Sampling technology
Taxonomic composition
Exposure risk assessment

ABSTRACT

As people desire contact with the natural environment and comfortable living, modern buildings are increasingly incorporating the design of courtyard spaces. Hence, it is of interest to investigate the fungal air quality of courtyard spaces. This study aimed to investigate seasonal patterns of airborne fungal concentration, particle-size distribution, and taxonomic composition by using air microbial samplers and combining culture and molecular techniques. The results indicated that fungal concentration varied between seasons, peaking in autumn (825 ± 260 CFU/m³) and being the lowest in winter (617 ± 197 CFU/m³). Spearman's correlation analysis showed that fungal concentrations were positively correlated with relative humidity and wind speed. The relative humidity and wind speed supported fungal dispersion, whereas temperature negatively affected fungal dispersion in a multiple linear regression model. The fungal concentrations corresponding to "not excellent" in the Air Quality Index (AQI: >50) were higher than those corresponding to "excellent" (AQI: 0–50). The airborne fungal particle sizes in the range 1.1–3.3 μ m (>58%) were predominant in all four seasons. In all four seasons, the airborne fungi had count median diameters ranging from 1.60 μ m to 1.80 μ m, allowing the fungi to penetrate the terminal bronchi. *Verticillium* (75.9%) and *Cladosporium* (27.4%) were the most abundant fungal genera in spring and summer, respectively. *Flammulina* was predominant in autumn and winter, accounting for 35.2% and 31.3%, respectively. Moreover, 14 fungal genera that contained allergenic or pathogenic species were identified in all four seasons. The highest mean average daily dose rates of airborne fungi was found in autumn. These findings will provide information for creating a more reasonable, comfortable, and healthier courtyard space air environment.

1. Introduction

The rapid modern developments in economics, science, and technology have accelerated urbanization (Deng, 2010; Shi and Sun, 2013). As a consequence, urban spaces have blocked the access of human beings to nature. To some extent, the addition of courtyard spaces has met people's demand for contact with nature (Jiao, 2007), and thus has become a trend in modern architecture. A courtyard space is a "the building without a roof," typified by an outdoor space that is enclosed within the building walls, where sun, wind, rain, and snow can be experienced, and there are plants, flowers, and other auxiliary facilities within it (Deng, 2010; Jiao, 2007; Li, 2004). A courtyard space not only

meets people's preferences for lifestyle, leisure, communication, and viewing but also plays an important role in solving problems of indoor and outdoor air exchange, temperature, and humidity (Deng, 2010; Jiao, 2007). Wang et al. (2007) emphasized the necessity and importance of courtyard spaces on university campuses. Hence, due to the complexity of the internal environment and the importance and function of courtyard spaces, it is meaningful to investigate its fungal air quality.

Due to the fact that the courtyard space is finite, exposure to a space with a high concentration of airborne fungi may cause airborne-related diseases (Fung and Hughson, 2003; Kim et al., 2017). Exposure to airborne fungi may be associated with respiratory symptoms such as coughing, asthma, bronchitis (Reboux et al., 2009, 2019), allergies,

* Corresponding author.

E-mail address: liag@xauat.edu.cn (A. Li).

<https://doi.org/10.1016/j.apr.2020.10.014>

Received 27 August 2020; Received in revised form 17 October 2020; Accepted 17 October 2020

Available online 19 October 2020

1309-1042/© 2020 Turkish National Committee for Air Pollution Research and Control. Production and hosting by Elsevier B.V. All rights reserved.

hypersensitivity diseases (Fung and Hughson, 2003), and even cancer (Dvorackova et al., 1981). The deposition of fungal spores in the human respiratory tract depends on particle size (Horner et al., 1995). More importantly, fungal particles smaller than 5 μm are more likely to penetrate the lower respiratory system (Kim and Kim, 2007), possibly leading to allergies or asthma (Horner et al., 1995). Thus, investigating the fungal concentrations and particle size in courtyard spaces is important for human health.

Airborne fungal taxonomic compositions are important in determining human health risk levels due to the presence of pathogens and allergens in the airborne fungi (China, 2006; Horner et al., 1995; Simon-Nobbe et al., 2008). Horner et al. (1995) found that more than 80 fungal genera were associated with symptoms of respiratory tract allergies. There are 59 fungal taxonomy lists in the directory of pathogenic microorganisms in human infection promulgated by the Ministry of Health of the People's Republic of China (2006). Thus, to understand composition of airborne fungi in depth, high-throughput sequencing technology has been employed as an effective method for detecting airborne fungal diversity (Qi et al., 2020; Wei et al., 2015; Wu et al., 2019) and for determining the taxonomic composition of fungal populations (Peccia et al., 2011). This method can perform hundreds to thousands of identifications on each sample (Dannemiller et al., 2014). Thus, the identification of fungal diversity by high-throughput sequencing is more advanced and more accurate than the previous identification of species by microscopy (Asif et al., 2019; Fang et al., 2005; Kim and Kim, 2007; Li et al., 2012; Li et al., 2016; Mashat, 2015).

The courtyard space is a transition area between the interior of the building and the natural environment. It is also a place where people often rest and relax; however, few studies have evaluated the fungal air quality of courtyard spaces. Hence, in this study, we provide firsthand information concerning airborne fungi in a courtyard space. The analysis combines air microbial samplers, existing culture methods, and emerging DNA-based methods to evaluate airborne fungi during a one-year period. The seasonal variations in airborne fungal concentration, particle size distribution, and taxonomic composition were determined. Furthermore, the relationships between airborne fungal concentrations and environmental factors were analyzed.

2. Materials and methods

2.1. Sampling site

Airborne fungal samples were collected at the courtyard space of the library building in the Xi'an University of Architecture and Technology

(108°97'31.63" N, 34°24'37.36" E) located in the inner city of Xi'an, Shaanxi Province, China. The building is surrounded by plants and classroom buildings. There are no industrial pollution sources surrounding the building. The courtyard space is in the middle of the library building, between its indoor and outdoor environments, and is subjected to the outside weather conditions (such as rain, snow, and direct sunlight). The studied courtyard space is a place where teachers, students, and library staff rest, read, and communicate. During our sample collection, there were always 7–8 people walking around the corridor and the courtyard space. Trees, potted plants, herbs, and flowers surrounded the courtyard space. There are two restrooms and some lidded trash cans (250 L) at the corners of the courtyard space. The sampling point was placed in the center of the courtyard space (Fig. 1).

2.2. Airborne fungal sampling strategy

Airborne fungal samples were taken using a viable Andersen sampler (FA-3, Xi'an, China) and BioSampler (SKC Inc., Eighty Four, PA, USA) from September to November 2018 (autumn); December 2018 to January 2019 (winter); March to May 2019 (spring); and June to July 2019 (summer). The sampling was performed between 9:00 and 11:30 a.m. and 14:00 and 16:30 p.m. on each sampling day. The sampling duration for the viable Andersen sampler was 30 min (including the period when the samplers were sterilized with 75% ethanol, collection of three air samples, and sealing the plates); for the BioSampler, the duration was 110 min (collecting three air samples) at each time point. A total of 936 plates were collected using the viable Andersen sampler, and a total of 60 phosphate-buffered saline (PBS) samples were taken using the BioSampler. The details concerning the air sample collection are shown in Table S1 (Supplementary material).

A six-stage Andersen sampler with different size ranges (I: $>7.0 \mu\text{m}$, II: $4.7\text{--}7.0 \mu\text{m}$, III: $3.3\text{--}4.7 \mu\text{m}$, IV: $2.1\text{--}3.3 \mu\text{m}$, V: $1.1\text{--}2.1 \mu\text{m}$, VI: $0.65\text{--}1.1 \mu\text{m}$) was employed to collect the airborne fungal samples, operating at an airflow rate of 28.3 L/min for 5 min. Three consecutive replicates were collected at each time point using the viable Andersen sampler to calculate the mean value. Potato dextrose agar with chloramphenicol (which restricts the growth of bacteria) was used to culture the collected airborne fungi. The BioSampler (airflow: 12.5 L/min) was filled with 20 mL of PBS and operated for 30 min. To represent the human breathing zone, both samplers were set at 1.5-m above the ground. The airborne fungal concentrations and particle sizes were obtained using the samples collected with the viable Andersen sampler. The BioSampler was used to determine the taxonomic composition of airborne fungi (Fig. S1, supplementary material).

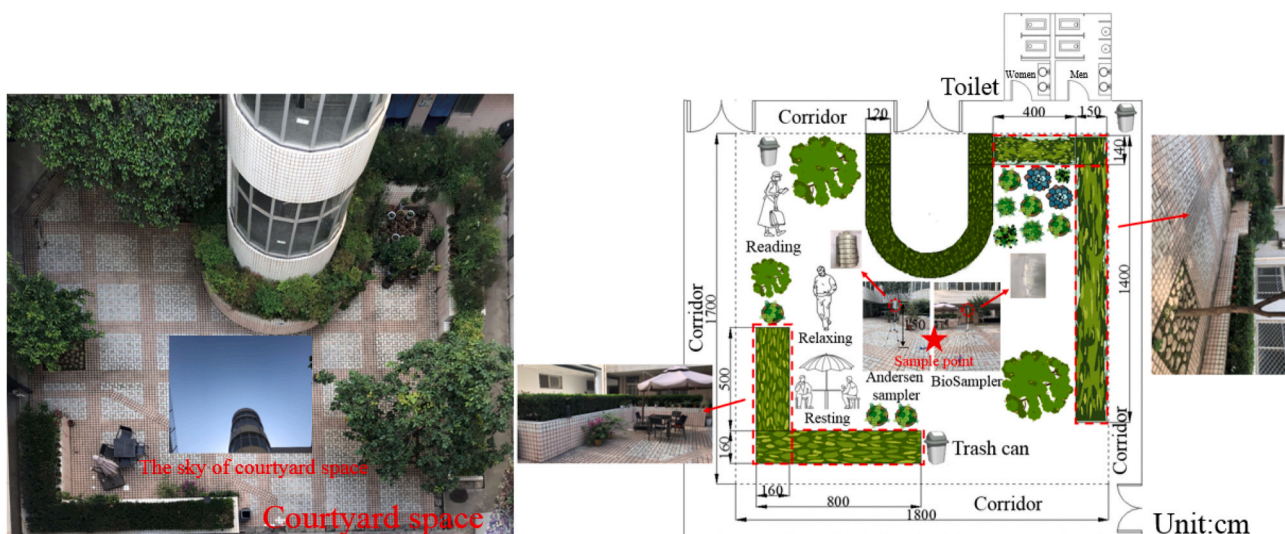


Fig. 1. Schematic diagram of the courtyard space and sample collection location.

After sampling, the plates and PBS samples were transferred to the laboratory. Plates were incubated at 28 °C for 3–5 days. CFU values were corrected using the positive hole correction table as previously described (Andersen, 1958). The fungal concentrations obtained were expressed as CFU/m³ (Kozdroj et al., 2019). The calculation equations of the proportions of airborne fungi at each stage and the airborne fungal concentrations are provided in Section S1 (Supplementary material). The collected PBS samples were deposited into sterilized 50-mL tubes and stored at –80 °C for subsequent DNA extraction and amplification.

During the each viable Andersen sampling period (5 min at each time point), without affecting the measurement of airborne fungal sample, the concentrations of particulate matter (PM_{2.5} and PM₁₀) were recorded every 30 s using a DUSTTRAKTM II-8531 aerosol monitor (TSI, USA). Temperature, relative humidity, and wind speed were recorded at every 1 min using a multichannel portable tester-8386A (TSI, USA).

2.3. Count median diameter of fungi

The count median diameter (CMD) of airborne fungi (μm) was calculated as described previously (Hu et al., 1994). Briefly, particle size was converted to logarithms (X), and the probability unit (Y) corresponding to the cumulative percentage of particle numbers at all levels was calculated using the probit table. Then, the regression coefficient B was calculated according to equation (1):

$$B = \frac{\sum XY - (\sum X)(\sum Y)/N}{\sum X^2 - (\sum X)^2/N} \quad (1)$$

The CMD was calculated according to equation (2)

$$CMD = 10^{(5 - Y_{mean} + BX_{mean})/B} \quad (2)$$

where N is the number of groups of particles, X_{mean} is the logarithmic mean, and Y_{mean} is the average of the probability units.

2.4. Treatment of BioSampler collected samples – DNA extraction and processing

All collected PBS samples using the BioSampler in each season were thoroughly mixed for DNA extraction. The genomic DNA was extracted using splitting and phenolic chloroform extraction, and then the DNA was checked by running the samples on 1.2% agarose gels. After the DNA extraction, fungal DNA was amplified by the polymerase chain reaction (PCR) using the following set of primers: fungi-ITS1 forward primer (5'-CTTGTCATTTAGAGGAAGTAA-3') and ITS1 reverse primer (5'-GCTGCGTTCTTCATCGATGC-3'); the ITS1 is the target of taxonomic positioning and taxonomic composition analysis in fungal ecology research (An and Yamamoto, 2016). The PCR products were purified using an AxyPrep DNA gel extraction kit (Axygen, USA) and quantified using FTC-3000 TM real-time PCR (Shanghai Funlyn Biotech Co., Ltd., China). Then, the purified PCR products from different samples were pooled in equimolar amounts to complete the library construction and paired-end sequenced (2 × 300 bp) on an Illumina Miseq platform. Reads from all samples were run through Trimmomatic (version 0.35) to remove low-quality reads using the parameters Slidingwindow: 50:20, Minlen: 50, and trimmed reads were then further merged using the FLASH program (version 1.2.11). Finally, the fungal ITS sequences were assignment for taxonomy against the Unite database. Raw sequencing data were deposited at the NCBI Sequence Read Archive with the accession number PRJNA667152.

2.5. The evaluation of health risk

In order to assess the health risk to people (using ages between 20 and 40) in the courtyard space, the average daily dose rates (ADD) of airborne fungi by inhalation were estimated based on the model recommended by the United States Environmental Protection Agency. The

ADD through inhalation were evaluated using equation (3) (Yang et al., 2019a, b).

$$ADD = \frac{C \times IR \times EF \times ED}{BW \times AT} \quad (3)$$

where ADD is the average daily doses of exposure (CFU/(kg·d)); C is the airborne fungal concentration (CFU/m³); IR is the inhalation rate (m³/d); EF is the exposure frequency (d/yr); ED is the duration of exposure duration; BW is the body weight (kg), and AT is the average lifetime (d). The detailed values of each parameter in our study are shown in Table S2 in the supplementary material.

2.6. Statistical analysis

The mean values, standard deviations, and ranges of the airborne fungi and environmental factors were calculated. The data were analyzed by Origin 2016 (OriginLab, USA) and SPSS 25 (IBM Corp., USA). The Kolmogorov–Smirnov test was used for normality testing. As the data distributions were generally skewed, the multi-sample Kruskal–Wallis H test was used to analyze seasonal variation in airborne fungal concentration, environmental factors, count median diameter, and average daily dose of exposure. The Mann–Whitney U test was used for classification of airborne fungal concentrations based on the air quality index. Spearman's correlation analysis was used to assess the relationships between the airborne fungal concentration and environmental factors. Additionally, a multiple linear regression model was used to analyze the effects of several variables (temperature, relative humidity, particulate matter, and wind speed) on airborne fungal concentration.

3. Results and discussion

3.1. Seasonal variation in airborne fungal concentration

The sampling campaigns occurred during the four seasons of spring, summer, autumn, and winter in the courtyard space, and the airborne fungal concentrations are presented in Table 1. The airborne fungal concentrations varied by season (the multi-sample Kruskal–Wallis H test). These results are in agreement with previous studies that reported seasonal variation in airborne fungal concentrations (Fang et al., 2005; Li et al., 2011, 2017b; Yamamoto et al., 2012). The airborne fungal concentrations decreased from spring to summer, increased again in autumn, then decreased toward winter. In general, the concentrations of airborne fungi in autumn (825 ± 260 CFU/m³) were higher than in spring (719 ± 299 CFU/m³) and summer (661 ± 327 CFU/m³), while the lowest concentrations occurred in winter (617 ± 197 CFU/m³). Therefore, students, teachers, and staff relaxing in the courtyard space were more exposed to potential health risks of airborne fungi during autumn. The seasonal variability of airborne fungal concentration in this study was consistent with the findings by Li et al. (2017b) for the roof of a building in Xi'an, by Li et al. (2011) for the roof of a building on a campus in Qingdao, and by Yamamoto et al. (2012) for the rooftop of a five-story building in New Haven. Previous studies have shown that fungal growth was influenced by UV light, nutrient levels, solar radiation, and weather conditions, among other factors (Fang et al., 2008; Jones and Harrison, 2004; Zhai et al., 2018), and that these factors are not independent; rather, there are interactions. Hence, some vegetation withered or grew slowly in winter in the courtyard space, and this may be the cause of low airborne fungal concentrations in winter (Kozdroj et al., 2019; Li et al., 2017b) consistent with previous work (Fang et al., 2005; Li et al., 2017b). Additionally, strong solar radiation and UV light provide unfavorable conditions for the growth and survival of airborne fungi in the summer (Karra and Katsivela, 2007; Zhai et al., 2018).

Due to the fact that environment of courtyard space is similar to outdoor space, this study compared the concentrations of airborne fungi

Table 1

Seasonal variation in the airborne fungal concentration and environmental parameters.

Item	Season								<i>p</i> -value ^b
	Spring		Summer		Autumn		Winter		
	Mean±SD ^a	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Fungal concentration (CFU/m³)	719 ± 299	184–1329	661 ± 327	360–1385	825 ± 260	388–1279	617 ± 197	254–905	0.001
Relative humidity (%)	48 ± 18	25–74	61 ± 12	45–83	54 ± 13	36–72	38 ± 15	22–73	0.010
Temperature (°C)	22.4 ± 4.6	13.2–29.7	28.9 ± 1.8	25.0–31.2	22.5 ± 3.8	15.7–27.6	5.9 ± 2.4	2.9–9.4	0.000
PM _{2.5} (µg/m ³)	50 ± 30	5–106	38 ± 20	6–80	44 ± 17	17–81	92 ± 79	7–257	>0.05
PM ₁₀ (µg/m ³)	90 ± 51	7–168	63 ± 35	7–126	77 ± 33	23–135	160 ± 140	16–467	>0.05
Wind speed (m/s)	0.17 ± 0.11	0.02–0.47	0.10 ± 0.06	0.02–0.18	0.19 ± 0.22	0.01–0.60	0.05 ± 0.06	0.01–0.20	0.004

^b The multi-sample Kruskal–Wallis *H* test was used to examine seasonal variation.^a Mean: mean fungal concentration. SD: standard deviation.

with outdoor airborne fungi in several other areas. The annual mean concentration of airborne fungi in the courtyard space was 706 ± 279 CFU/m³, higher than that found in the studies performed in Xi'an (399 CFU/m³) (Li et al., 2017b), the Qingdao coastal region (99 CFU/m³) (Li et al., 2011), the outside an eatery in India (282 CFU/m³) (Priyamvada et al., 2018) or in the industrial urban area of south Cairo (216 CFU/m³) (Abdel Hameed et al., 2012). The higher airborne fungal concentrations found in this study may be due to the limited outdoor space in the courtyard, the surrounding plants and flowers (Kozdroj et al., 2019; Zhai et al., 2018), the toilets (Ohagim et al., 2017) and/or the garbage cans (Lis et al., 2004) as sources. However, the mean airborne fungal concentrations were lower than the outdoor fungal concentrations at the Research Center for EcoEnvironmental Sciences (1357 CFU/m³), the Beijing Botanical Garden (1332 CFU/m³) (Fang et al., 2005) and an outdoor area in Xinxiang (12729 CFU/m³) (Yan et al., 2019). Hence, diverse environmental conditions and different environmental parameters around different sampling locations led to different concentrations of airborne fungi (Zhai et al., 2018).

3.2. Relationship between fungal concentration and environmental parameters

Zhai et al. (2018) reported that airborne fungal concentrations change with environmental factors. Table 1 shows mean values and ranges of the relative humidity, temperature, particulate matter, and wind speed, each of which differed according to the season. Distinct fluctuations of relative humidity were observed for the four seasons. The highest relative humidity was 61% in summer, and the lowest was 38% in winter. Similar temperatures were measured in spring and autumn,

and the mean temperature dropped from 28.9 °C in summer to 5.9 °C in winter. For the particulate matter, the concentrations of PM_{2.5} and PM₁₀ measured in spring, summer, and autumn fluctuated slightly, while they increased to 92 µg/m³ and 160 µg/m³ in winter. The wind speed in all four seasons varied between 0.01 and 0.60 m/s.

Spearman's correlation coefficients of these parameters with the measured fungal concentrations are shown in Table 2. The relationships between total airborne fungal concentrations and respirable fungal concentrations with environmental parameters showed similar results in our study. There was a positive correlation between total airborne fungal concentration and relative humidity ($r = 0.686$, $p = 0.000$), as well as a positive correlation between respirable fungal concentrations and relative humidity ($r = 0.648$, $p = 0.000$). Many related studies have reported that there was a positive correlation between airborne fungal concentrations and relative humidity (Abdel Hameed et al., 2012; Frankel et al., 2012; Ponce-Caballero et al., 2013; Wu et al., 2020). However, there were no correlations between total fungal concentration and temperature, PM_{2.5}, or PM₁₀ ($p > 0.05$). Likewise, correlations were not found between respirable fungal concentrations and temperature, PM_{2.5} or PM₁₀ ($p > 0.05$). Previous studies have shown that the effect of relative humidity on fungal concentrations was greater than those of temperature and particulate matter (Abdel Hameed et al., 2012). Our study corroborated the findings of Ponce-Caballero et al. (2013) who reported that relative humidity was the environmental parameter that had the highest correlation with airborne fungal concentration; high relative humidity favors fungal growth, resulting in elevated fungal concentration (Li et al., 2017b). There were positive correlations between wind speed and total airborne fungal concentration ($r = 0.471$, $p = 0.000$) and respirable airborne fungal concentration ($r = 0.490$, $p =$

Table 2

Spearman's correlation between fungal concentration and environmental factors, and fungal concentrations and environmental factors in a multiple linear regression model.

Item		Environmental factors					<i>R</i> ²
		Relative humidity (%)	Temperature (°C)	PM _{2.5} (µg/m ³)	PM ₁₀ (µg/m ³)	Wind speed (m/s)	
Spearman's correlation	Total fungal concentrations (CFU/m ³)	$r = 0.686$ $p = 0.000$	$r = -0.108$ $p > 0.05$	$r = 0.214$ $p > 0.05$	$r = 0.241$ $p > 0.05$	$r = 0.471$ $p = 0.000$	–
	Respirable fungal concentrations ^a (CFU/m ³)	$r = 0.648$ $p = 0.000$	$r = -0.113$ $p > 0.05$	$r = 0.187$ $p > 0.05$	$r = 0.213$ $p > 0.05$	$r = 0.490$ $p = 0.000$	–
Multiple linear regression model	Total fungal concentrations (CFU/m ³)	$B = 11.142$ $\beta = 0.665$ $p = 0.000$	$B = -9.592$ $\beta = -0.306$ $p = 0.009$	$B = -0.030$ $\beta = -0.005$ $p > 0.05$	n. a.	$B = 760.224$ $\beta = 0.375$ $p = 0.000$	0.668 $F =$ 23.599 $p = 0.000$
	Respirable fungal concentrations (CFU/m ³)	$B = 8.849$ $\beta = 0.609$ $p = 0.000$	$B = -8.265$ $\beta = -0.304$ $p = 0.011$	$B = -0.235$ $\beta = -0.045$ $p > 0.05$	n. a.	$B = 760.439$ $\beta = 0.432$ $p = 0.000$	0.651 $F =$ 21.931 $p = 0.000$

n. a.: To avoid multicollinearity in the independent variables of the multiple linear regression model, PM₁₀ was deleted, and other independent variables were confirmed to be independent.

r: Spearman's correlation coefficient, *B*: Unstandardized coefficient, β : Standardized coefficient, *R*²: Goodness of fit.

^a Sum of fungal concentrations measured at stage III: 3.3–4.7 µm, stage IV: 2.1–3.3 µm, stage V: 1.1–2.1 µm and stage VI: 0.65–1.1 µm (Kim and Kim, 2007).

0.000). High wind speed increases the number of fungal spores brought from plants, trees and the ground into the atmosphere and favors the suspension of fungi, leading to a higher concentration of fungi (Zhai et al., 2018).

The effects of relative humidity, temperature, PM_{2.5} and wind speed on airborne fungal concentrations were also established using a multiple linear regression model. Table 2 shows the unstandardized coefficient, standardized coefficient, and *p*-value for each parameter and the *R*² value for each regression model. The values of *R*² were 0.668 (*p* = 0.000) for total airborne fungal concentration and 0.651 (*p* = 0.000) for respirable airborne fungal concentration in the multiple linear regression model. Similar to the Spearman's correlation, using the regression model we observed that relative humidity was positively correlated with airborne fungal concentration. There was also a positive correlation between fungal concentration and wind speed. Furthermore, temperature had negative effects on outdoor fungal concentration in the regression models, consistent with the findings of Awad et al. (2018). The relationship between fungal concentration and relative humidity and temperature in the regression model was consistent with the findings of Green et al. (2003) and Tseng et al. (2011). To some degree, meteorological parameters lead to characteristic fungal concentrations (Zhai et al., 2018) and have a large effect on airborne fungal concentration (Frankel et al., 2012). Wind speed plays a predominant role in the characteristics of airborne fungi (Zhai et al., 2018). Moreover, relative humidity, temperature and wind speed act in synergy on airborne fungi (Zhai et al., 2018).

According to the definition of the air quality index (AQI) in the Technical Regulations on Ambient Air Quality Index (HJ 633–2012) (China, 2012) and concentrations of PM_{2.5} and PM₁₀, in this study we divided the airborne fungal concentrations into two categories: excellent (AQI: 0–50) and others (AQI: >50) (Fig. 2). According to the AQI classification of PM_{2.5}, the airborne fungal concentrations of others (757 CFU/m³ in AQI (PM_{2.5})) was higher than excellent (568 CFU/m³ in AQI (PM_{2.5})) (*p* = 0.021). Similar to PM_{2.5}, the airborne fungal concentrations of others (756 CFU/m³ in AQI (PM₁₀)) was higher than excellent (559 CFU/m³ in AQI (PM₁₀)) (*p* = 0.015). Li and colleagues also found that the concentrations of airborne fungi tended to increase with the level of pollution in Xi'an (Li et al., 2017a). This can be explained by the fact that airborne particles constitute nutrients for airborne fungi, supporting their growth and reproduction (Zhai et al., 2018). In addition, PM and fungi complement each other, and fungal bioaerosols can also contribute to PM.

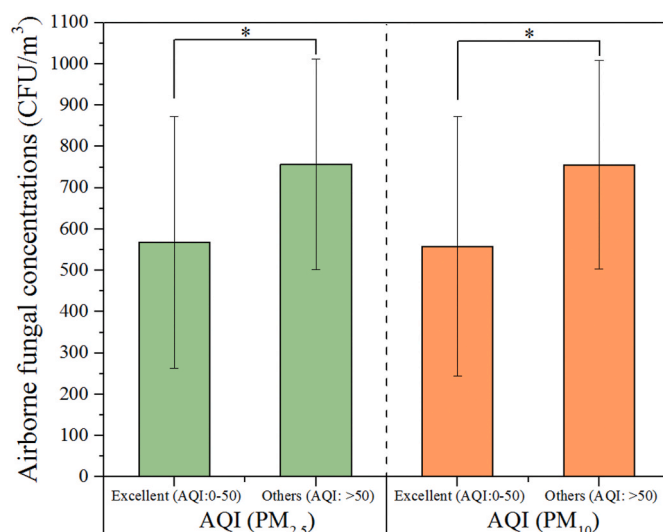


Fig. 2. Airborne fungal concentrations at different AQI in the courtyard space.

3.3. Size distribution of airborne fungi in the courtyard space

Certain particle sizes of airborne fungi are associated with the development of symptoms of human respiratory disease (Horner et al., 1995). Thus, it is helpful to know the particle size distribution of airborne fungi to prevent the associated health risks. The particle size distribution patterns of airborne fungi presented a single-peak distribution during all four seasons (Fig. 3 A and B). Similar results were found by Fang et al. (2008) and Raisi et al. (2013). The highest proportions of airborne fungi were detected in stage IV (2.1–3.3 μm) in spring (41.9%, 291 CFU/m³), summer (38.5%, 246 CFU/m³), and autumn (36.3%, 303 CFU/m³), and in stage V (1.1–2.1 μm) in winter (29.5%, 178 CFU/m³). The lowest proportion of airborne fungi was found in stage VI (0.65–1.1 μm) in all four seasons (spring: 2.2%, summer: 2.3%, autumn: 3.8%, winter: 5.5%). The results of our study were similar to those reported for Qingdao for the four seasons (Li et al., 2011), in summer and autumn for Qingdao (Xu et al., 2011), and for Beijing for the four seasons (Fang et al., 2005). In general, seasonal variation affects airborne fungal concentration at each stage; however, this has less effect on particle size distribution trends. In our study, small particle sizes (1.1–3.3 μm) were predominant during each season. More importantly, the proportions of stages IV and V (1.1–3.3 μm) of airborne fungi in each season exceeded 58%. Furthermore, 82% of the airborne fungi in the courtyard space could be deposited in the lower respiratory tract (Kim and Kim, 2007) and possibly trigger diseases such as allergies or asthma (Horner et al., 1995).

Count median diameter (CMD) refers to the airborne fungal size of the upper and lower 50% of the value (Hu et al., 1994). The count median diameters of the four seasons are illustrated in Fig. 4. The particle size in spring was 1.76 μm and decreased to 1.74 μm, 1.66 μm, and 1.61 μm in summer, autumn, and winter (*p* = 0.003, the multi-sample Kruskal–Wallis *H* test), respectively. Zhang (2013) found that the highest fungal count median diameter occurred in spring. Similarly, Fang and colleagues found that fungi particle sizes in spring, summer, and autumn were larger than those in winter (Fang et al., 2013). The count median diameter of fungi ranged from 1.60 to 1.80 μm in the four seasons, being smaller than the particle size of airborne fungi found in Beijing (2.00 μm) (Fang et al., 2008) and in Xi'an (2.50 μm) (Zhang, 2013). The fungal particle size in the courtyard space was small, possibly because the particles of airborne fungi existed as single conidia or small aggregates (Fang et al., 2013; Kellogg and Griffin, 2006). Based on the schematic diagram of the human respiratory system, the average-sized airborne fungi in all seasons could reach the terminal bronchi (Miao, 2010; Ye, 2019), corresponding to high risk of disease. In addition, the viable Andersen sampler was used to collect airborne fungal sample in this study, and we used CFU as a measure of fungal quantity. A possible problem is that this measure yields the same quantity irrespective of particle size, even though a larger particle likely contains more fungal cells, while a smaller particle likely contains fewer fungal cells. This leads to underestimation of fungal quantities in larger particle sizes relative to the quantities in smaller particle sizes, resulting in a shift of the peak of fungal particle size distribution to the smaller particle sizes.

3.4. Airborne fungal taxonomic composition in PBS samples

Next, we analyzed internal transcribed spacer (ITS) regions to identify the microbes present, aiming at accurately understanding the taxonomic composition of airborne fungal samples, including fungal genera that contain allergenic or pathogenic species. At the phylum level, the most abundant taxa were Ascomycota and Basidiomycota (Fig. 5). Similar results have been reported in previous studies (Bowers et al., 2013; Du et al., 2018; Qi et al., 2020; Woo et al., 2013). The relative abundance of Ascomycota in spring and summer accounted for 94% and 78%, respectively, and the relative abundance of Basidiomycota was 56% and 56% in autumn and winter, respectively.

Considering the taxonomic composition of airborne fungi at the

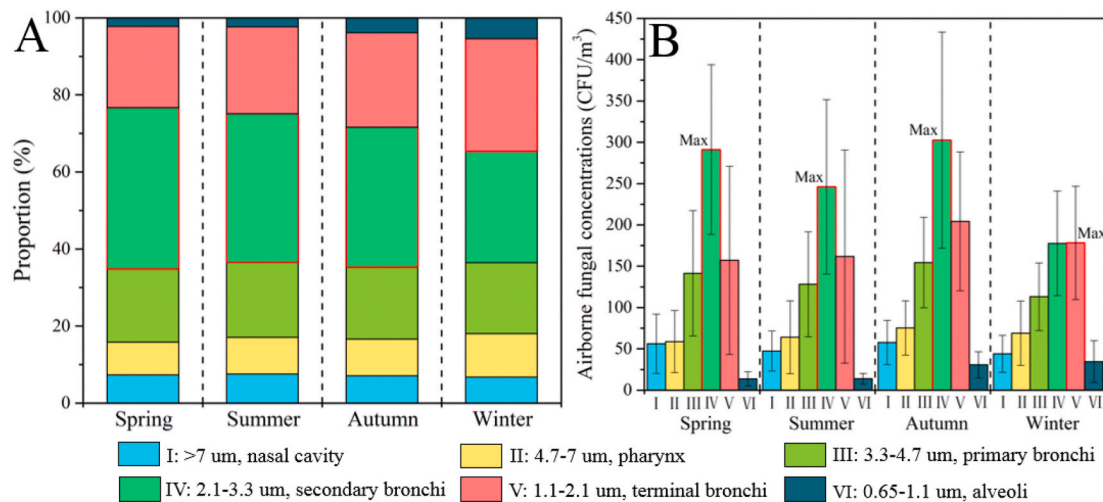


Fig. 3. Size distribution of airborne fungi in different seasons. (A) percentage of airborne fungi at each size stage; (B) airborne fungal concentrations at each size stage. Mean and SD are shown. Size stages: I: >7.0 μm , II: 4.7–7.0 μm , III: 3.3–4.7 μm , IV: 2.1–3.3 μm , V: 1.1–2.1 μm , VI: 0.65–1.1 μm .

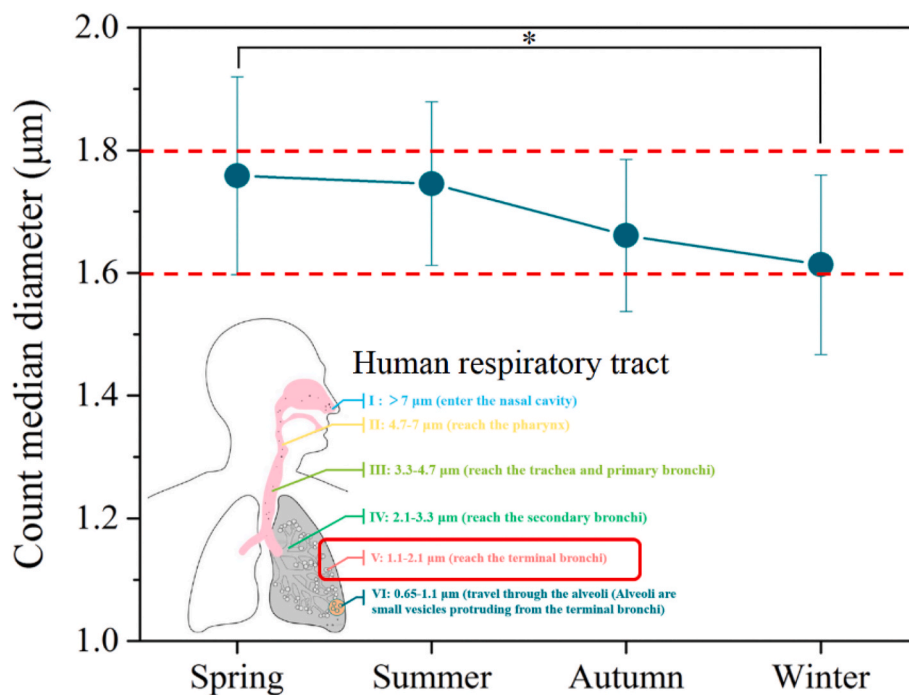


Fig. 4. Seasonal variation in the count median diameter of the airborne fungi and schematic diagram of the human respiratory system (Miao, 2010; Ye, 2019).

genus level, the top six fungal genera represented in terms of relative abundance are shown in Table 3. In general, the composition at the genus level varied from season to season, which might be related to the differences in environmental factors and sampling conditions in different seasons (Fang et al., 2005; Kozdrój et al., 2019; Qi et al., 2020). The top six genera in spring were *Verticillium* (75.9%), *Plectosphaerella* (9.6%), *Ilyonectria* (1.7%), *Articulospora* (0.6%), *Alternaria* (0.3%), and *Talaromyces* (0.2%), while in summer, fungal genera were represented by *Cladosporium* (27.4%), *Alternaria* (15.1%), *Neurospora* (8.3%), *Cutaneotrichosporon* (7.2%), *Selenophoma* (5.0%), and *Trichosporon* (4.8%). The predominant fungal genera in autumn were *Flammulina* (35.2%), *Fusarium* (22.3%), *Pleurotus* (18.6%), *Leptosphaerulina* (12.1%), *Alternaria* (7.7%), and *Rhodotorula* (2.5%). The primary fungal genera in winter were *Flammulina* (31.3%), *Fusarium* (11.6%), *Pleurotus* (11.3%), *Bjerkandera* (9.1%), *Neurospora* (4.6%), and *Penicillium* (3.6%). The dominant genus of fungi in the four seasons was different from other

studies in Xi'an (Qi et al., 2020) and Beijing (Fang et al., 2005). Such differences might be due to the use of different sampling instruments (BioSampler, six-stage sampler, and air particulate matter sampler) or to different sampling regions. Peng and Bau (2012) also found that the highest concentrations of *Cladosporium* and *Alternaria* occurred in summer. This is because the conidia of *Cladosporium* and *Alternaria* were often abundant due to copious sunshine (Fang et al., 2005). Additionally, *Penicillium* was only found in the winter due to a negative correlation between *Penicillium* growth and temperature (Xu et al., 2017). *Flammulina* had a higher abundance in autumn and winter, mainly because *Flammulina* appears in the cold season (Dong, 2012).

As human immunodeficiency and infection increase, the presence of fungal pathogens increases human health risks (Andrianopoulos, 2002); therefore, it is important to link fungal taxonomic composition with human illnesses. In the current study, 14 fungal genera that contain allergenic or pathogenic species (China, 2006; Qi et al., 2020;

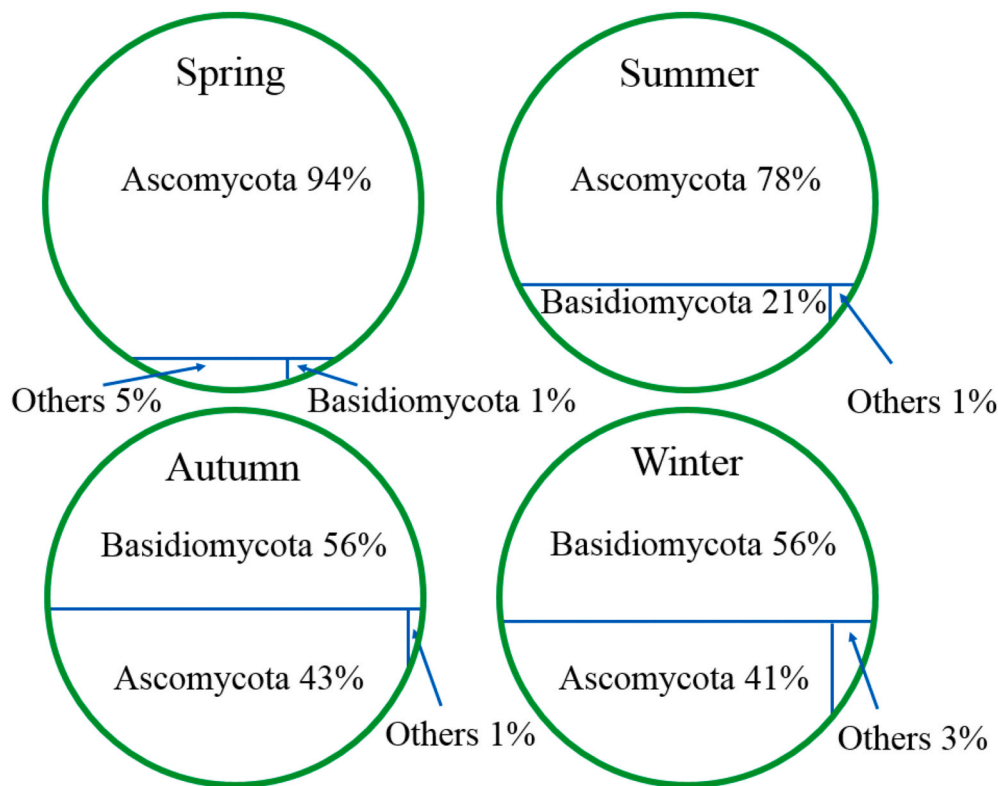


Fig. 5. Seasonal variation at the phylum level of airborne fungi.

Table 3

The top represented genera of airborne fungi collected by the BioSampler.

Season	Genera of the fungi (% Sequences)	Fungal genera that contain allergenic or pathogenic species
Spring	<i>Verticillium</i> 75.9% <i>Plectosphaerella</i> 9.6% <i>Ilyonectria</i> 1.7% <i>Articulospora</i> 0.6% <i>Alternaria</i> 0.3% <i>Talaromyces</i> 0.2% Unclassified 9.2% Others 2.5%	<i>Alternaria</i> 0.3% <i>Fusarium</i> 0.2% <i>Talaromyces</i> 0.2% <i>Saccharomyces</i> 0.12% <i>Malassezia</i> 0.04% <i>Aspergillus</i> 0.01%
Summer	<i>Cladosporium</i> 27.4% <i>Alternaria</i> 15.1% <i>Neurospora</i> 8.3% <i>Cutaneotrichosporon</i> 7.2% <i>Selenophoma</i> 5.0% <i>Trichosporon</i> 4.8% Unclassified 15.3% Others 16.9%	<i>Cladosporium</i> 27.4% <i>Alternaria</i> 15.1% <i>Neurospora</i> 8.3% <i>Trichosporon</i> 4.8% <i>Wallemia</i> 1.3% <i>Malassezia</i> 0.9% <i>Aspergillus</i> 0.8%
Autumn	<i>Flammulina</i> 35.2% <i>Fusarium</i> 22.3% <i>Pleurotus</i> 18.6% <i>Leptosphaerulina</i> 12.1% <i>Alternaria</i> 7.7% <i>Rhodotorula</i> 2.5% Unclassified 0.1% Others 1.5%	<i>Fusarium</i> 22.3% <i>Pleurotus</i> 18.6% <i>Alternaria</i> 7.7% <i>Rhodotorula</i> 2.5% <i>Cladosporium</i> 1.3%
Winter	<i>Flammulina</i> 31.3% <i>Fusarium</i> 11.6% <i>Pleurotus</i> 11.3% <i>Bjerkandera</i> 9.1% <i>Neurospora</i> 4.6% <i>Penicillium</i> 3.6% Unclassified 4.5% Others 24.0%	<i>Fusarium</i> 11.6% <i>Pleurotus</i> 11.3% <i>Neurospora</i> 4.6% <i>Penicillium</i> 3.6% <i>Talaromyces</i> 2.8% <i>Aspergillus</i> 2.6% <i>Rhodotorula</i> 2.0% <i>Alternaria</i> 1.1% <i>Wallemia</i> 0.8% <i>Cladosporium</i> 0.2% <i>Candida</i> 0.05% <i>Malassezia</i> 0.04%

Simon-Nobbe et al., 2008) were detected in all air samples collected in the courtyard space during all four seasons (Table 3). Fungal genera that contain allergenic or pathogenic species were detected during each season; in particular, the most abundant genus of fungi in summer was an opportunistic pathogenic fungus (*Cladosporium*, 27.4%). *Cladosporium* can cause allergic reactions in humans (Simon-Nobbe et al., 2008). *Aspergillus* has been linked to respiratory infections, allergies, and lung disease (Brown et al., 2012), and the toxin produced by *Aspergillus* (aflatoxin) is carcinogenic (Wu et al., 2019). Moreover, *Alternaria* has been associated with asthma (Halonen et al., 1997), and *Penicillium* can have toxic effects on cell lines, with an average mortality rate of up to 50% (Smith et al., 1992). Other pathogenic fungi such as *Fusarium*, *Neurospora*, *Pleurotus*, *Trichosporon*, *Rhodotorula*, *Malassezia*, *Candida*, *Talaromyces*, *Saccharomyces* and *Wallemia* can also cause allergic symptoms and other diseases (China, 2006; Fung and Hughson, 2003; Qi et al., 2020; Simon-Nobbe et al., 2008). Although the diseases caused by these opportunistic fungi are not immediately harmful, if people are exposed to these pathogenic fungi for long periods, the fungi can cause a series of diseases. Therefore, it is particularly important to understand whether there are pathogenic fungi in the courtyard space. Additionally, *Verticillium* fungi cause vascular wilts of plants, thereby preventing plant growth in the courtyard space (Klosterman et al., 2009).

3.5. Exposure risk assessment for airborne fungi

As the above results have demonstrated that 82% of the airborne fungi can be deposited in the lower respiratory tract (particle size < 4.7 μm) and have shown the presence of fungal genera that contain allergenic or pathogenic species, the exposure risk of airborne fungi should not be ignored. Since most of the airborne fungi pose health risks to people through inhalation (Yang et al., 2019a), inhaled ADD was calculated in the present study.

As shown in Fig. 6, the ADD for airborne fungi changed with the seasons ($p = 0.002$, the multi-sample Kruskal–Wallis H test). In general, the mean ADD decreased from spring (mean $\pm$ SD: 6.5 ± 2.7 CFU/

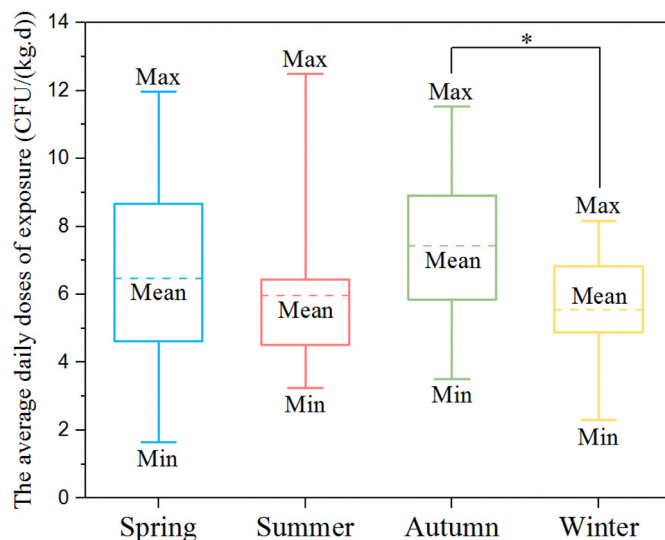


Fig. 6. Seasonal variation in the average daily doses of exposure to airborne fungi.

(kg·d) to summer (mean $\pm$ SD: 6.0 ± 2.9 CFU/(kg·d)), increasing again in autumn (mean $\pm$ SD: 7.4 ± 2.3 CFU/(kg·d)), followed by a decrease toward winter (mean $\pm$ SD: 5.6 ± 1.8 CFU/(kg·d)). The highest mean ADD values were found in autumn, with a range from 3.5 to 11.5 CFU/(kg·d), 1.32-fold the lowest mean ADD in winter. Therefore, more attention should be paid to the high levels of airborne fungi in autumn. At present, airborne fungi are known to be a threat to public health (Fung and Hughson, 2003; Kim et al., 2017). However, few studies have focused on the connection between airborne fungi and ADD; the present study provides the motivation for future research concerning the health effects of airborne fungi.

4. Conclusion

In this study, airborne fungal concentration, particle size distribution, and taxonomic composition were measured in a courtyard space during the course of a year. The present study showed that airborne fungal concentrations were high in autumn and spring. Spearman's correlation analysis and multiple linear regression models both showed that increases in relative humidity and wind speed were associated with increases in airborne fungal concentrations, while the temperature negatively affected airborne fungal concentration. The concentrations of airborne fungi increased along with the AQI. Seasonal variation affected the fungal concentration of each size stage and had little effect on the size distribution characteristics, whereas seasonal variation produced differences in count median diameter. The airborne fungal taxonomic composition varied among the four seasons. The most abundant fungal genera that contained allergenic or pathogenic species were *Cladosporium*, but *Aspergillus*, *Alternaria*, *Fusarium*, *Neurospora*, *Trichosporon*, *Pleurotus*, *Rhodotorula*, *Penicillium*, *Malassezia*, *Candida*, *Talaromyces*, *Saccharomyces* and *Wallemia* were also detected. The average daily dose of exposure to airborne fungi changed with the seasonal variation. Furthermore, in the future, continuous sampling of the courtyard space should be performed on a shorter time scale to further understand the transient changes in airborne fungi.

Author statement

Dingmeng Wu: Methodology, Investigation, Formal analysis, Data Curation, and Writing Original Draft. Ying Zhang: Conceptualization and Formal analysis. Wenwen Qin: Investigation and Data Curation. Yu Tian: Investigation. Angui Li: Conceptualization, Supervision, Writing,

Project administration and Funding acquisition. Li'an Hou: Editing and Funding acquisition. Yicun Hou: Investigation. Bingyang Han: Investigation. Tianqi Wang: Investigation. Jing Xiong: Formal analysis. Ran Gao: Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the Ministry of Science and Technology of the People's Republic of China (No. 2017YFC0702800) and the Ministry of Science and Technology of the People's Republic of China (No. 2020YFC0842500).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apr.2020.10.014>.

References

- Abdel Hameed, A.A., Khoder, M.I., Ibrahim, Y.H., Saeed, Y., Osman, M.E., Ghanem, S., 2012. Study on some factors affecting survivability of airborne fungi. *Sci. Total Environ.* 414, 696–700.
- An, C., Yamamoto, N., 2016. Fungal compositions and diversities on indoor surfaces with visible mold growths in residential buildings in the Seoul Capital Area of South Korea. *Indoor Air* 26, 714–723.
- Andersen, A.A., 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. *J. Bacteriol.* 76, 471.
- Andrianopoulos, A., 2002. Control of morphogenesis in the human fungal pathogen *Penicillium marneffei*. *International Journal of Medical Microbiology* 292, 331–347.
- Asif, A., Zeeshan, M., Jahanzaib, M., 2019. Assessment of indoor and outdoor microbial air quality of cafeterias of an educational institute. *Atmospheric Pollution Research* 10, 531–536.
- Awad, A.H.A., Saeed, Y., Hassan, Y., Fawzy, Y., Osman, M., 2018. Air microbial quality in certain public buildings, Egypt: a comparative study. *Atmospheric Pollution Research* 9, 617–626.
- Bowers, R.M., Clements, N., Emerson, J.B., Wiedinmyer, C., Hannigan, M.P., Fierer, N., 2013. Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. *Environ. Sci. Technol.* 47, 12097–12106.
- Brown, G.D., Denning, D.W., Gow, N.A., Levitz, S.M., Netea, M.G., White, T.C., 2012. Hidden killers: human fungal infections. *Sci. Transl. Med.* 4.
- China, M.o.E.a.E.o.t.P.s.R.o., 2012. Technical Regulation on Ambient Air Quality Index. Ministry of Ecology and Environment of the People's Republic of China.
- China, M.o.H.o.t.P.s.R.o., 2006. Directory of Microbes Infecting Humans (Beijing).
- Dannemiller, K.C., Lang-Yona, N., Yamamoto, N., Rudich, Y., Peccia, J., 2014. Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations. *Atmos. Environ.* 84, 113–121.
- Deng, J., 2010. The Research on the Traditional Courtyard Space in the View of Modern Culture. Hunan University, Hunan (In Chinese).
- Dong, X., 2012. The Effects of Different Light Qualities on Commodity Traits and Yield of Yellow and White Flammulina Velutipes Strains. Jilin Agricultural University, Jilin.
- Du, P., Du, R., Ren, W., Lu, Z., Fu, P., 2018. Seasonal variation characteristic of inhalable microbial communities in PM_{2.5} in Beijing city, China. *Sci. Total Environ.* 610, 308–315.
- Dvorackova, I., Stora, C., Ayraud, N., 1981. Evidence for aflatoxin B 1 in two cases of lung cancer in man. *J. Canc. Res. Clin. Oncol.* 100, 221–224.
- Fang, Z., Ouyang, Z., Hu, L., Wang, X., Zheng, H., Lin, X., 2005. Culturable airborne fungi in outdoor environments in Beijing, China. *Sci. Total Environ.* 350, 47–58.
- Fang, Z., Ouyang, Z., Zheng, H., Wang, X., 2008. Concentration and size distribution of culturable airborne microorganisms in outdoor environments in Beijing, China. *Aerosol. Sci. Technol.* 42, 325–334.
- Fang, Z., Sun, P., Ouyang, Z., Liu, P., Sun, L., Wang, X., 2013. Studies on the size distribution of airborne microbes at home in Beijing. *Environmental Science* 34, 2526–2532. In Chinese.
- Frankel, M., Beko, G., Timm, M., Gustavsen, S., Hansen, E.W., Madsen, A.M., 2012. Seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity, and air exchange rate. *Appl. Environ. Microbiol.* 78, 8289–8297.
- Fung, F., Hughson, W.G., 2003. Health effects of indoor fungal bioaerosol exposure. *Appl. Occup. Environ. Hyg* 18, 535–544.
- Green, C.F., Scarpino, P.V., Gibbs, S.G., 2003. Assessment and modeling of indoor fungal and bacterial bioaerosol concentrations. *Aerobiologia* 19, 159–169.

- Halonen, M., Stern, D.A., Wright, A.L., Taussig, L.M., Martinez, F.D., 1997. Alternaria as a major allergen for asthma in children raised in a desert environment. *Am. J. Respir. Crit. Care Med.* 155, 1356–1361.
- Horner, W., Helbling, A., Salvaggio, J., Lehrer, S., 1995. Fungal allergens. *Clin. Microbiol. Rev.* 8, 161–179.
- Hu, Qingxuan, Lu, Jianchun, Xu, Xiuzhi, Che, Fengxiang, Cai, Zenglin, Lu, Zhixin, Wen, Qingli, 1994. Relationship between indoor air bacteria and fungal particles in Shenyang. *Arid Environmental Monitoring* 8, 100–104 (In Chinese).
- Jiao, Y., 2007. The Chinese Traditional Courtyard under the Concept of Shi Zhong. Harbin Institute of Technology, Harbin (In Chinese).
- Jones, A.M., Harrison, R.M., 2004. The effects of meteorological factors on atmospheric bioaerosol concentrations—a review. *Sci. Total Environ.* 326, 151–180.
- Karra, S., Katsivela, E., 2007. Microorganisms in bioaerosol emissions from wastewater treatment plants during summer at a Mediterranean site. *Water Res.* 41, 1355–1365.
- Kellogg, C.A., Griffin, D.W., 2006. Aerobiology and the global transport of desert dust. *Trends Ecol. Evol.* 21, 638–644.
- Kim, K., Kabir, E., Jahan, S.A., 2017. Airborne bioaerosols and their impact on human health. *Journal of Environmental Sciences-China* 67, 23–35.
- Kim, K.Y., Kim, C.N., 2007. Airborne microbiological characteristics in public buildings of Korea. *Build. Environ.* 42, 2188–2196.
- Klosterman, S.J., Atallah, Z.K., Vallad, G.E., Subbarao, K.V., 2009. Diversity, pathogenicity, and management of *Verticillium* species. *Annu. Rev. Phytopathol.* 47, 39–62.
- Kozdrój, J., Frączek, K., Ropek, D., 2019. Assessment of bioaerosols in indoor air of glasshouses located in a botanical garden. *Build. Environ.* 166, 106436.
- Li, M., Qi, J., Zhang, H., Huang, S., Li, L., Gao, D., 2011. Concentration and size distribution of bioaerosols in an outdoor environment in the Qingdao coastal region. *Sci. Total Environ.* 409, 3812–3819.
- Li, W., 2004. Design of the Courtyard-Space in Colleges. Beijing Forestry University, Beijing (In Chinese).
- Li, W., Rui, L., Xie, Z., Wang, J., Fan, C., Liu, P., Li, Y., 2017a. Concentration and size distribution characteristics of culturable bioaerosols at various air quality levels during fall and winter in Xi'an, China. *Environ. Sci.* 38, 4494–4500.
- Li, A.G., Liu, Z.J., Liu, Y., Xu, X.X., Pu, Y.L., 2012. Experimental study on microorganism ecological distribution and contamination mechanism in supply air ducts. *Energy and Buildings* 47, 497–505.
- Li, Y., Lu, R., Li, W., Xie, Z., Song, Y., 2017b. Concentrations and size distributions of viable bioaerosols under various weather conditions in a typical semi-arid city of Northwest China. *J. Aerosol Sci.* 106, 83–92.
- Li, A.G., Xiong, J., Yao, L.Z., Gou, L., Zhang, W.R., 2016. Determination of dust and microorganism accumulation in different designs of AHU system in Shaanxi History Museum. *Building and Environment* 104, 232–242.
- Lis, D.O., Ulfvig, K., Wlazlo, A., Pastuszka, J.S., 2004. Microbial air quality in offices at municipal landfills. *J. Occup. Environ. Hyg.* 1, 62–68.
- Mashat, B.H., 2015. Indoor and outdoor microbial aerosols at the holy mosque: a case study. *Atmospheric Pollution Research* 6, 990–996.
- Miao, Z., 2010. Fungal aerosol in rabbit houses and its spread to the environment. Shandong Agricultural University. Shandong (In Chinese).
- Ohagim, P., Ikon, G., Matthew, P., Ohagim, G., 2017. Microbiological assessment of indoor air in public toilets across selected motor parks in Owerri Metropolis, Nigeria. *J. Microbiol. Exp* 5, 00166.
- Peccia, J., Hospodsky, D., Bibby, K., 2011. New directions: a revolution in DNA sequencing now allows for the meaningful integration of biology with aerosol science. *Atmos. Environ.* 45 (1994), 1896–1897.
- Peng, W., Bau, T., 2012. A review on community diversity of urban airborne fungi. *Journal of Fungal Research* 10, 49–55.
- Ponce-Caballero, C., Gamboa-Marrufo, M., López-Pacheco, M., Cerón-Palma, I., Quintal-Franco, C., Giacomani-Vallejos, G., Loria-Arcila, J.H., 2013. Seasonal variation of airborne fungal propagules indoor and outdoor of domestic environments in Mérida, Mexico. *Atmósfera* 26, 369–377.
- Priyamvada, H., Priyanka, C., Singh, R.K., Akila, M., Ravikrishna, R., Gunthe, S.S., 2018. Assessment of PM and bioaerosols at diverse indoor environments in a southern tropical Indian region. *Build. Environ.* 137, 215–225.
- Qi, Y., Li, Y., Xie, W., Lu, R., Mu, F., Bai, W., Du, S., 2020. Temporal-spatial variations of fungal composition in PM_{2.5} and source tracking of airborne fungi in mountainous and urban regions. *Sci. Total Environ.* 708, 135027.
- Raisi, L., Aleksandropoulou, V., Lazaridis, M., Katsivela, E., 2013. Size distribution of viable, cultivable, airborne microbes and their relationship to particulate matter concentrations and meteorological conditions in a Mediterranean site. *Aerobiologia* 29, 233–248.
- Reboux, G., Bellanger, A., Roussel, S., Grenouillet, F., Sornin, S., Piarroux, R., Dalphin, J., Millon, L., 2009. Indoor mold concentration in Eastern France. *Indoor Air* 19, 446–453.
- Reboux, G., Rocchi, S., Laboissière, A., Ammari, H., Bochaton, M., Gardin, G., Rame, J. M., Millon, L., 2019. Survey of 1012 moldy dwellings by culture fungal analysis: threshold proposal for asthmatic patient management. *Indoor Air* 29, 5–16.
- Shi, T., Sun, J., 2013. Study on the oxygen environment courtyard space of Shenyang Construction University. In: The 9th China (Beijing) International Garden Expo (Beijing, p. In Chinese).
- Simon-Nobbe, B., Denk, U., Pöhl, V., Rid, R., Breitenbach, M., 2008. The spectrum of fungal allergy. *Int. Arch. Allergy Immunol.* 145, 58–86.
- Smith, J.E., Anderson, J.G., Lewis, C.W., Murad, Y.M., 1992. Cytotoxic fungal spores in the indoor atmosphere of the damp domestic environment. *FEMS Microbiol. Lett.* 100, 337–343.
- Tseng, C.-H., Wang, H.-C., Xiao, N.-Y., Chang, Y.-M., 2011. Examining the feasibility of prediction models by monitoring data and management data for bioaerosols inside office buildings. *Build. Environ.* 46, 2578–2589.
- Wang, C., Cao, J., Zhao, L., 2007. Researches on space environment of college courtyard. *Shanxi Architecture* 33, 42–43 (In Chinese).
- Wei, M., Yu, Z., Zhang, H., 2015. Molecular characterization of microbial communities in bioaerosols of a coal mine by 454 pyrosequencing and real-time PCR. *Journal of Environmental Sciences-China* 30, 241–251.
- Woo, A.C., Brar, M.S., Chan, Y., Lau, M.C., Leung, F.C., Scott, J.A., Vrijmoed, L.L., Zawar-Reza, P., Pointing, S.B., 2013. Temporal variation in airborne microbial populations and microbially-derived allergens in a tropical urban landscape. *Atmos. Environ.* 74, 291–300.
- Wu, D., Zhang, Y., Li, A., Kong, Q., Li, Y., Geng, S., Dong, X., Liu, Y., Chen, P., 2019. Indoor airborne fungal levels in selected comprehensive compartments of the urban utility tunnel in Nanjing, Southeast China. *Sustainable Cities and Society* 51, 101723.
- Wu, D., Zhang, Y., Tian, Y., Li, A., Li, Y., Xiong, J., Gao, R., 2020. On-site investigation of the concentration and size distribution characteristics of airborne fungi in a university library. *Environ. Pollut.* 114138.
- Xu, C., Wei, M., Chen, J., Zhu, C., Li, J., Lv, G., Xu, X., Zheng, L., Sui, G., Li, W., 2017. Fungi Diversity in PM_{2.5} and PM₁₀ at the Summit of Mt. Tai: Abundance, Size Distribution, and Seasonal Variation.
- Xu, W., Qi, J., Jin, C., Gao, D., Li, M., Li, L., Huang, S., Zhang, H., 2011. Concentration distribution of bioaerosol in summer and autumn in the Qingdao coastal region. *Huan jing ke xue= Huanjing kexue*. 32, 9–17.
- Yamamoto, N., Bibby, K., Qian, J., Hospodsky, D., Rismaniyazdi, H., Nazaroff, W.W., Peccia, J., 2012. Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. *ISME J.* 6, 1801–1811.
- Yan, X., Qiu, D., Zheng, S., Yang, J., Sun, H., Wei, Y., Han, J., Sun, J., Su, X., 2019. Distribution characteristics and noncarcinogenic risk assessment of culturable airborne bacteria and fungi during winter in Xinxiang, China. *Environ. Sci. Pollut. Control Ser.* 26, 36698–36709.
- Yang, K., Li, L., Wang, Y., Xue, S., Han, Y., Liu, J., 2019a. Airborne bacteria in a wastewater treatment plant: emission characterization, source analysis and health risk assessment. *Water Res.* 149, 596–606.
- Yang, K., Li, L., Wang, Y., Xue, S., Han, Y., Liu, J., 2019b. Emission level, particle size and exposure risks of airborne bacteria from the oxidation ditch for seven months observation. *Atmospheric Pollution Research* 10, 1803–1811.
- Ye, X., 2019. Research on PM_{2.5} Concentration Distribution and Indoor-Outdoor Correlation in Urban Residential Areas in Severe Cold Regions. Harbin Institute of Technology Harbin, China (In Chinese).
- Zhai, Y., Li, X., Wang, T., Wang, B., Li, C., Zeng, G., 2018. A review on airborne microorganisms in particulate matters: composition, characteristics and influence factors. *Environ. Int.* 113, 74–90.
- Zhang, Y., 2013. Study on Distribution Characteristics of Microorganism in. University Campus Environment.

Supplementary information

Assessment of seasonal variations in concentration, particle-size distribution, and taxonomic composition of airborne fungi in a courtyard space

Dingmeng Wu^{a, b}, Ying Zhang^b, Wenwen Qin^b, Yu Tian^b, Angui Li^{b*}, Li'an Hou^c,
Yicun Hou^b, Bingyang Han^b, Tianqi Wang^b, Jing Xiong^{a, b}, Ran Gao^b

^a School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an, Shaanxi 710055, PR China

^b School of Building Services Science and Engineering, Xi'an University of Architecture and Technology, Xi'an, Shaanxi 710055, PR China

^c Rocket Force University of Engineering, Xi'an, Shaanxi 710025

*Corresponding author: Angui Li

Email: liangui@xauat.edu.cn

Present/permanent address: School of Building Services Science and Engineering,
Xi'an University of Architecture and Technology, Xi'an, Shaanxi 710055, China

List of Sections

Section S1. The calculation equations of the proportion of airborne fungi at each stage and the airborne fungal concentrations

List of Tables

Table S1 Sample collection description.

Table S2 Parameters used in the average daily dose rates (ADD).

List of Figures

Fig. S1. Scheme of sample management used in this study.

Section S1. The calculation equations of the proportion of airborne fungi at each stage and the airborne fungal concentrations

The airborne fungal concentrations were calculated using equation (1):

$$C = \frac{P_r \times 1000}{5 \text{ min} \times 28.3 \text{ L} / \text{min}} \quad (1)$$

where, C is the airborne fungal concentrations (CFU/m³) and P_r is the corrected colony count (Liu et al., 2014).

The proportion of airborne fungi at each stage was calculated using equation (2):

$$R = \frac{C_i}{C} \times 100\% \quad (2)$$

where R is the proportion of airborne fungi at each stage (%), C_i is the i^{th} stage concentrations for the airborne fungi (CFU/m³), and C is the total airborne fungal concentration (CFU/m³) (Wu et al., 2020).

Table S1

Sample collection description.

Number of air samplers														
Air sampler	Replicates	Samples are used in the study	Each season				Time of day		Each particle size range					
			Spring	Summer	Autumn	Winter	AM	PM	I	II	III	IV	V	VI
Viable Andersen sampler	156	52 ^a	48 ^c	36 ^c	36 ^c	36 ^c	78	78	156	156	156	156	156	156
BioSampler	60	4 ^b	18 ^d	12 ^d	18 ^d	12 ^d	30	30						

^a Three consecutive replicates were collected to calculate the mean value, thus, a total of 52 samples are used in the study.

^b All the samples from each season are thoroughly mixed for DNA extraction, thus, a total of 4 samples are used in the study.

^c Spring: sampling 16 times, three samples were collected at each time, total of 48 samples. Summer: sampling 12 times, three samples were collected at each time, total of 36 samples. Autumn: sampling 12 times, three samples were collected at each time, total of 36 samples. Winter: sampling 12 times, three samples were collected at each time, total of 36 samples.

^d Spring: sampling six times, three PBS samples were collected at each time, total of 18 samples. Summer: sampling four times, three PBS samples were collected at each time, total of 12 samples. Autumn: sampling six times, three PBS samples were collected at each time, total of 18 samples. Winter: sampling four times, three PBS samples were collected at each time, total of 12 samples.

Table S2

Parameters used in the average daily dose rates (ADD).

Parameters	Units	Values	References
Inhalation Rate	m ³ /d	16.7	(Protection, 2016)
Exposure Frequency	day/year	36.5 ^a	
Exposure Duration	year	25	(Yang et al., 2019)
Body Weight	Kg	61.9	(Protection, 2016)
Average Life Time	day	74.83×365	(Protection, 2016)

^a Due to people spend approximately 90% of their time indoors (Long et al., 2001), therefore, this study assumes that people spend the remaining 10% of their time in the outdoor courtyard space for rest and relax, thus, $EF=365 \times 0.1 = 36.5$.

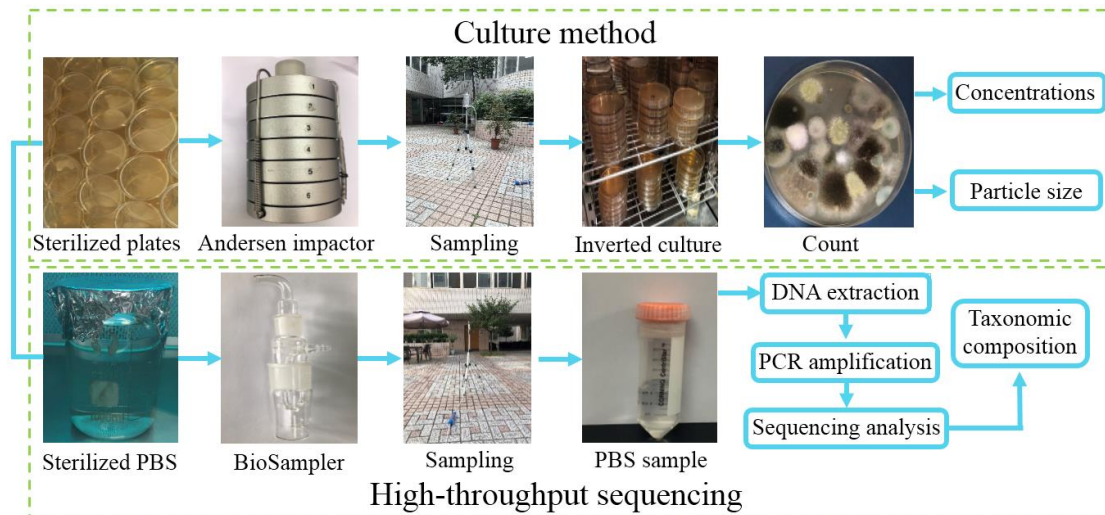


Fig. S1. Scheme of sample management used in this study.

References

- Liu, Z., Li, A., Hu, Z., Sun, H., 2014. Study on the potential relationships between indoor culturable fungi, particle load and children respiratory health in Xi'an, China. *Building and Environment* 80, 105-114.
- Long, C.M., Suh, H.H., Catalano, P.J., Koutrakis, P., 2001. Using time-and size-resolved particulate data to quantify indoor penetration and deposition behavior. *Environmental science & technology* 35, 2089-2099.
- Protection, M.o.E., 2016. Exposure Factors Handbook of Chinese Population (Adult volume). China (In Chinese).
- Wu, D., Zhang, Y., Tian, Y., Li, A., Li, Y., Xiong, J., Gao, R., 2020. On-site investigation of the concentration and size distribution characteristics of airborne fungi in a university library. *Environmental Pollution*, 114138.
- Yang, K., Li, L., Wang, Y., Xue, S., Han, Y., Liu, J., 2019. Airborne bacteria in a wastewater treatment plant: Emission characterization, source analysis and health risk assessment. *Water research* 149, 596-606.