



Spatiotemporal distribution and the passive dispersal of fungal spores through HVAC systems

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Abstract Heating, ventilation and air conditioning (HVAC) systems are widely used to regulate indoor temperature and air quality of modern buildings. The central supply and exhaust system cause the dispersal and removal of bioaerosols. This study presents results from *ex situ* experiments conducted to a better understanding of the spatiotemporal distribution, passive dispersal and removal of fungal spores through HVAC systems. The study was conducted in a 50 square meters by 3 m high instrument room in a research building with HVAC systems. Plates with *Aspergillus flavus* colony were attached upside down on the edge of the ceiling vent to imitate the mildew growing. Fungal spores were released for 10 min, collected and counted by the settle plate method in 2 h. Results show that *A. flavus* spores dispersed to 3.6 m in 2 min and were evenly distributed in the room within 8 min. The concentration of spores decreased from 48 to 3–6 CFU/plate after 60 min and was lower than 1% after 120 min. Our results suggest that airborne fungal spores disperse and remove by HVAC systems efficiently. Without the external and internal

source, the HVAC system could greatly reduce the fungal amount in the indoor air to the background level within one hour. This study provided the observed data of the transmission and retention of internal or external biological contaminants through HVAC system.

Keywords Sick building · Allergenic fungi · *Aspergillus* · Spatio-temporal variations · Indoor aerosolized fungi

1 Introduction

People who live a modern lifestyle spend 85–90% of their time in indoor environments (Spengler et al., 2001). Respectively, the microbial contaminations of indoor environments have caught more and more attention since the increase of sick building syndrome (Sahlberg et al., 2013) and building-related illnesses (Squinazi, 1990). Fungi are ubiquitous in the environment, and their spores are found widely in the air. Lots of studies in different geographical areas found consistent associations between mold and respiratory or allergic health effects (WHO (World Health Organization) Europe, 2009). Evident mold or dampness had consistent positive associations with multiple allergic and respiratory effects (Mendell et al., 2011). Human health may be negatively affected by exposure to indoor fungal contaminants in their environments (Cramer et al., 2014; Lin et al., 2016).

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Fungi can grow on almost any building material including a central air conditioning system, wallpaper, walls, concrete bricks and ceiling tiles if there were enough available water (Campana et al., 2020; Prussin & Marr, 2015). These materials provided good substrates for fungal growth and sporulation and as a result, deterioration of human health occurred. The release and dispersal of fungal spores in a building could be driven by air currents and gravity (Mensah-Attipoe & Toyinbo, 2019). Besides, changes to abiotic parameters, such as temperature, relative humidity, air velocity and vibration have influenced the spatial and temporal distribution of indoor microbial composition.

In modern buildings, heating, ventilation and air conditioning (HVAC) systems are widely used to regulate indoor temperature and humidity. The HVAC systems could cause the passive release and dispersal of fungal spores and fragments. Many reports demonstrated that HVAC systems provide favorable conditions for microbial growth (Batterman & Burge, 1995; Sibanda et al., 2021). Some reports focus on the mathematical models to describe the spore flux after dispersal (Gopalakrishnan et al., 2019), whereas some studies focus on the spatial distribution of fungal flora in indoor or outdoor environments (Lin et al., 2016, 2018). However, the spatiotemporal distribution and the passive dispersal of fungal spores through the HVAC systems in a building were less reported.

Aspergillus spp. is a dominant allergenic fungal genus in indoor air and is the dominant culturable fungi in HVAC segments (Liu et al., 2019). Some species of *Aspergillus*, such as *A. flavus*, *A. fumigatus*, *A. sydowii* and *A. niger* grow well in indoor environments (Lin et al., 2016; Mousavi et al., 2016). Inhaling a complex mixture of their hyphal fragments and spores has been reported to cause severe asthma with fungal sensitization (Agarwal & Gupta, 2011), allergic diseases or aspergillosis (Bains & Judson, 2012; Janahi et al., 2017; Pearson et al., 2015). Dispersal of *Aspergillus* through the HVAC systems caused public health concerns. Therefore, the growth and presence of *Aspergillus* and their subsequent discharge into ambient air is essential for the public health issue. However, most studies focused on the fungal diversity in HVAC systems. Fewer reports mentioned the dispersal process with empirical data. Here, we present the process of passive spore transmission and

their spatiotemporal distribution through the HVAC systems in a building.

2 Materials and methods

2.1 Study site

In tropical and subtropical areas, cooling in buildings is essential for thermal comfort, particularly in public buildings such as schools, offices, supermarkets, etc. To simulate dispersal of the internal or external biological contaminants through an HVAC system, the study was conducted in an instrument room in a research building with an HVAC system at Tunghai University, Taiwan. It is 8.6 m × 5.8 m by 3 m high, the temperature was 24 °C and the relative humidity was 70%–80%. The vent was in the ceiling at 3-m high. There was obviously mildew growing on the gypsum panel ceiling board around the air outlets. The condensation caused by cold air increased the humidity of the building material and result in mold growth and mildew. Typically, this happens in buildings with ventilation and air conditioning systems in tropical areas. We kept a supply vent and a return vent only (Fig. 1A) and sealed the others with plastic. The airflow was from inside to the door. The airflow rates of the supply vent are 1.9–2.0 m/s. The airflow rate of the tested laboratory was non-detectable (AVM-01, Tai Shi).

2.2 The airborne fungal flora

before and after treatment of portable HEPA filtration units

To understand the microbial community in the study site, the airborne fungal community was investigated through the plate count method in the beginning. To reduce background noise, two HEPA filtration units (17,005, Honeywell) were used in the laboratory for 30 min. The airborne fungal community after filtration was also investigated. Four sampling sites, S1 to S4, were set up from the supply vent to the return vent and the distance between sites was 1.2 m (Fig. 1A). At each sampling site, three replica plates contained malt extract agar (MEA) were placed on the floor and at the height of 1.2- and 2.2-m height (Fig. 1B), respectively. Plates were transferred to the laboratory for the culturable count, fungal isolation and identification.

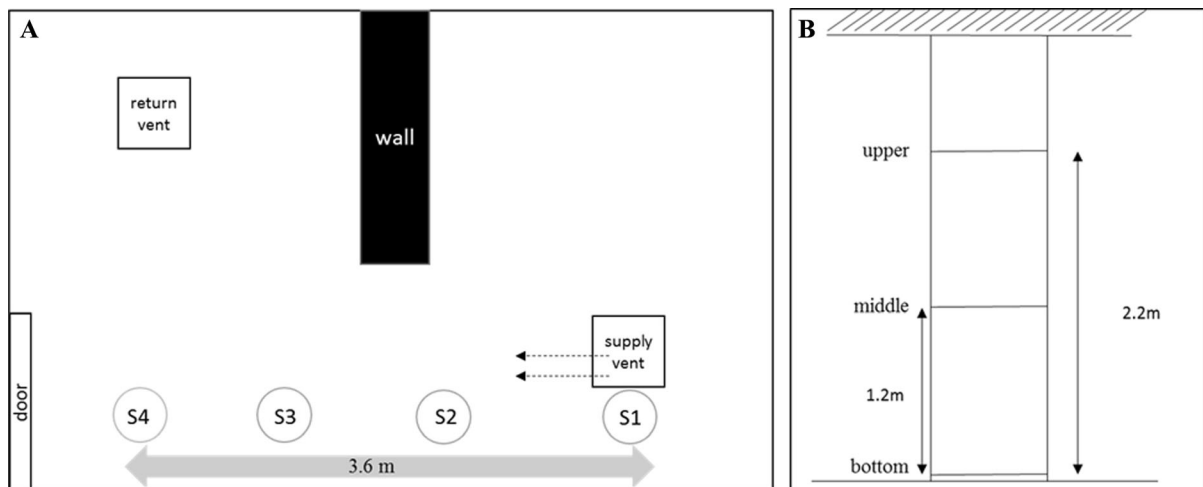


Fig. 1 **A** Schematic of the experimental setup (top view) for the measurement of the airborne flora and spore emission of *Aspergillus flavus* which isolated from environments; **B** at each

sampling site (S1–S4), three replica plates contained malt extract agar were placed on the floor and at the height of 1.2 and 2.2 m height (side view)

2.3 Preparation of test materials and evaluation of dispersal

In the examined room, *Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium* and *Alternaria* were the dominant genera. *Aspergillus flavus* is one of the dominant fungal species present in ambient air in Taichung (Lin et al., 2018), and it was important for clinical and public health practice. Because of the importance of *A. flavus* and its presence in the examined room, the isolate was chosen for observation of spore transmission as an indicator of biological contaminants. We cultured this strain on Malt Extract Agar (MEA) at 25 °C for 7 days.

Three plates with *A. flavus* colony with matured spores were attached upside down on the edge of the air conditioner outlet to providing its spores for 10 min. To observe the horizontal transfer and deposition of fungi, the top and bottom layers were located at a 2.2-m height and on the floor. Human exposure to aeroallergens is usually at approximately 1.2 to 1.5 m. Therefore, the medium layer was set at a height of 1.2 m. The distance between the supply vent and the return vent was 3.6 m. In order to investigate the vertical transfer of the fungi by the return vent, four sampling sites were set up from the supply vent to the return vent and the distance between sites was 1.2 m (Fig. 1A). Fungal spores were collected by settle plates for 2 min from four sites and three heights (on the floor, at the height of 1.2- and 2.2-m) at start time,

5th, 8th, 60th, 120th and 180th minute since release spores (Fig. 1B). The plates were transferred to the laboratory for the culturable count.

2.4 Statistical analysis

The fungal flora in each sample before and after treatment of portable HEPA filtration units was recorded as a binary matrix. The binary matrix was used to calculate the Bray–Curtis Similarity Index and construct nonparametric multi-dimensional scaling (MDS) plots using Primer 6 software (version 6. 1. 15; Primer-E Ltd., United Kingdom) (Clarke et al., 2014). Effects of treatment of portable HEPA filtration units on the airborne fungal community were analyzed by one-way analysis of similarity (ANOSIM) by PRIMER (Clarke et al., 2014). For effects of treatment of portable HEPA filtration units on total fungal concentrations were analyzed by Wilcoxon Signed-Rank test. Shapiro–Wilk test was used to check the normality of data distribution. We found that strong evidence of non-normality and used Kruskal–Wallis test to analyze the effects of sampling sites, heights and investigated time on the culturable counts of *A. flavus*.

3 Results

3.1 The airborne fungal flora

before and after treatment of portable HEPA filtration units

We investigated the background fungal flora of the study site and used HEPA filtration units to reduce the biological interferences in the next experiment. There were 138 CFU belong to 52 morph-species of airborne fungi were recorded. On average, 6.67 CFU/plate was recorded before treatment of portable HEPA filtration units. Before treatment of portable HEPA filtration units, *Penicillium* species were the dominant fungi. Some *Cladosporium* species and *Aspergillus fumigatus* were recorded. Only 1 colony of *A. flavus* was recorded. After HEPA filtration, the fungal concentration decreased to 4.83 (CFU/plate/30 min) on average. The abundance of *Penicillium* and *Cladosporium* decreased and *A. flavus* was not detected. Treatment of portable HEPA filtration units decreased the fungal concentration in the air, however, the differences were not significant ($P = 0.1583$). In MDS plotting (Fig. 2), the compositions of fungal communities were grouped separately according to the treatment of portable HEPA filtration units. However, ANOSIM results show that the compositions of fungal communities before treatment of portable HEPA

filtration units were not significantly different from those after treatment ($P = 0.06$).

3.2 The passive release and spatiotemporal distribution of fungal spores

Table 1 shows the concentrations of *A. flavus* measured in four sampling sites on the floor (bottom) and at 1.2- (middle) and 2.2-meters (upper) above the floor since the passive release of fungal spores. During the first 2 min, there were on average 12.33–22.33, 17.33–37.00, 20.67–33.00 and 19.00–30.33 CFU/plate from S1 to S4 (Table 1; Fig. 3A). This indicated that air condition releases and disperses *A. flavus* spores to 3.6 m away in 2 min. During the 3rd to the 5th minute, there were on average 8.67–10.00, 23.33–48.67, 15.33–18.67 and 13.00–15.00 CFU/plate from S1 to S4 (Table 1; Fig. 3B). The highest concentration of *A. flavus* presented in the plate at the height of about 2.2 m above the floor (upper layer) at sampling site S2 (Table 1; Fig. 3B). The airflow spreads horizontally, therefore the highest amount of spores appears at the upper layer which is closed to the air outlet (S2), not below the air outlet (S1). According to this data, the peak of spore release appeared in 3–5 min. During the 6th to the 8th minute, there were on average 14.00–17.67, 13.00–16.33, 15.00–19.33 and 17.67–20.33 CFU/plate from S1 to S4 (Table 1; Fig. 3C), and they were not different significantly

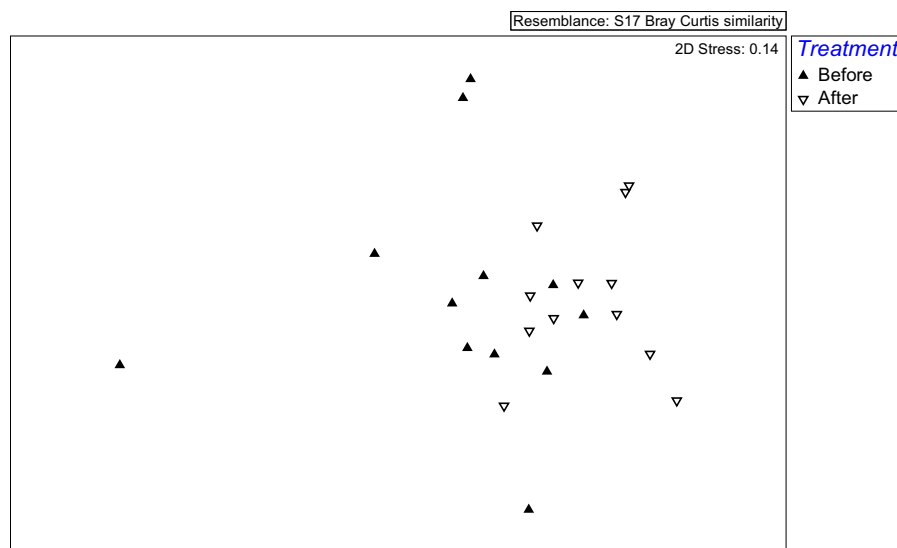


Fig. 2 Multi-dimensional scaling of airborne fungal community before (▲) and after (▽) treatment of portable HEPA filtration units

Table 1 Concentrations of *Aspergillus flavus* (CFU/plate) measured in four sampling sites (S1, S2, S3 and S4) at the height of about 2.2 (upper) and 1.2 m (middle) above the floor and on the floor (bottom) at second, 5th, 8th, 60th, 120th and 180th min after hanging plates

Place	Layer	Time (min)	S1			S2			S3			S4		
			Upper	Middle	Bottom	Upper	Middle	Bottom	Upper	Middle	Bottom	Upper	Middle	Bottom
		2	12.33±2.08 ^a	22.33±5.86 ^a	22.33±4.04 ^a	37.00±5.29 ^a	31.33±6.51 ^a	17.33±3.79 ^a	24.33±1.53 ^a	20.67±3.21 ^a	33.00±4.36 ^a	19.00±4.36 ^a	26.67±2.31 ^a	30.33±5.03 ^a
		5	8.67±1.15 ^b	8.67±4.51 ^b	10.00±4.58 ^b	48.67±11.15 ^b	29.67±3.06 ^b	23.33±6.11 ^b	15.33±3.21 ^b	18.67±2.89 ^b	18.33±2.52 ^b	14.33±7.02 ^b	15.00±1.73 ^b	13.00±0.00 ^b
		8	14.00±3.61 ^b	17.67±3.79 ^b	17.67±4.16 ^b	15.67±3.51 ^b	16.33±4.04 ^b	13.00±3.00 ^b	16.00±3.61 ^b	15.00±1.73 ^b	19.33±5.51 ^b	20.33±3.51 ^b	18.67±4.51 ^b	17.67±4.16 ^b
		60	3.00±1.00 ^c	4.33±2.31 ^c	4.67±1.15 ^c	5.00±2.65 ^c	3.67±3.51 ^c	4.67±0.58 ^c	2.33±1.53 ^c	2.00±0.00 ^c	3.67±1.15 ^c	3.33±1.15 ^c	3.67±2.52 ^c	3.67±0.58 ^c
		120	2.00±1.00 ^d	0.67±1.15 ^d	1.67±1.53 ^d	2.00±1.00 ^d	1.33±1.15 ^d	1.33±1.15 ^d	2.00±1.00 ^d	2.33±2.08 ^d	1.00±0.00 ^d	1.00±1.00 ^d	2.33±1.15 ^d	0.33±0.58 ^d
		180	2.00±0.00 ^d	0.33±0.58 ^d	1.00±1.00 ^d	0.33±0.58 ^d	1.00±1.00 ^d	1.33±0.58 ^d	1.67±1.53 ^d	1.50±0.71 ^d	1.00±1.00 ^d	0.00±0.00 ^d	1.33±1.53 ^d	1.33±2.31 ^d

The numbers in the cell indicated the means and standard deviations of *A. flavus* concentration and the means with different alphabetic superscript indicated significantly different among investigated time at 5% levels according to Kruskal–Wallis post hoc test

among sites. These results indicated the spores were evenly distributed indoors in 6–8 min. At the 60th, 120th and 180th minute after hanging plates, the concentration of *A. flavus* among four sampling sites was less than 5 CFU/plate on average (Table 1; Fig. 3D–3F). After one hour, the number of spores decreased to the background value.

The temporal distribution of *A. flavus* in the building was illustrated in Fig. 4. At the upper layer, the highest concentration appeared in the S2 plate at the 5th minute (Fig. 4A). At the middle and bottom layer, the concentrations of *A. flavus* at the 2nd, 5th and 8th minute were higher than that after 8 min (Fig. 4B, 4C). The results indicated most spores in the plates were released and evenly suspended in the air after 8 min. The concentration of spores was decreased to 3–6 CFU/plate after 60 min, 0–3 CFU/plate after 120 min and 0–4 CFU/plate after 180 min (Table 1; Fig. 4), which indicated that short-term biological contamination in a room with exhaust facilities decreased to the background value in one hour. Concentration of *A. flavus* was different significantly among investigated time (Kruskal–Wallis chi-squared = 175.73, P -value < 0.05).

4 Discussion

The dispersal pattern of fungal spores during 0–2, 3–5 and 6–8 min through air conditioning was presented in this study. We found that airborne fungal spores were dispersed by HVAC systems efficiently. At the first 2 min, lots of spores were detected in the plates near the outlet. At the 2nd minute, site S2 which was 1.2 m away from the spore sources had the highest concentration of *A. flavus* at upper and middle layers. At the 2nd minute, site S3 had the highest concentrations at the bottom layer. Nearly 90% of the spore transmission occurred within minutes of exposure to airflow. After 8 min, the spore concentration in the room became homogenized. The results suggested that air conditions facilitated the homogenization of spore concentration in a room in six minutes. After 10 min, the external source of spore release was removed. Most spores were removed by the exhaust system and deposited on the floor after 120 min. The indoor fungal contaminants were the critical factor to determine the influences of air conditions. If fungal contaminants existed in the ducts for central air

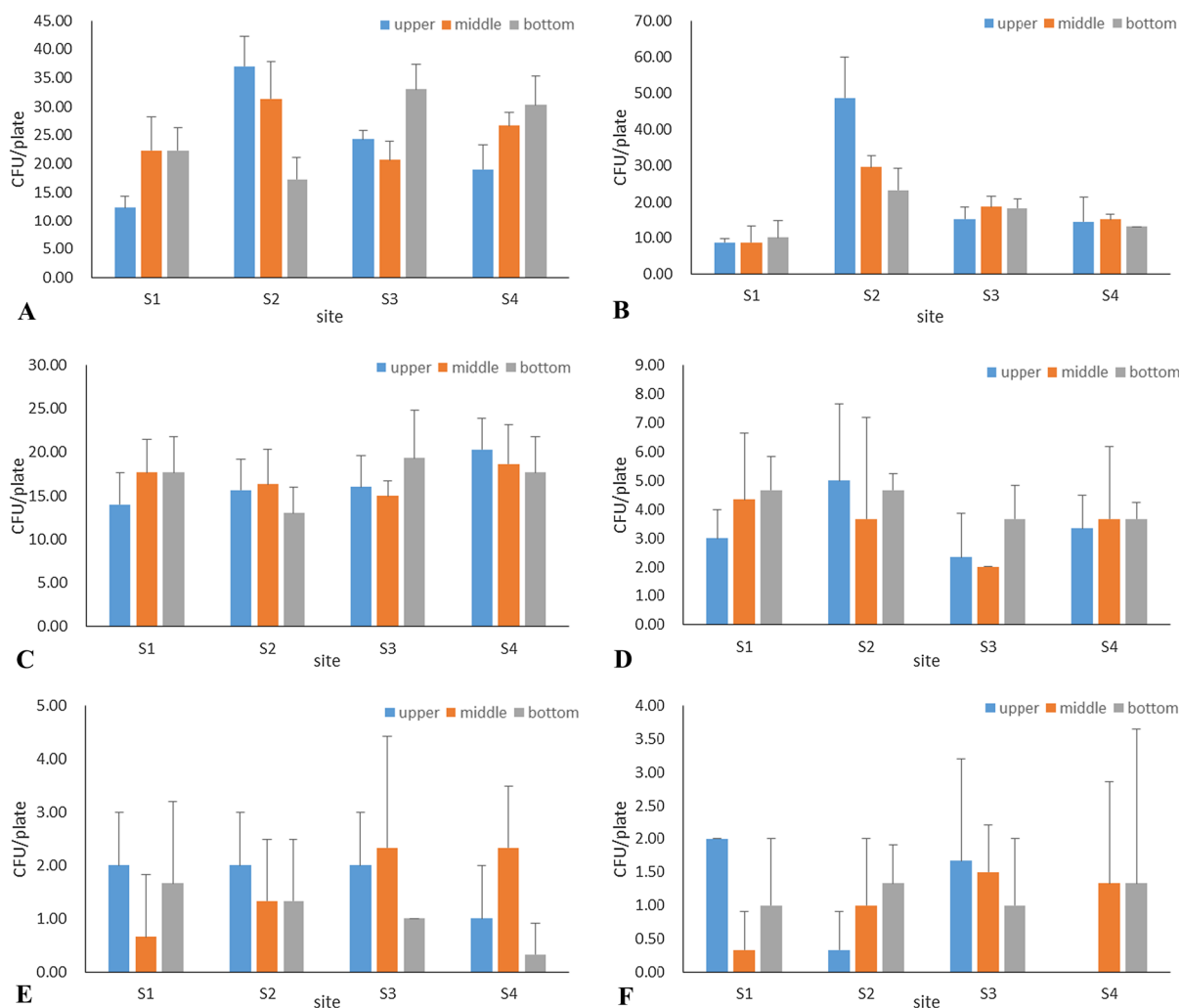


Fig. 3 Spatial distribution of *Aspergillus flavus* in four sampling sites at three layers at second (A), 5th (B), 8th (C), 60th (D), 120th (E) and 180th min (F) since spore released

conditioning, the airborne fungal spores could be dispersed by HVAC systems efficiently. If not, the HVAC systems could facilitate the removal of particles through the exhaust system. The mildew growing on the ducts makes central air conditioning a source and dispersal of fungal contaminants.

This study provided the field data quantitatively describing fungal dispersal through HVAC systems with an external fungal spore source. The temporal and spatial distribution patterns of the fungal spores were illustrated. We found that a large proportion of *A. flavus* spores was released and evenly suspended in the air in 8 min. This was also reported by Górny et al. (2001) and Sivasubramani et al. (2004) in the

aerosolization chamber or tube. Górny et al. (2001) investigated the release of *A. versicolor* spores from agar in an aerosolization chamber and found that 71–88% spores became airborne during the first 10 min. Sivasubramani et al. (2004) also found a similar released pattern in a laboratory tube. Buttner et al. (1999) reported that the highest concentrations of *Penicillium chrysogenum* spores were dispersed at the initial start-up of the system and a rapid decline was observed. The evidence suggested that a large proportion of spores was released during the first 8–10 min effectively.

We also found that the passive release of *A. flavus* spores through the ceiling vent was as a function of

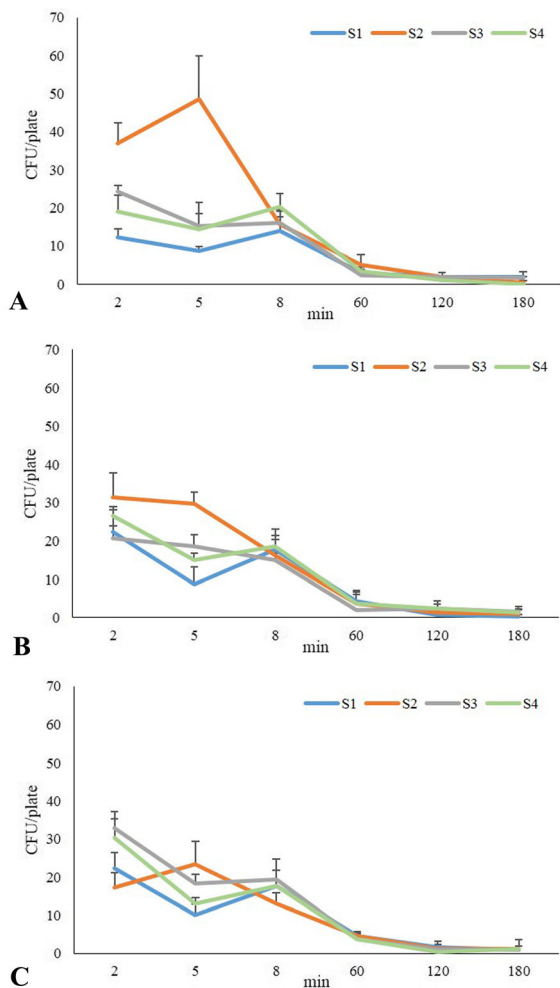


Fig. 4 Temporal distribution of *Aspergillus flavus* in four sampling sites at the height of about 2.2 (A) and 1.2 m (B) above the floor and on the floor (C)

dispersal distance in the beginning. *Aspergillus flavus* produces conidia ranging from 3 to 6 μm and they dispersed to 3.6 m through the air conditioning within 2 min. The spore was evenly distributed in the room in 8 min, which indicated the air current from the central air condition and the gravity influenced the spore deposition rate. The deposition of airborne fungal spores on indoor surfaces is one of the most important issues of particle dynamics. Ventilation is an important factor to influence the spore deposition rates indoor (Kanaani et al., 2008). They found that the deposition rates of fungal spores (*A. niger* and *Penicillium* species) were similar, especially at very low air exchange rates (Kanaani et al., 2008).

In this study, the settle plate method was used to collect *A. flavus*. This method gathers bioaerosol through gravitational force (Kim et al., 2018). Compared to active sampling methods, the limitation of the settle plate method was the collection of smaller particles because the dry deposition of particles is a function of their mass, as well as the estimation of the CFUs counted from a certain amount of air (Wikuats et al., 2020). The advantage of this method included being economically feasible, easily applicable, and allows samplings from different places at the same time (Ghosh et al., 2015). The results derived from this method are reproducible and reliable, and describe the real conditions.

In this study, the release of *A. flavus* was from agar, not from ceiling tile surfaces. Rough ceiling tile has larger area from fungal growth because of its irregular, almost porous texture (Górny et al., 2001). The larger area might result in a higher release of spores (Górny et al., 2001). However, at the low air velocity, no statistically significant difference for the release was observed between agar and ceiling tile surfaces (Górny et al., 2001). In this study, the airflow rates of the supply vent are 1.9–2.0 m/s. The airflow rate of the tested laboratory was non-detectable. Under such low air velocity, the HVAC system could greatly reduce the number of spores in the indoor air within one hour. Our study provided valid results about the efficient removal of bio-contamination by the HVAC system in public environments.

5 Conclusion

This study presents results from ex situ experiments conducted to a better understanding of the spatiotemporal fluctuation and passive dispersal of airborne fungal spores by the airflow of HVAC systems. A good HVAC system could greatly reduce the number of spores in the indoor air within one hour. Conversely, with the fungal source from contaminated materials and niches, the airborne fungal spores were effectively spread within 2 min and evenly distributed indoors in 6 min.

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