

# Airborne fungal microbiota in indoor and outdoor classrooms of schools in Ho Chi Minh City: concentration and composition

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**Abstract:** Airborne microbial contamination is vital for evaluating air quality and its impact on human health. This study investigated airborne fungal concentrations and compositions in indoor and outdoor classrooms at a university and two secondary schools in Ho Chi Minh City, Vietnam, aiming to understand the factors influencing fungal presence and propose preventative measures for potential health risks. 120 air samples were collected over a semester using an active sampling method with a SKC Biostage (single stage bioaerosol impaction) and cultivated on Sabouraud Dextrose Agar (SDA). Fungal concentration was measured in air-conditioned (AC) and non-air-conditioned (non-AC) classrooms, both with and without students present, and compared to outdoor levels in schoolyards. Results revealed lower average fungal concentration in AC classrooms ( $178.31 \pm 47.32$  CFU/m<sup>3</sup>) compared to non-AC classrooms ( $236.11 \pm 57.24$  CFU/m<sup>3</sup>), while outdoor areas exhibited an average density of  $223.57 \pm 41.42$  CFU/m<sup>3</sup>. Correlation analysis indicated a positive relationship between fungal density and both temperature and humidity. Air conditioning was found to significantly reduce fungal density by regulating these factors and filtering fungal spores. Most indoor samples exhibited fungal concentrations below the World Health Organization (WHO) recommended limit of 500 CFU/m<sup>3</sup>. Identified fungal genera included *Aspergillus* (specifically *A. niger*, *A. terreus*, *A. fumigatus*, *A. oryzae*, *A. flavus*) and *Trichoderma* spp. This study provides insights into the airborne fungal microbiome within educational settings in Ho Chi Minh City, emphasizing the influence of environmental factors and human activity. The findings contribute to a better understanding of potential health risks associated with airborne fungi and inform strategies for improving indoor air quality in schools.

**Keywords:** bioaerosols; classroom, outdoor; fungal concentration; fungal composition

## 1. Introduction

Bioaerosols, comprising airborne particles of biological origin such as virus, bacteria, algae, archaea, fungi, fungal spores, pollen, particulate matter, and cellular by-products, represent a significant fraction (30% – 80%) of atmospheric particulate matter (Xie et al., 2018). Among these, infectious bioaerosols pose health risks, contributing to pathologies such as infectious diseases, asthma, acute toxic reactions, allergies, and cancer (Douwes et al., 2017; Jeong et al., 2022). Fungi, a major component of bioaerosols, are commonly used as an indicator of indoor air quality due to their environmental ubiquity and close association with human body (Haas et al., 2023; Wu et al., 2021). Fungal spores, easily dispersed in the air, pose significant health threats, particularly to vulnerable groups such as children and adolescents with developing immune systems (Lee et al., 2021). Prolonged exposure to mold-contaminated environments can increase the risk of respiratory diseases and chronic illnesses (Guo et al., 2021; Wu et al., 2020; Wu, et al., 2021).

Fungi are not only spread through the air but also adhere to surfaces such as tables, chairs, walls, curtains, other objects and from human activities. In addition, indoor airborne molds are also affected by outdoor sources (Wu et al., 2020). Students spend a significant amount of time in school, where exposure to airborne fungi can lead to health issues such as lung diseases, mucous membrane irritation, and fungal infections (Hussin et al., 2011; Limon et al., 2017; Portnoy et al., 2005). Indoor fungi, indicated by visible spots and odors, has been linked to an increased risk of children's pneumonia (Guo et al., 2021). Outdoors, elevated fungal spore concentrations are associated with allergic and asthmatic responses. Studies in Canada and Southern California found that fluctuations in ambient mold spore levels correlate with childhood asthma attacks, even in areas with relatively low spore concentrations ( $\sim 4,000$  spores/m<sup>3</sup>) (Dales et al., 2004; Delfino et al., 1997; Solomon et al., 2006). Additionally, molds can produce mycotoxins that are linked to a range of health problems, including skin rashes, immune suppression, organ damage, and

potential cancer risks. Volatile organic compounds (mVOCs) from molds, known for their strong odors, have been associated with headaches, nasal irritation, and nausea (Sandilli, 2002). Identifying the species composition of molds in the air is crucial for assessing human health risks (Wu et al., 2021). Species such as *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria*, that are known for their allergenic and pathogenic potential, while others are non-pathogenic (Haas et al., 2023; Hussin et al., 2011; Sautour et al., 2009; Shin et al., 2015). Identifying fungal species is therefore essential for assessing health risks and designing targeted preventive measures (Hospodsky et al., 2012).

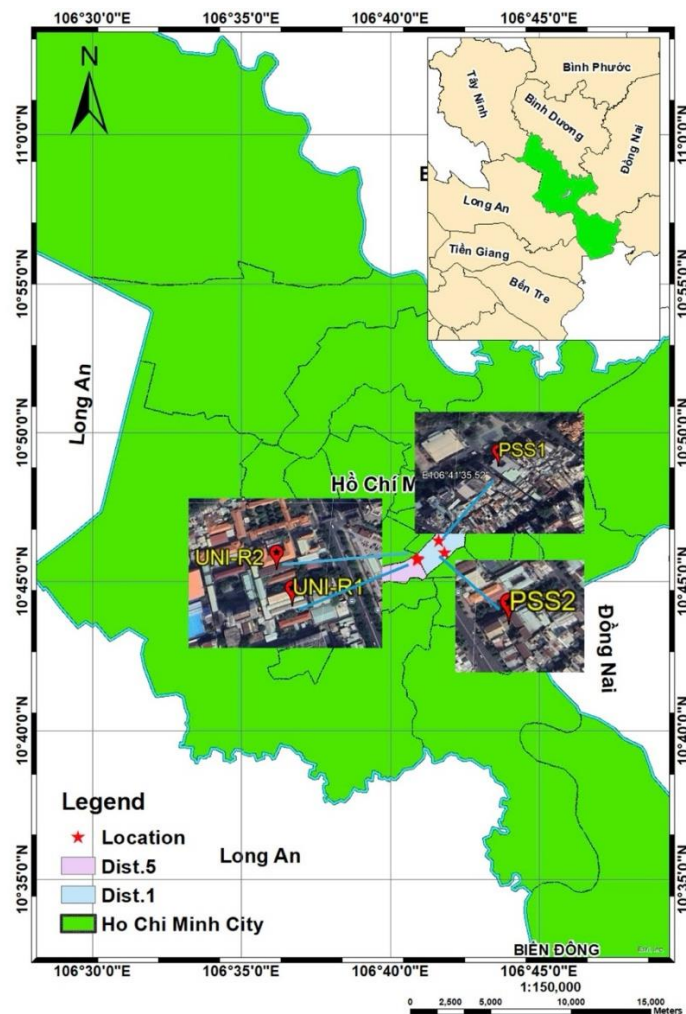
Ho Chi Minh City, with its rapidly urbanizing, industrializing environment, and tropical climate, provides an ideal environment for the growth and spread of airborne microorganisms, including molds (Hai et al., 2019). Although there are variety studies on air quality and airborne pathogens in the world, research on molds in classroom air in Vietnam is still very limited (Hai et al., 2019; Huy et al., 2022). Several studies on airborne fungal concentration and diversity in the world have been conducted in hospitals, schools, kindergartens, etc. At a hospital in France, Sautour et al. (2009) showed that the indoor fungal concentration was highest in summer (4.2 to 5.0 CFU/m<sup>3</sup>) and lowest in winter (2.7 to 3.1 CFU/m<sup>3</sup>) and the outdoor was highest in autumn (103.7 CFU/m<sup>3</sup>) and lowest in winter (49.0 CFU/m<sup>3</sup>). A study by (Guo et al., 2021) in China focused on the microbial concentration in buildings such as residences, classrooms, hospitals, and offices and found that the indoor fungal concentration was highest in residential areas (749 ± 726 CFU/m<sup>3</sup>). (Yogeswaran et al., 2023) reported fungal concentrations in air-conditioned laboratories in Malaysia, where the aquaculture laboratory exhibited the lowest fungal concentration at 30.87 ± 35.64 CFU/m<sup>3</sup>, while the virology laboratory recorded the highest level at 528.12 ± 36.13 CFU/m<sup>3</sup>. In South Korea, a study on indoor air quality at childcare centers by Oh et al. (2014) reported average indoor fungal concentrations ranging from 95.6 ± 34.2 CFU/m<sup>3</sup> to 269.6 ± 29.6 CFU/m<sup>3</sup>, which were higher than outdoor concentrations ranging from 176.0 ± 38.5 CFU/m<sup>3</sup> to 221.6 ± 23.5 CFU/m<sup>3</sup>.

This study addresses the gap by analyzing fungal concentrations in classrooms and outdoor spaces at schools in central Ho Chi Minh City. The objectives are to: (1) evaluate and compare indoor and outdoor fungal levels, (2) assess the impact of human activity on mold levels, and (3) identify mold species (mold belong to fungi) that may pose a health risk to humans. The findings in this study offer valuable scientific evidence for environmental managers to improve school infrastructure ventilation systems to support student health in Vietnam in the future.

## 2. Materials and methods

### 2.1. Sampling sites

Airborne fungal samples were collected from four classrooms (2 AC classes and 2 non-AC classes), and outdoor samples from schoolyards during one semester from February to June 2024 in Ho Chi Minh City, Vietnam. The city's climate is characterized by a tropical monsoon regime with two distinct seasons: the dry season and the rainy season. From November to April, the dry season brings minimal rainfall, accounting for only 10–15% of the total annual precipitation, along with high evaporation and temperatures averaging 29–30°C. In contrast, the rainy season, spanning May to October, contributes 85–90% of the annual rainfall, with recent years seeing totals ranging between 1,000 and 1,600 mm (Khoi et al., 2020).



**Fig. 1.** Sampling sites at one University and two PSS schools in Ho Chi Minh City

These samples were collected when the classrooms and schoolyards were occupied and unoccupied. The criteria for selecting classrooms in the study were ventilation, classroom area, and the number of students in the class were similar between classes with AC and non-AC. At the university, two representative classrooms were selected: one classroom with AC (UNI-R1) and one non-AC (UNI-R2). About 80~100 students for every class, and one outdoor sampling location was set in front of the classroom, mean schoolyard. At the two public secondary schools, samplers were selected in one classroom and one schoolyard at every school. The first public secondary school (PSS1) was categorized in AC class (mean PSS1) and schoolyard (out-PSS1) in and the second public secondary school (PSS2) was collected in non-AC class (mean PSS2) and schoolyard (out-PSS2) (Fig. 1).

## 2.2. Equipment and collecting of fungi in bioaerosol samplings

Fungi in bioaerosol samples were collected on SDA medium dishes placed in the single-stage bioaerosol (SKC Biosatge, USA) equipment with a total of 120 samples. Sampling was conducted using the active method at 9:00 a.m. every Friday. During each sampling session, two samples were collected simultaneously resulting in seven sampling areas. Samples were collected only twice at the same time of day. The active sampling method operated at a flow rate of 28.3 L/min for 5 minutes. Before each sampling, the SKC Biostage equipment was meticulously cleaned by disassembling it, immersing the components in an ultrasonic bath filled with Mili-Q water, and disinfecting the sampling site using a sterile swab soaked in 70% ethanol (Faridi et al., 2015). The sampling height was adjusted close to the breathing zone of humans, 1.5 m above the floor. The plates containing the samples were carefully covered with protective film, labeled and transported to the laboratory for the next analysis. During the sampling process, the number of students and the temperature and humidity of each sampling room were also recorded. Colonies of fungi were counted after 70–120 hours of incubation at  $37\pm 1$  °C for fungal qualitative and quantitative analysis and identification later (Hai et al., 2019).

Throughout the sampling process, meteorological parameters such as temperature (T), relative humidity (RH), CO<sub>2</sub>, and PM<sub>2.5</sub> were continuously recorded using the Aerobox outdoor sensor kit from National Central University, Taiwan, as part of the PM<sub>2.5</sub> @Asia project. The sensor kit is calibrated biannually by the research team at NCU to ensure measurement accuracy.

### 2.3. Methods for fungi quantifying and identifying

The quantity of fungal concentration was determined by counting the number of CFU/m<sup>3</sup>. Each mold sampling petri dish was counted the colony forming units (CFU), by the following formula:

$$A = a \times \frac{1000}{q \times t} \quad (1)$$

Where:

*A*: colony forming unit (CFU/m<sup>3</sup>)

*a*: counted colonies/ Petri dish

1000: conversion factor between liters and cubic meters (m<sup>3</sup>)

*t*: time (min)

*q*: flow (L/min)

The identification of fungal bioaerosols at genus and species levels relied on macroscopic and microscopic morphologies classification. Fungal colonies were classified based on their colony type, cell morphology, and various morphological characteristics, including the structure of spores, hyphae, and fruiting bodies, alongside biochemical and molecular methods. Tools like dichotomous keys and phylogenetic trees were utilized to assist in the identification process (Pitt & Hocking, 2009). Cultured fungi were analyzed and isolated for qualitative identification. A microscope was used to examine typical colony characteristics, followed by staining to assess the colonies' shape, arrangement, and color. Finally, biochemical identification tests and antibiogram techniques were performed.

### 2.4. Statistical analysis

Data analysis for this study was conducted using R software version 4.3.2. The Kruskal-Wallis H Test was applied to evaluate differences in fungal concentrations across sampling locations, with statistical significance defined as a p-value less than 0.05. Visualization of the data, including fungal composition, concentration, and correlations with microclimatic factors, was performed using the "ggplot2" package in R. The bootstrap regression analysis provided stable coefficient estimates, suggesting a strong and reliable relationship between the environmental factor variables and the outcome variable.

## 3. Results and discussion

### 3.1. Airborne Fungal concentration and meteorological factors in classrooms and schoolyards

This study measured aerosol fungal density at seven sampling locations at one university and two public secondary schools in Ho Chi Minh City. These locations consisted of four classrooms (two air-conditioned and two naturally ventilated without air conditioning) and three outdoor schoolyards. The mean and range of total airborne fungal density are presented in Table 1. The average fungal concentration in the indoor classrooms was  $117.98 \pm 84.67$  CFU/m<sup>3</sup> with a range from 28.27 to 295.70 CFU/m<sup>3</sup> both AC and non-AC rooms. The average fungal concentration in three schoolyards was  $88.29 \pm 74.77$  CFU/m<sup>3</sup> ranging from 21.20 to 284.95 CFU/m<sup>3</sup> at the university and two secondary schoolyards. In the occupied non-AC (UNI-R2 room), the mean concentration of culturable fungi was  $236.11 \pm 57.24$  CFU/m<sup>3</sup> higher than in AC (UNI-R1 room) ( $178.31 \pm 47.32$  CFU/m<sup>3</sup>). Similarly, at two PSSs, the mean concentration of culturable fungi in non-AC room PSS2 was  $53.71 \pm 3.87$  CFU/m<sup>3</sup> and in AC room PSS1 ( $45.94 \pm 10.72$  CFU/m<sup>3</sup>).

**Table 1.** The collected fungal concentration and environmental factors in classrooms and schoolyards during one semester

Characteristic	In								
	UNI			PSS1-AC			PSS2 non-AC		
	Overall $N = 16^l$	Non-Stu $N = 4^l$	Stu $N = 12^l$	Overall $N = 9^l$	Non-Stu $N = 3^l$	Stu $N = 6^l$	Overall $N = 7^l$	Non-Stu $N = 2^l$	Stu $N = 5^l$
Concentration (CFU/m <sup>3</sup> )	190.02 ± 60.36 (94.09 - 295.70)	138.44 ± 30.85 (96.77 - 166.67)	207.21 ± 58.46 (94.09 - 295.70)	41.62 ± 10.86 (28.27 - 63.60)	32.98 ± 4.08 (28.27 - 35.34)	45.94 ± 10.71 (35.34 - 63.60)	51.49 ± 5.34 (42.40 - 56.54)	45.94 ± 5.00 (42.40 - 49.47)	53.71 ± 3.87 (49.47 - 56.54)
Temperature (°C)	32.79 ± 1.82 (28.44 - 35.62)	32.83 ± 2.05 (30.61 - 35.53)	32.78 ± 1.84 (28.44 - 35.62)	28.30 ± 1.20 (26.85 - 30.35)	29.72 ± 0.55 (29.31 - 30.35)	27.59 ± 0.61 (26.85 - 28.40)	30.38 ± 2.07 (26.67 - 33.67)	30.10 ± 0.04 (30.07 - 30.13)	30.49 ± 2.53 (26.67 - 33.67)
Relative Humidity (%)	62.99 ± 7.59 (46.45 - 72.34)	60.77 ± 7.00 (53.76 - 70.15)	63.73 ± 7.92 (46.45 - 72.34)	61.19 ± 9.77 (47.75 - 74.69)	61.50 ± 12.32 (47.75 - 71.54)	61.03 ± 9.59 (47.91 - 74.69)	66.45 ± 4.05 (62.55 - 72.84)	68.37 ± 6.33 (63.89 - 72.84)	65.68 ± 3.47 (62.55 - 70.15)
CO <sub>2</sub> (ppm)	550.11 ± 69.42 (410.41 - 676.10)	531.85 ± 57.64 (457.80 - 598.47)	556.20 ± 74.19 (410.41 - 676.10)	836.08 ± 322.87 (431.12 - 1,375.89)	450.06 ± 17.00 (431.12 - 464.00)	1,029.09 ± 180.45 (878.96 - 1,375.89)	677.57 ± 122.11 (464.85 - 802.81)	572.09 ± 151.65 (464.85 - 679.32)	719.76 ± 93.97 (560.25 - 802.81)

<sup>l</sup>Mean ± SD (Min-Max)

Characteristic	Out								
	UNI			PSS1			PSS2		
	Overall $N = 6^l$	Non-Stu $N = 3^l$	Stu $N = 3^l$	Overall $N = 12^l$	Non-Stu $N = 6^l$	Stu $N = 6^l$	Overall $N = 10^l$	Non-Stu $N = 5^l$	Stu $N = 5^l$
Concentration (CFU/m <sup>3</sup> )	200.27 ± 58.47 (142.48 - 284.95)	150.54 ± 8.06 (142.48 - 158.60)	250.00 ± 32.59 (220.43 - 284.95)	49.47 ± 13.48 (21.20 - 63.60)	41.23 ± 14.43 (21.20 - 56.54)	57.72 ± 5.32 (49.47 - 63.60)	53.71 ± 7.60 (42.40 - 63.60)	49.47 ± 7.07 (42.40 - 56.54)	57.95 ± 5.91 (49.47 - 63.60)
Temperature (°C)	33.30 ± 0.98 (31.84 - 34.34)	33.37 ± 0.86 (32.38 - 33.96)	33.24 ± 1.28 (31.84 - 34.34)	32.22 ± 2.63 (27.20 - 35.89)	32.00 ± 2.86 (27.20 - 35.89)	32.44 ± 2.64 (27.96 - 35.79)	31.85 ± 1.44 (29.21 - 33.39)	31.52 ± 1.53 (29.21 - 33.35)	32.18 ± 1.44 (29.83 - 33.39)
Relative Humidity (%)	64.01 ± 7.77 (49.89 - 71.99)	61.94 ± 10.46 (49.89 - 68.61)	66.07 ± 5.35 (61.57 - 71.99)	56.49 ± 8.55 (44.45 - 68.69)	57.07 ± 8.77 (46.47 - 68.69)	55.91 ± 9.11 (44.45 - 67.04)	60.80 ± 5.52 (53.51 - 67.89)	61.50 ± 5.98 (53.66 - 67.89)	60.09 ± 5.62 (53.51 - 66.14)
CO <sub>2</sub> (ppm)	538.52 ± 61.92 (422.86 - 603.61)	516.72 ± 81.28 (422.86 - 563.77)	560.31 ± 39.40 (526.56 - 603.61)	497.78 ± 49.55 (439.64 - 597.53)	478.03 ± 31.90 (439.64 - 521.95)	517.52 ± 58.72 (457.52 - 597.53)	561.06 ± 60.66 (497.55 - 670.52)	543.43 ± 63.93 (497.55 - 655.74)	578.68 ± 58.45 (511.64 - 670.52)

<sup>l</sup>Mean ± SD (Min-Max)

Note: UNI : University AC : Air-Conditioning Rooms  
 Stu : Student presence Non-AC: Non-Air-Conditioning Rooms  
 Non-Stu: without student PSS1 : First Publish Secondary School  
 In : Classroom PSS2 : Second Publish Secondary School.  
 Out : Schoolyard

When unoccupied, the mean fungal value in AC classrooms was  $115.69 \pm 4.95$  CFU/m<sup>3</sup> in UNI-R1 and  $32.98 \pm 4.08$  CFU/m<sup>3</sup> in PSS1. Both of these figures were lower than the mean fungal value in non-AC classrooms, such as UNI-R2 ( $161.29 \pm 1.41$  CFU/m<sup>3</sup>) and PSS2 ( $45.94 \pm 5.00$  CFU/m<sup>3</sup>). These results emphasize the role of air conditioning in reducing fungal presence by facilitating air circulation and filtration (Wu et al., 2021). It minimized the dispersion of fungal particles and maintained stable temperature and humidity, thereby limiting the conditions for fungi growth. Both UNI-R1 and PSS1 were AC classrooms; however, UNI-R1 exhibited a higher fungal concentration. The larger sizes of university classrooms explained this difference compared to those in the secondary schools, as well as the significant variation in student numbers - approximately 100 students in the university classroom versus around 40 students in each secondary school classroom. This study also explains that UNI-R2 and PSS2 are similar in non-AC classrooms.

In the university schoolyard, the mean concentration of environmental fungi while unoccupied was  $150.54 \pm 28.36$  CFU/m<sup>3</sup> and increased to  $223.57 \pm 41.42$  CFU/m<sup>3</sup> when occupied by students. Meanwhile, at schoolyards of PSS-OD1 and PSS-OD2, the mean concentrations of environmental fungi with presented students were  $57.71 \pm 5.32$  CFU/m<sup>3</sup> and  $57.95 \pm 5.91$  CFU/m<sup>3</sup> respectively. This suggested that human presence may contribute to the diffusion and increase in fungi concentration. The environment of outdoor spaces, where air circulation is continuous, aids in dispersing and reducing the airborne fungi. When students were present, the movement of air could stir up deposited fungal spores, leading to an increase in fungi density. This indicates that human activity may significantly influence fungal levels (Wu et al., 2020).

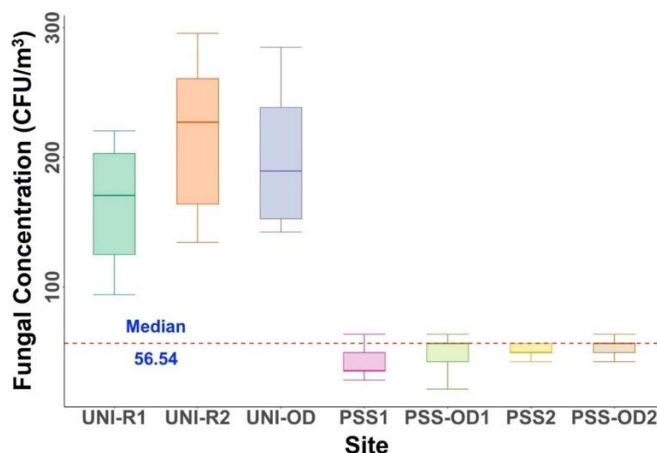
When comparing the fungal concentration between non-AC classrooms (UNI-R2 and PSS2) and the ambient air outdoors, the results showed that the outdoor fungal concentration was correspondingly lower in an environment of schoolyards. This indicated that the air entering non-AC classrooms was not filtered, causing more fungi to accumulate on objects and desks. (Wu et al., 2021). In this study, the outdoor airborne fungal particles in the sampling areas was lower than in some previous studies. Fang et al. (2005) also reported that fungal concentrations ranged from 24 to 13,960 CFU/m<sup>3</sup>. In another research, fungal level was from 21,000 to 102,000 spores/m<sup>3</sup> of outdoor air in New Orleans, Louisiana, USA by Solomon et al. (2006). A study by Sautour et al. (2009) reported that the concentration of fungi in the air was below 500 CFU/m<sup>3</sup>. In addition, the study by (Wu et al., 2020) found the fungal number to be  $721 \pm 249$  CFU/m<sup>3</sup>. A study by Dalles et al. (2020) of children aged 8 to 18 at the Children's Hospital in Ottawa found that asthma symptom scores increased by 10%–30% for every 1000 spores/m<sup>3</sup> increase in airborne mold concentration. These findings emphasize the critical importance of maintaining low fungal densities in school environments to safeguard respiratory health.

Environmental factors such as high temperature and high humidity in non-AC classrooms could facilitate fungi growth and spread more than in AC classrooms. Meanwhile, schoolyards mean the open spaces and frequent wind help dilute the concentration of airborne fungi originating from planted surroundings and road dust in the air (Uk Lee et al., 2016). On the other hand, Lee et al. (2021) showed that fungi density did not differ significantly between indoors and outdoors, which was consistent with our study results.

### **3.2. Effect of human presence on fungal concentration**

The results from this study showed that the concentration of fungi in the classroom air with people was significantly higher than that in classrooms without people. This aligns with the findings of Hussin et al. (2011) research, which indicates that people were one of the factors contributing to increased fungal concentrations in indoor environments. The samples were collected at seven locations during the study to assess the variation in fungal density. The Kruskal-Wallis H Test showed a significant difference (p-value) in fungal concentrations at indoor and outdoor sites over the entire study period. However, significant variation ( $p < 0.05$ ) was observed in fungal concentrations on monitored sampling sites in a variety of conditions AC, non-AC and OD, and people, which can be explained by the number of fungal concentrations were relatively higher indoor and outdoor with occupied compared to non-occupied.

The study assessed the variation of spatial fungal concentration with sample sizes for each condition ( $n = 17$  for AC,  $n = 15$  for non-AC and  $n = 28$  for OD) (Figure 2). The data showed that human presence increasing air fungal count in AC and OD samplings, where the fungal bioaerosol levels difference between groups was insignificant. Notably, fungal concentration varied significantly in outdoor conditions with people, displaying instability. These findings highlighted that human presence could impact fungal bioaerosols in AC or non-AC classrooms, within the context of indoor air quality studies. In this case, humans were determined one of the factors to the increase fungal bioaerosols. Additionally, other factors inside such as furniture (e.g., tables and chairs), walls, curtains and items brought indoors by humans also played a role.



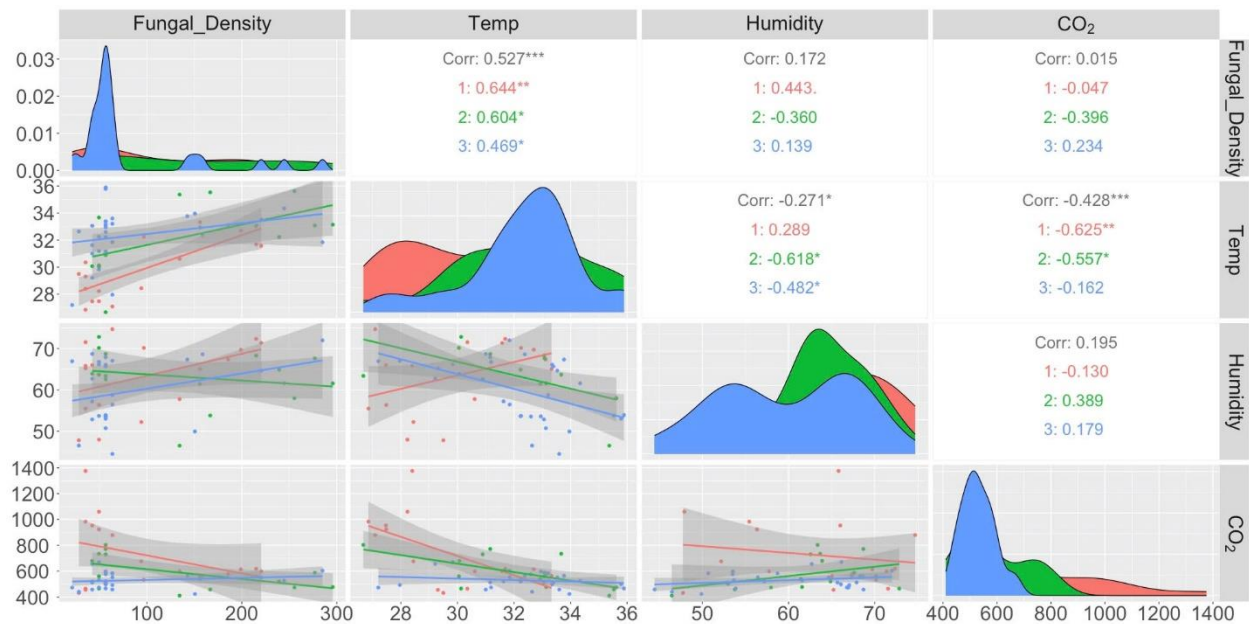
**Fig. 2.** Distribution of fungal concentration at different sampling locations

According to Sandilli et al. (2020), the Environmental Protection Agency (EPA) states had reports that half of the nation’s 115,000 schools face indoor air quality issues. Children are particularly vulnerable due to the extended time spent in school facilities, prompting the EPA to create the “Indoor Air Quality Tools for Schools” kit to help identify and address mold problems. In Connecticut, 68% of schools reported air quality issues, a rise in asthma rates among children (with one in ten affected) and the health problems of teachers such as asthma, chronic sinusitis, and headaches. Research showed that an increase of 1,000 fungal spores/m<sup>3</sup> can raise asthma symptom scores by 10–30%, and exposure to mold and moisture damage in schools exacerbates respiratory problems in children.

In addition, the fungal concentration in the schoolyards did not differ significantly between times with and without students, indicating that outdoor factors, such as wind and sunlight, played one more important role in maintaining stable fungal density than human factors. This may indicate that the sources of fungi in the schoolyard mainly came from the natural environment, and the impact of humans on the spread of fungi outdoors was comparatively limited as compared to closed environments.

### 3.3. Effect of environmental conditions on fungal concentrations

Indoor and outdoor microclimatic conditions affect the growth rate of indoor microbiological contaminants (Jones & Harrison, 2004). The regression lines reveal trends within each group, providing insights into the variability and interactions among variables under specific conditions (Fig.3).



**Fig. 3.** Correlation matrix among fungal concentrations and microclimatic conditions

The correlation matrix among fungal concentration, temperature (Temp), humidity (Humidity), and CO<sub>2</sub> concentration (CO<sub>2</sub>) displayed a relatively strong positive correlation between bacterial density and temperature ( $p < 0.001$ ), suggesting that fungal spores tends to increase as temperature rises. Additionally, there was a weak positive correlation between fungal concentration and humidity. CO<sub>2</sub> concentration had no evident effect on fungi within the examined conditions. Environmental parameters including temperature, relative humidity, and CO<sub>2</sub> concentration had important impacts on the level of fungi in an indoor environment (Wu et al., 2020).

Temperature was an important factor that directly affects the growth of airborne fungi (Ponce-Caballero et al., 2013). The average temperature in classrooms and outdoor areas was recorded to range from  $29.70 \pm 0.50^\circ\text{C}$  to  $35.53 \pm 1.66^\circ\text{C}$ , respectively. Meanwhile, UNI-R2 room with non-AC had the highest temperature, reaching  $35.53 \pm 1.66^\circ\text{C}$ , while air-conditioned classrooms such as PSS1 had an average temperature of  $31.54 \pm 0.70^\circ\text{C}$ . Excessively high temperatures increase the rate of water evaporation, resulting in drier air and consequently reducing the growth and dispersion of fungi. In contrast, warm temperatures, especially when accompanied by high humidity, promote fungal growth (Wu et al., 2020). This study showed that in non-AC rooms where temperatures were higher, fungal densities were also significantly higher.

As evidenced by the UNI-R2 room, the fungal density was  $178.32 \pm 53.28 \text{ CFU/m}^3$ , while in the AC-UNI-R1 room, the fungal density was only  $161.29 \pm 47.32 \text{ CFU/m}^3$ . This shows that the lower temperatures maintained by the air-conditioning system helped limit fungal growth, while the higher temperatures in the non-air-conditioned classrooms created ideal conditions for their proliferation (Jeong et al., 2022).

Humidity significantly affects air fungal count, supported by high humidity levels and environmental factors like trees and traffic near the university (Ponce-Caballero et al., 2013). In contrast, middle schools with fewer trees and less traffic had lower fungal density, showing humidity isn't the sole factor.

CO<sub>2</sub> levels indicate air quality, with higher levels in poorly ventilated spaces (Guo et al., 2021). AC-UNI-R2 had higher CO<sub>2</sub> (607.72 ppm) than non-AC-UNI-R2 (565.56 ppm) due to better ventilation in the latter. Outdoor CO<sub>2</sub> was lower, reflecting improved air circulation and air quality.

Fungi in the ambient air are more easily dispersed by wind than in the enclosed spaces of classrooms (Haas et al., 2023). The interaction between temperature, humidity, and CO<sub>2</sub> concentration was an important factor in determining the density of airborne fungi (Ponce-Caballero et al., 2013). Air-conditioned

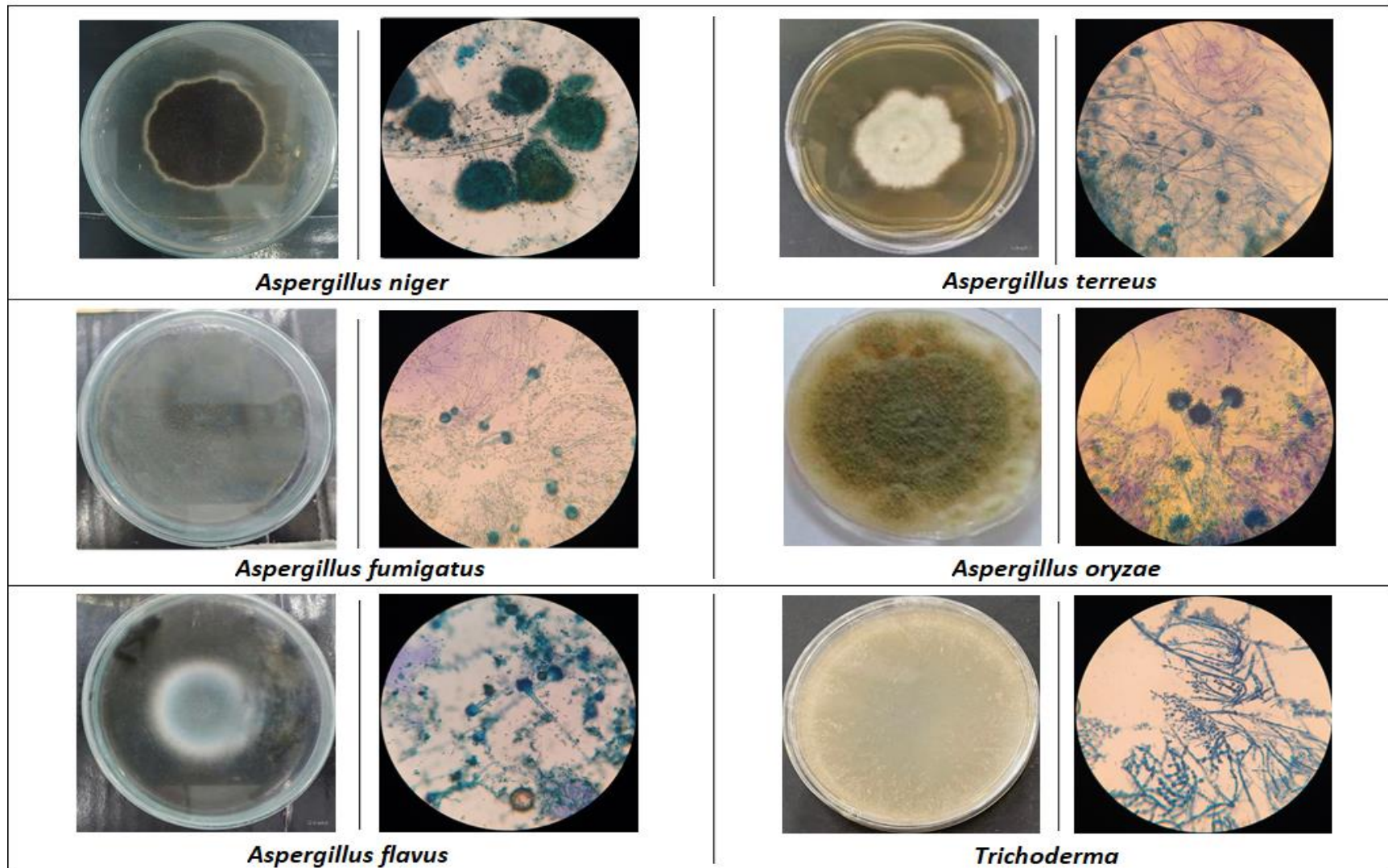
classrooms, such as UNI-R1 and PSS1, had low temperatures and higher CO<sub>2</sub> concentrations, which could lead to increased fungal concentrations due to poor ventilation and limited spore dispersal. In ambient environments, influenced by wind and natural air exchange, non-air-conditioned classrooms, such as UNI-R2 and PSS2, showed significantly higher mold densities than air-conditioned ones. This disparity is primarily attributed to the lack of effective air circulation in non-AC classrooms, where stagnant air allows mold particles to accumulate, especially in the absence of an air filtration system.

Airborne fungi were influenced by environmental factors (Zhai et al., 2018). The microclimatic conditions, including temperature, humidity, and CO<sub>2</sub> concentration, as outlined in Table 1 above, were analyzed for their correlation with airborne fungal concentration using Spearman analysis (Fig. 3). Correspondingly, high temperature contributes to increased airborne mold density because it facilitates their growth (Uk Lee et al., 2016). Many studies have shown a positive correlation between temperature and airborne mold density (Frankel et al., 2012; Hai et al., 2019; Wu et al., 2020). However, some research has shown no significant association between temperature and airborne mold density (Hai et al., 2019; Saadati et al., 2022). There was a slight positive correlation between airborne mold density and humidity, but it was not statistically significant ( $p > 0.05$ , CI = [0.10, 0.41]). This aligns with findings of Lee et al. (2021) although Hai et al. (2019) reported a negative correlation between humidity and mold density in ambient air. There was no significant correlation between mold density and CO<sub>2</sub> concentration ( $p > 0.05$ , CI = [-0.25, 0.27]). However, some studies have shown that there is a negative correlation between mold density and CO<sub>2</sub> concentration (Hai et al., 2019; Madureira et al., 2015).

To evaluate the reliability of the regression model, a nonparametric bootstrap method was applied with 1,000 iterations. The results showed that the initial ( $R^2$ ) coefficient of the model is 0.4871, indicating that approximately 48.71% of the variance in the dependent variable is explained by the independent variables in the model. Additionally, the bias between the ( $R^2$ ) value from the original sample and the mean ( $R^2$ ) from the bootstrap samples is 0.0430. This bias reflects a slight difference between the original model and the repeated models, indicating a relatively stable estimate. The standard error of ( $R^2$ ) from the bootstrap samples is 0.0773, showing the variability of the coefficient of determination across the iterations. These results suggest that the model has a high degree of reliability, with the variation in ( $R^2$ ) across the bootstrap samples within an acceptable range. The results of the bootstrap regression model suggested that fungal concentration was a significant predictor of microclimatic conditions, supporting our hypothesis and previous literature.

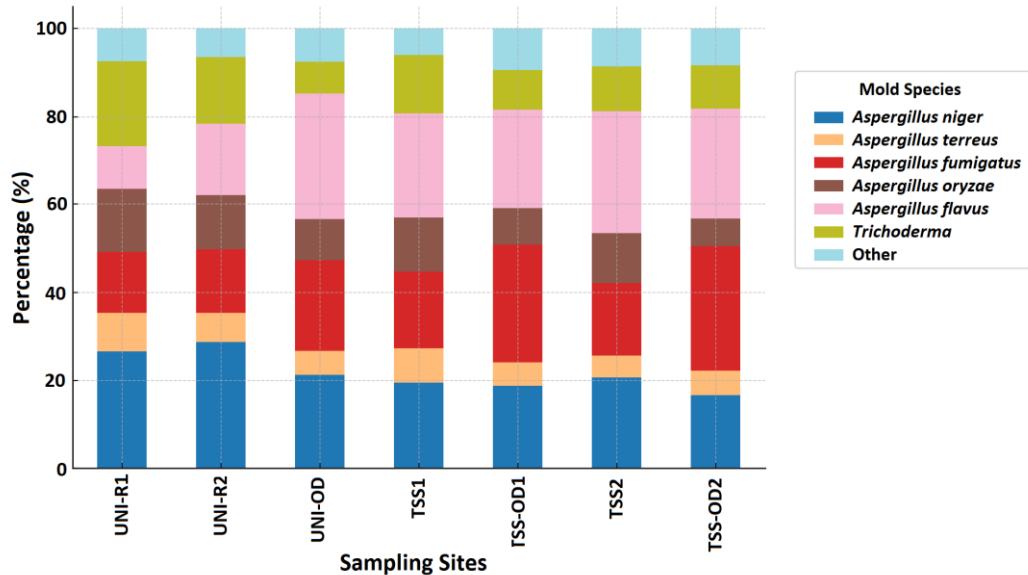
### **3.4. The detected fungal genera and species-level identification**

The identification of fungal species under indoor and outdoor classroom conditions, including the presence or absence of students, was shown in Figure 4.



**Fig. 4.** The detected fungal species identification

Among these, the genus *Aspergillus* dominates, with five species identified, while the remaining species belong to the genus *Trichoderma*. Within university classrooms, *A. niger* showed the highest prevalence, accounting for 28.74% at non-AC UNI-R2 and 26.67% at AC UNI-R1. In contrast, *A. oryzae* was more prevalent in middle school classrooms, with proportions of 23.73% at TSS1 and 27.81% at TSS2. Conversely, in outdoor environments, *A. fumigatus* and *A. flavus* were predominant. The prevalence of *A. fumigatus* was 20.46% at UNI-OD, 26.61% at TSS-OD1, and 28.17% at TSS-OD2, while *A. flavus* accounted for 28.74% at UNI-OD, 22.56% at TSS-OD1, and 25.14% at TSS-OD2 (Fig. 5). This data indicated that *A. fumigatus* and *A. flavus* thrive in natural environments rich in decomposing organic matter, such as soil, decaying leaves, and humid areas.



**Fig. 5.** Proportions of six dominant fungal species isolated from seven sampling sites

It is evident that *A. niger* and *A. oryzae* predominantly grow in enclosed spaces like classrooms, where high humidity and nutrient sources from building materials and accumulated dust are present. On the other hand, *A. fumigatus* and *A. flavus* are more prevalent in outdoor environments, influenced by natural factors such as wind, sunlight, and moisture from rain and dew. This divergence highlights the biological adaptation of mold species to specific environmental conditions, ranging from natural to artificial habitats.

Future studies should prioritize the identification of *Aspergillus* species at the species level, given that certain species, such as *A. flavus*, *A. fumigatus* could cause allergies, infections, mycotoxin production, and transmissible diseases (Hussin et al., 2011; Wu et al., 2021). Moreover, most fungal infections predominantly affect immunocompromised individuals. Overall, pathogenic or allergenic fungal species were primarily detected in outdoor environments.

Other researchers on airborne molds indicated that most species isolated in this study are consistent with findings from other studies, except for *Trichoderma spp.*, which was uniquely identified in this research. Several previous studies have summarized the distribution of mold species isolated from indoor and outdoor environments. A study conducted in Styria using the MAS-100NT® air sampler and Alphasense OPC-N3 particle counter revealed the predominance of *Aspergillus sp.* and *A. fumigatus* in outdoor environments (Haas et al., 2023). Research on indoor environments, such as hospitals, schools, and libraries, also highlights the widespread presence of *Aspergillus spp.* Across most surveyed locations (Sautour et al., 2009; Tormo-Molina et al., 2012; Cabo Verde et al., 2015).

Species of *Aspergillus* and *Trichoderma* exert a wide array of effects on human health, ranging from pathogenicity to industrial utility. Many *Aspergillus* species are associated with allergic responses,

respiratory conditions, and mycotoxin production, posing significant health risks (Gerardi et al., 2024). For instance, *A. flavus* synthesizes aflatoxins, potent hepatocarcinogens linked to liver cancer (Alameri et al., 2023), while *A. fumigatus* is a leading cause of invasive aspergillosis in immunocompromised populations (McCormick et al., 2010). *A. niger* may trigger hypersensitivity pneumonitis and asthma in vulnerable individuals (Khan et al., 2024). Conversely, *A. oryzae* and *Trichoderma* species are generally considered non-pathogenic and are extensively utilized in food fermentation, enzyme production, and sustainable agricultural practices (Jin et al., 2021; Woo et al., 2022).

#### 4. Conclusions

This study demonstrated that air conditioning significantly reduces fungal concentration in classrooms compared to non-air-conditioned spaces and outdoor environments within Ho Chi Minh City schools. Furthermore, human occupancy was identified as a contributing factor to increased fungal concentrations indoors. Temperature and humidity were positively correlated with fungal concentration, highlighting the influence of environmental factors. *Aspergillus*, specifically *A. niger*, *A. terreus*, *A. fumigatus*, *A. oryzae*, and *A. flavus*, along with *Trichoderma spp.*, were the dominant fungal genera identified. These findings underscore the importance of optimizing ventilation and environmental control, including temperature and humidity regulation, to mitigate potential health risks associated with airborne fungi in classrooms. While limited by a relatively small sample size, the study provides valuable preliminary data on airborne fungal communities in Vietnamese schools and emphasizes the need for further research to expand our understanding and develop targeted interventions for improved indoor air quality.

#### Acknowledgments

The authors thank Vietnam National University Ho Chi Minh City supported the finance for research. We gratefully thank the Air and Water Pollution - Public Health - Climate Change research group in the Faculty of Environment of the University of Science. We kindly thank Prof. Sheng-Hsiang (Carlo) Wang from National Central University, Taiwan for supporting us with the Aerobox device during for this study.

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