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Microbiological contamination of indoor air in university classrooms (Case study: University of Science - Vietnam National University, Ho Chi Minh city)

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Abstract:

This study was conducted to preliminarily assess microbial contamination of the indoor air inside classrooms at the University of Science - Ho Chi Minh city. At the same time, this study demonstrates the scientific basis of air quality assessment in a school environment and develops long-term direct or indirect solutions to protect the environment and ensure student health. In this project, sampling and quantitative analysis were performed to identify the airborne bacteria and fungi. Samples were collected from classrooms over a period of 4 months (03-06/2019) according to Koch's sedimentation method. The sampling plates were placed 1 m above the floor and the sampling time was 15 min. The colonies were counted after 24-48 h of incubation at 37±1°C for the bacteria and 70-120 h at 25±1°C for moulds. The results showed bacterial and fungal densities ranging from 359.6-2,427.3 CFU/m^3 and 106.1-928.9 CFU/m^3, respectively. The bacterial density was 2-3 times higher than the fungal density for all survey sites likely because the origin of these microorganisms is human activity. In addition, the density of bacteria and fungi in the air were also affected by weather and environmental factors. 5 isolated bacteria were identified as *Bacillus atrophaerus*, *Acinetobacter baumannii*, *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus cereus*. The fungi isolates included *Aspergillus tamarii*, *Aspergillus niger*, and *Fuligo septica*. They were all related to human and plant diseases.

Keywords: **airborne microorganisms, classroom, indoor air.**

Classification number: **5.1**

Introduction

In recent years, indoor air pollution has been of increasing interest to scientists as well as environmental management authorities as most people are indoors about 80-90% of their time [1, 2]. Hence, indoor air quality is of greater significance to human health due to the greater exposure time of indoor air than outdoor air. An average person inhales around 6-10 l/min and needs 15 m^3 of air per day [3]; thus, it is critical that indoor air be studied and evaluated. According to research by the United States Environmental Protection Agency (USEPA), indoor air pollution is one of the top five public health risks [4] contributing to an increase in the likelihood of cardiovascular diseases, lung diseases, and respiratory diseases [5].

Research by U.S. scientists (Earth and Sciences Division) has shown that the air people normally breathe has 1,800 types of bacteria including non-harmful and harmful ones, both of which affect human health. The human nasal cavity and the oral cavity contain millions of microorganisms that are found in indoor air. Some types of bacteria such as *Streptococcus*, *Mycoplasma*, and *Staphylococcus* cause skin-related diseases, respiratory system diseases, and allergies, which leads to an increase in the proportion of people infected [6, 7]. Human activities, indoor furniture, and equipment are the main factors contributing to the accumulation and spread of microbiological contaminants in indoor air [8, 9]. Human activities such as talking, sneezing, coughing, walking, and washing can produce biological dust in the air. Food, houseplants, house dust, clothing, carpets, wood materials, and furniture can also occasionally release various types of microorganisms into indoor air. Bacteria and fungi also come from outdoor sources such as soil or plants that are transported indoors as dust by wind transport [10, 11].

An increasing amount of research is also being

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conducted on indoor air bioaerosols. Series of studies on air microbiology in classrooms, libraries, and lecture halls of several universities have been carried out. Research by D. Guan, et al. [12] in China compared the bacterial density in the classroom between two sampling methods, of which the density was 209-838 CFU/m^3 for the passive sampling method (Natural sedimentation method - NSM) and 353-1,932 CFU/m^3 for the active sampling method (Air planktonic bacteria sampling method). In Poland, B. Ewa, et al. (2018) [13] focused on the viable bacterial concentration in an office, which was found to be in the range of 540-1,360 CFU/m^3. There was a huge difference in bacterial density between the two types of working rooms, specifically, the air-conditioned rooms were found to have a bacterial density 1.5 times higher than non-air-conditioned rooms. In addition, another study in Ethiopia found that the bacterial concentrations in some libraries at Jimma University ranged from 367-2,595 CFU/m^3 [14]. It can be easily recognised that these studies have around a 200-300 CFU/m^3 starting threshold of bacterial density, which is fairly high.

Meanwhile, studies on airborne viable bacteria and fungi from indoor air have not been paid much attention in Vietnam. Over the last decade, there have been several studies on airborne microorganisms in hospitals, food stock, and outdoor air. N.Q. Tuan (2010) [14] performed some sampling for a survey of air microbiology in the recovery and operating rooms of 13 hospitals and found variable microorganism densities ranging between 64.2-1,247.8 CFU/m^3 and higher concentrations obtained at around 200-500 CFU/m^3. The authors referred to the EU clean room classification standards EU GMP 1997, WHO 2002, and Merck's operating room standard (2009) when conducting this study. At that time, the control of air quality and air pollution in the hospitals of Vietnam did not have any standards for the limits of microbiological contamination in recovery and operating rooms. A study of airborne microorganisms in food storage warehouses was conducted by T.N.L. Tuyen, and N.T. Luan (2014) [15] and it showed that the airborne bacteria concentration was greater than the concentration of fungi and then proposed safety measures. In this work, the density of microorganisms in air was found to decrease from morning to afternoon and increased from the dry season to the rainy season. The authors compared the data with the National Technical Regulation on Biosecurity and Poultry Farm Conditions (QCVN 01-15:2010/BNNPTNT) and the Safir standard to assess the aerobic bacteria density in the air because there were no national regulations on limiting the level of microbiological contamination in the air for food storage.

In addition, characteristics of airborne bacteria and fungi in the atmosphere around Ho Chi Minh city from 2014 to 2016 was studied by V.D. Hai, et al. (2019) [16]. There have been remarkable records of the difference in the number of microorganisms between inland and suburban locations. The authors found the average microbial density on weekdays was higher than on the weekends. This study acknowledged that Vietnam did not yet have a set of microbiological standards for the air environment. In addition, the research had found that passive sampling methods often got higher density results than active sampling methods. They also found that the number of airborne microorganisms in the dry season was usually higher than in the rainy season.

Ho Chi Minh city is the most populous city in Vietnam and one of the most important economic centres in the country. This results in a lot of environmental stresses, but, so far, no research on airborne microorganisms has been conducted in the classroom of educational institutions. Most research on the microbiological contamination of indoor and outdoor air are evaluated based on standards and referenced standards of countries around the world. At present, there are not many research papers focusing on environmental pollution by microorganisms in indoor air.

Therefore, this study provides information on the current density of bacteria and fungi at several different classrooms in two campuses of the University of Science, Vietnam National University, Ho Chi Minh city and the impact of environmental factors on the growth of microorganisms in indoor air. The density of microorganisms is one of the most important criteria to be considered for building a healthy and safe space for students and teachers.

Methods and materials

Sampling sites

The air samples of the bacteria and fungi were collected in several classrooms of the University of Science, Vietnam National University, Ho Chi Minh city. The university has 2 campuses, one in the city centre and one in the suburb of the city, which has about 20,000 students enrolled. In this study, the samples were taken from the two campuses at the following locations. From the first campus, three locations including room C32 of ~100 students, room E401 of ~50-80 students, and lecture hall 1 of ~300 students. This campus is near a densely populated area and the main road of the district (Nguyen Van Cu street, district 5). From the second campus, room F106 of ~100 students and lecture hall B of ~300 students. This area is located in the suburbs and covered with many trees around. There is a long distance between the blocks of the classrooms (Linh Trung ward, Thu Duc district).

The different sampling locations provided a variety of microbial densities in the classrooms of each campus. The

criteria for selecting the classroom to be surveyed was to represent upper and lower floors, the classroom area, and the number of students in the classroom.

The sample collection time was fixed on the Tuesdays of each week with 2 weeks per period at the 5 locations mentioned. There were 4 sampling periods and at each period samples of bacteria, fungi, and a blank were collected. For each sampling period, the samples were collected twice per day at 9:00 and 14:00 for 4 months from March 2019 to June 2019. Therefore, the total number of expected samples was 5×4 periods×5 locations=100 samples.

Sampling was conducted by a passive method known as the natural sedimentation method (NSM) or Koch's sedimentation method [17]. The sampling height was adjusted to be near the human breathing zone, which was 1 m above the floor. Each dish was exposed to air for 15 min (after conducting a pre-survey to select the appropriate sampling time). During the sampling process, the number of students, temperature, and humidity for each sampled room was also recorded.

Sampling procedure

Bacteria and fungi were collected on Nutrient Agar and Czapek-dox Agar, respectively. After the air exposure, the plates were carefully packed, labelled, and taken to the laboratory for analysis of the total aerobic bacteria and total fungi. The colonies were counted after 24-48 h incubation at 37±1°C for bacteria and 70-120 h incubation at 25±1°C for moulds [18]. The samples were finally subjected to qualitative, quantitative, and microbiological analyses.

Once the colony forming units (CFU) were determined, the CFU/m^3 values were calculated using the following equation described by Omeliansky [19-21]:

$$N = \frac{a \times 100 \times 100}{\pi r^2 \times t\frac{1}{5}}$$

where N is the microbial counts in CFU/m^3 of indoor air, a is the number of colonies per Petri dish, πr^2 is the dish surface area (cm^2), and t is the exposure time (min).

The samples of air-borne microorganisms were then compared to the European Protection Agency's cleanliness classification standards - EUR 14988 EN [22].

This study used protein mass spectrometry (MALDI-TOF) to identify the microorganisms by molecular markers. Any similarities of protein spectra from the target microorganisms were determined from a database of nearly 6,000 different microorganisms. The Maldi biotyper allows the accurate identification of the species of microorganisms including G(+) and G(–) bacteria, anaerobic-aerobic bacteria, yeast, mycobacteria, and mycelium. MALDI-TOF is a fast, simple, and high throughput proteomic technique used to identify many types of bacteria [23].

Results

The indoor air microbial loads of the 5 classrooms were determined by taking 100 samples. The concentration of bacterial and fungi aerosol present in the investigated classrooms are presented in Table 1. The results indicate that the highest bacterial air density in CFU/m^3 were obtained at 14:00 h in room E401 at campus 1, while the lowest bacterial CFU/m^3 air values were obtained at 14:00 h in room F106 at campus 2. The highest fungal air values were recorded at 9:00 h in room E401 at campus 1 with a value of 928.9 CFU/m^3, while the lowest fungal air density was recorded at 9:00 h in lecture hall B on campus 2, which was 106.1±81.3 CFU/m^3.

Table 1. Number of bacteria and fungi in air (CFU/m^3) at 5 points and their sampling time.

Microorganism	Sampling time	Campus 1			Campus 2	
		Lecture hall I (n=4)	*Room C32 (n=4)*	*Room E401 (n=4)*	*Lecture hall B (n=4)*	*Room F106 (n=4)*
Bacteria	9:00	1,077±854.2	1,598.8±1,958.3	2316±1,826.9	484.7±413.4	785.9±188.1
	14:00	1,771.7±776.1	1,625.6±1,285.4	2,427.3±1,906.8	668.1±701.6	359.3±92.4
Fungi	9:00	928.8±398.1	663.5±223.3	928.9±355.3	106.1±81.3	231.5±188.1
	14:00	527.5±54.3	729.8±325.1	746.4±627.9	262.6±276.7	121.7±92.4

The bacterial and fungal densities of the 2 campuses are shown in Fig. 1. The density of microorganisms on campus 1 was higher than that of campus 2 and the bacterial density was higher than the fungal density.

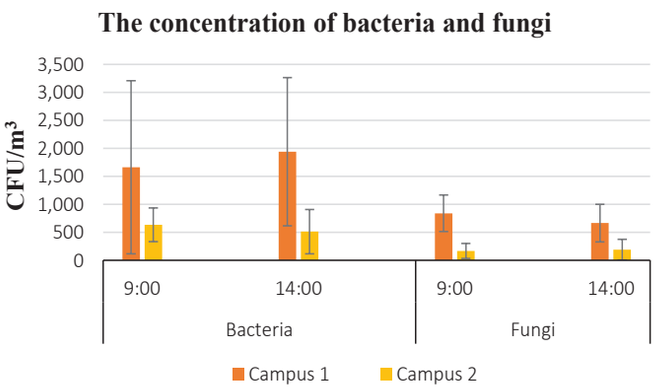

Fig. 1. Microbiological density in campus 1 and 2.

Microbial air quality scenario of classrooms is indicated in Table 2. All survey sites were contaminated with micro-pollutants from low to very high. Room E401 was the most heavily polluted than the rest of the classrooms.

Table 2. Evaluation of microbiological air quality (According to the standards of the European Protection Agency - EUR 14988 EN, 1993 [22]).

Microorganism	Range of values (CFU/m³)	Pollution degree	Campus 1						Campus 2			
			Lecture hall I		*Room C32*		*Room E401*		*Lecture hall B*		*Room F106*	
			9:00	*14:00*	*9:00*	*14:00*	*9:00*	*14:00*	*9:00*	*14:00*	*9:00*	*14:00*
Bacteria	<50	Very low	–	–	–	–	–	–	–	–	–	–
	50-100	Low	–	–	–	–	–	–	–	–	–	–
	100-500	Intermediate	–	–	–	–	–	–	✓	–	–	✓
	500-2,000	High	✓	✓	✓	✓	–	–	–	✓	✓	–
	>2,000	Very high	–	–	–	–	✓	✓	–	–	–	–
Fungi	<25	Very low	–	–	–	–	–	–	–	–	–	–
	25-100	Low	–	–	–	–	–	–	–	–	–	–
	100-500	Intermediate	–	–	–	–	–	–	✓	✓	✓	✓
	500-2,000	High	✓	✓	✓	✓	✓	✓	–	–	–	–
	>2,000	Very high	–	–	–	–	–	–	–	–	–	–

(✓) in the range; (–) not in the range.

As seen in Table 3, the microbial isolates included 5 bacteria and three fungi. *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus cereus* are common bacteria in the indoor air. They were present across all sampling locations. *Aspergillus tamarii* and *Aspergillus niger* were two common fungi appearing in all classrooms except lecture hall B.

Table 3. The distribution of microorganisms.

Microorganism		Campus 1			Campus 2	
		Lecture hall I	*Room C32*	*Room E401*	*Lecture hall B*	*Room F106*
Bacteria	*Bacillus atrophaerus*	+	–	–	+	+
	Acinetobacter baumannii	–	–	+	+	–
	Bacillus pumilus	+	+	+	+	+
	Bacillus subtilis	+	+	+	+	+
	Bacillus cereus	+	+	+	+	+
Fungi	*Aspergillus tamarii*	+	+	+	+	+
	Aspergillus niger	+	+	+	–	+
	Fuligo septica	–	–	–	+	+

(+) exist; (–) does not exist.

Discussion

Microbiological analysis

The concentration of bacteria in the classrooms of the two campuses ranged from 359.6±92.4 CFU/m³ to 2,427.3±1,906.8 CFU/m³ and tended to increase as morning progressed to afternoon. In campus 1, the average bacterial concentration in the room E401 was the highest during the survey and the lowest concentration in lecture hall I with 1,077±854.2 CFU/m³. Meanwhile in campus 2, the bacterial density in hall B and room F106 tended to be opposite in the morning and afternoon (Table 1). This is mainly explained by the fixed schedule of the week, the same number of students during the sampling period, the area of the classroom and the ventilation, outside air entering the classrooms (temperature, humidity) [24, 25]. Room E401 is small and has poor ventilation but the number of students in class is about 100. The remaining rooms have the same area, which is double the size of the E401 room, so the collected bacterial density was lower. A 2012 study by D. Hospodsky, et al. [26] in the United States also showed that the bacteria present in indoor air were derived primarily from humans. Therefore, the number of occupants in an indoor environment greatly affects the density of the bacteria in the air.

Similar to bacteria, the concentration of fungi at campus 1 was lowest at 527.5±54.3 CFU/m³ in lecture hall I and the highest concentration was 928.9±355.3 CFU/m³ in room E401. This was significantly different from the concentration values found at campus 2, which ranged only from 106.1±81.3 CFU/m³ to 262.6±276.7 CFU/m³. However, the difference in fungal density among the classrooms in each campus was quite small, only about 200 CFU/m³ at campus 1 and 120 CFU/m³ at campus 2 (Table 1). This shows that people are not the main source of fungi in the indoor air. The room's facilities, high humidity, and poor cleanliness of the rooms are conditions that support the strong growth of fungi. Correlation studies of microorganisms inside buildings were conducted by S.A. Wamedo, et al. (2012) [25] and G.S. Graudenz, et al. (2005) [27], which were in agreement with this view. The concentration of fungi in the morning tended to be higher than in the afternoon because the temperature in the morning was around 30°C, which is suitable for fungi growth [28].

From the data in Table 1, the bacterial density was 2-3 times higher than the fungal density at all survey locations. The temperature in Ho Chi Minh city is quite high (32-37°C) during the days, which is more suitable for the growth of bacteria rather than fungi (the optimal temperature range of fungi is 28-30°C) [16]. In summary, the origin of bacteria in the indoor air is mainly from humans while the origin of the fungi is from the condition of the facilities as well as environmental factors. Samples collected in this study were themselves the cause of large density difference between bacteria and fungi concentration [24].

The concentration of microorganisms between 2 campuses

The concentrations of bacteria and fungi were found to be significantly different between the sampling locations of both campuses. Fig. 1 shows that the average density of bacteria at

campus 1 in the morning and afternoon was 2-3 times higher than that of campus 2. The bacterial concentration in the afternoon was the highest at 1,941.5 CFU/m^3 while the highest bacterial concentration in campus 2 was only 635.3 CFU/m^3. Similarly, the highest fungal density was 840.4 CFU/m^3 at campus 1, which is 4-5 times higher when compared with campus 2. One explanation for this result is that campus 1 in the city centre where many high-rise buildings are situated nearby. The area of campus 1 is small and the natural ventilation in classrooms (they are without air-conditioners) is not good, which leads to high humidity. The roads in this area are less green and contain a high density of vehicles that generate dust suspended in the air, which easily attaches to the human body. At the same time, the number of students at this facility is quite large, so the air quality in the poorly-ventilated classrooms are worse. campus 2 is located on the outskirts of the city with greener roads and its area is three times larger than campus 1. However, campus 2 is located apart from major road systems and as a result there is less traffic on the roads. The class sizes of campus 2 are much less than the capacity of the classroom, which results in less dust-containing microorganisms. Research by W. Fabian, et al. (2016) [29] also has stated that environmental and outdoor factors (vegetation, urbanization, airborne particulate matter) affect the growth of mould and bacteria in the indoor air.

Assessment of microbiological contamination

This research was conducted based on the European Environmental Protection Agency's standard of microbiological pollution (EUR 14988 EN, 1993 [22] as the standard for assessing the quality of air microbiology in classrooms. In campus 1, lecture hall I and room C32 were infested with bacteria and fungi at a very high level. The bacterial densities were 1,771.7 CFU/m^3 and 1,625.6 CFU/m^3 for lecture hall I and room C32, respectively. The fungal densities were 928.7 CFU/m^3 and 729.8 CFU/m^3 for lecture hall I and room C32, respectively. Meanwhile, a considerable extent of contamination in room E401 was observed for bacteria (2,427.3 CFU/m^3) and fungi (928.9 CFU/m^3) as well. In campus 2, all classrooms had lower levels of microbiological contamination than campus 1 (Table 2). Bacterial pollution ranged from an intermediate to high level while fungi pollution was intermediate. This shows that the microbiological air quality in campus 1 is inferior to campus 2 and this result is completely consistent with the results of a study by H. Shokri, et al. (2010) [30]. Thus, it is necessary to arrange a suitable number of students for each class, design better ventilation systems, and clean classrooms regularly to improve air quality in the classrooms.

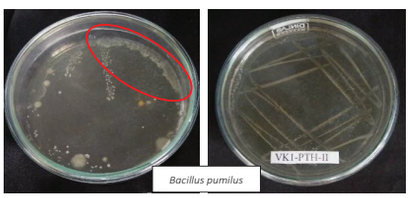

Fig. 2. Bacillus pumilus bacteria before and after isolation.

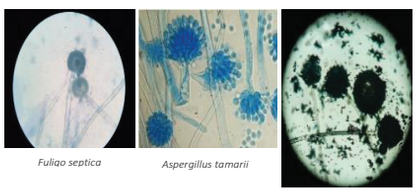

Fig. 3. Types of fungi under microscope.

Identification of microorganisms

The morphological characteristics of the colonies were selected as a representative of the dominant microorganisms isolated and identified by the MALDI-TOF method. The results showed 5 types of bacteria and 3 types of fungi were predominant over all the sampling sites. The dominant bacteria were *Bacillus atrophaerus*, *Acinetobacter baumannii*, *Bacillus pumilus* (Fig. 2), *Bacillus subtilis*, and *Bacillus cereus*. They were all gram-positive bacteria except for *Acinetobacter baumannii*, which is gram-negative, and aerobic spores. These bacteria are also found in soil, water, and some other habitats. *Bacillus atrophaerus* is a spore-forming bacillus that does not cause disease. This bacterium has been used as biological indicators for disinfection in biological research and method studies as well as in disinfectant studies, disease treatment evaluation, and potential assistance or means for vaccines. The remaining types of bacteria are related to skin diseases, pneumonia, sepsis, incision infection, urinary tract infection, and purulent meningitis after neurosurgery or food poisoning [31, 32]. *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus cereus* existed at all sampling sites (Table 3) indicating that they are popular bacteria in indoor air.

The dominant fungi included *Aspergillus tamarii*, *Aspergillus niger*, and *Fuligo septica* (Fig. 3). Two common fungi of the genus *Aspergillus* exist mainly in soil and are associated with a number of human diseases such as keratitis, allergies, and rhinitis [33]. They were all present in every classroom except hall B. *Fuligo septica* is the culprit of many types of root, stem, or leaf rot diseases of moistophilic plants, especially vegetables such as kohlrabi, cabbage, and many other fruits and vegetables [34]. *Fuligo septica* only appeared at campus 2 because there are many plants growing on campus (Table 3).

Conclusions

This study has provided important information on the status of microorganisms in the air and a preliminary evaluation of the microbiological air quality in indoor air at schools. Along with identifying the species, this study helped elucidate the impacts from these microorganisms in the air. The concentrations of bacteria in the air depend on factors such as weather, ventilation, as well as human activities. A limited classroom area with crowded human presence has a strong impact on the density and concentrations of bacteria in the air, which causes them to be high. The fungi concentration depends on environmental factors, the quality of facilities, and sanitation in the classroom. The inferior air quality of campus 1 compared to campus 2 is likely caused by differences of geographical locations. The classrooms at campus 1 have high-to-very high microbiological pollution while campus 2 has an average pollution level. To improve air quality, it is necessary to arrange a suitable number of students for each class, appropriately design ventilation, and to clean the classrooms regularly.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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