



## Analysis of the Abundance and Morphological Characteristics of Airborne Microorganisms in an Environmental Engineering Laboratory

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**Abstract:** This study aimed to analyze the abundance and morphology of airborne microorganisms in the Environmental Engineering Laboratory and to relate their distribution to differences in room conditions and sampling time. Sampling was conducted in two rooms, Room A and Room B, during the morning and afternoon using a microbiological air sampler with Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Plate Count Agar (PCA) media. Statistical analysis showed that room conditions strongly influenced the distribution of airborne microorganisms and were also affected by indoor airflow patterns. The findings revealed higher concentrations in Room B than in Room A (morning averages: 60.75 vs. 23.75 CFU/m<sup>3</sup>; afternoon averages: 44.5 vs. 24.25 CFU/m<sup>3</sup>), with a statistically significant difference (Mann–Whitney, p-value < 0.05). These results indicate that optimizing airflow direction, scheduling cleaning activities, and conducting routine bioaerosol monitoring are necessary as indicators of control performance. Morphological analysis identified Gram-positive bacteria such as *Staphylococcus* and *Bacillus*, as well as fungi such as *Aspergillus* and *Trichoderma*, all of which may affect health and indoor air quality. This study underscores the importance of laboratory indoor air quality management in reducing health risks associated with airborne microorganisms.

**Keywords:** Microorganisms; air; laboratory; room; time

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### INTRODUCTION

Microorganisms are distributed not only in aquatic environments but also in the air. Owing to their extremely small size, microorganisms are very light and can be readily transported by wind. Once airborne, they eventually return to the ground under the influence of gravity. However, unlike solid objects such as balls that fall directly, microbial particles settle very slowly and may remain suspended in the atmosphere for extended periods (Griffin et al., 2017). These biological particles are referred to as bioaerosols and include bacteria, fungi, viruses, and other biological fragments that can disperse over long distances and persist in the atmosphere for a certain period. Bioaerosols continue to be carried by air currents, many of which are generated by human activities, and may subsequently enter indoor environments. Indoor environmental conditions, such as temperature and humidity, can further influence microbial growth and proliferation (Chen et al., 2020). The microorganisms commonly present in both outdoor and indoor air include bacteria, fungi, and viruses. Some of these microorganisms are pathogenic and may adversely affect human health, causing effects ranging from respiratory tract irritation to allergic reactions and immune system disorders (US EPA, 2024).

Over the past two decades, indoor air quality (IAQ) has been increasingly recognized as a global public health concern because most of the world's population spends more than 80% of their time inside enclosed buildings. Previous studies have shown that IAQ is strongly influenced by bioaerosol dynamics associated with human activity, ventilation systems, and the physical characteristics of indoor environments. Molecular studies by Prussin and Marr (2015) emphasized that human activity is a major source of airborne bacterial emissions through respiration, speaking, and movement, which may subsequently accumulate in poorly ventilated spaces (Adams et al., 2015). Janczarek et al. (2024) further demonstrated that the intensity of indoor activities correlates with increased bioaerosol concentrations, particularly in highly occupied facilities. This relationship between human activity and ventilation conditions indicates that bioaerosol dynamics result from the interaction between emission sources and the ability of a room to dilute airborne contaminants. In addition, increased ventilation rates have been shown to significantly reduce indoor airborne bacterial and fungal concentrations, whereas relative humidity above 60% may enhance bioaerosol viability (Kwan et al., 2020; Li et al., 2021). Such unfavorable humidity and ventilation conditions are frequently encountered in laboratory facilities.

Although numerous studies have examined IAQ in healthcare facilities, offices, and commercial buildings (Chen et al., 2020; Janczarek et al., 2024), studies specifically focusing on environmental engineering laboratories remain limited. Most previous research has concentrated on buildings located in temperate regions, leaving the characteristics of bioaerosols in educational laboratories in tropical areas largely unexplored. In fact, environmental engineering laboratories possess distinct characteristics, including microbial culture activities, waste treatment processes, and the use of organic materials, all of which may serve as additional potential sources of bioaerosols. Laboratories are generally enclosed spaces and therefore allow exposure to various contaminants. Academic and research activities conducted in laboratories may also facilitate the distribution of microorganisms within indoor environments. Accordingly, air quality in laboratories is an important factor that requires careful attention. Several factors influence the presence of microorganisms in laboratory rooms, including ventilation, cleanliness, and the use of laboratory equipment, all of which can affect indoor air quality (Karim et al., 2024). These factors must be properly considered and understood to prevent poor indoor air quality, particularly under the influence of climatic conditions.

Tropical climatic conditions such as those in Indonesia, with average temperatures ranging from 25 to 32°C and relative humidity frequently exceeding 70%, may create an environment favorable for the growth and survival of airborne microorganisms (Li et al., 2021). The combination of laboratory activities and such climatic conditions may increase both the concentration and diversity of bioaerosols, including opportunistic microorganisms such as *Staphylococcus* and *Aspergillus* spp. Therefore, studies focusing on environmental engineering laboratories in tropical regions are important for generating contextual understanding of bioaerosol exposure risks and for formulating air quality management strategies suited to local conditions. Measurement of airborne microorganism concentrations in CFU/m<sup>3</sup> is a standard method widely used in microbiological air quality assessment and building risk management (Kwan et al., 2020). Culture-based approaches enable quantitative evaluation of microbial viability and comparison with environmental health threshold values. However, several important parameters must also be considered, as temperature, humidity, and lighting conditions may affect microbial concentrations expressed as CFU/m<sup>3</sup>.

Therefore, this study aims to measure the concentrations of airborne bacterial and fungal microorganisms (CFU/m<sup>3</sup>) in two environmental engineering teaching laboratories at two sampling times, identify the morphological characteristics of the detected bacteria and fungi, and statistically analyze differences in microbial concentrations based on room conditions and sampling time. In addition, this study evaluates the relationship between physical room parameters and microbiological air quality in order to formulate technical recommendations for improving ventilation and controlling humidity in environmental engineering laboratories.

## METHOD

This study was conducted in the Environmental Engineering Laboratory of an educational institution in Sleman, Yogyakarta, on June 12, 2025. It employed an observational, descriptive-analytic cross-sectional design. Air sampling was performed in two laboratory rooms at two different time points to compare the concentration and characteristics of airborne microorganisms under different room conditions and sampling times.

### Instruments and Materials

The instruments and materials used in this study included a microbiological air sampler, 37°C incubator, 25°C incubator, Petri dishes, inoculating loop, Nutrient Agar (NA), Plate Count Agar (PCA), Potato Dextrose Agar (PDA), and 75% alcohol.

### Media Preparation

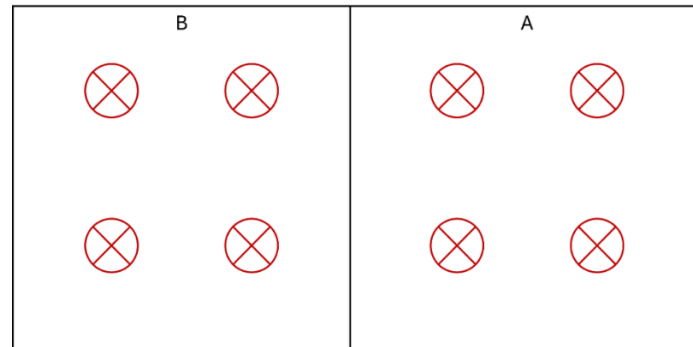
The first step of the study was the preparation of culture media for microbial growth. The media used were Potato Dextrose Agar (PDA) at a concentration of 3.9 g/L and Plate Count Agar (PCA). The media were dissolved and heated using a magnetic stirrer on a hot plate. The pH of the media was then measured and adjusted with acid (HCl) or base (NaOH) to an optimal range for microbial growth, namely pH 6.5-7.0. The media were sterilized in an autoclave at 121°C for 15 minutes to prevent contamination (Brock et al., 2019). Subsequently, 10 mL of medium was poured into each of 16 Petri dishes and allowed to solidify at room temperature. NA was used for bacterial growth, PDA for fungal growth, and PCA for total plate count (TPC) determination.

### Air Microbiological Sampling

Air sampling was performed to collect microorganisms present in the indoor air. The sampling parameters and calculation of airborne microorganism concentrations in this study followed SNI 9099:2024, the standard method for testing biological factors in workplace air. This standard includes the use of active sampling methods, regulation of airflow rate (L/min), sampling duration, calculation of air volume (m<sup>3</sup>), valid colony count ranges, and reporting of results in CFU/m<sup>3</sup> with statistical correction using the Feller table.

Air microbiological sampling was conducted using a microbiological air sampler with an impactor at predetermined times and at several points within the laboratory rooms. Sampling was carried out at 08:00 and 13:00 WIB in the Water Quality Laboratory (Room A) and the Environmental Biotechnology Laboratory (Room B). Four sampling points were selected in each room, resulting in a total of eight sampling locations. These points, including the center of the room and areas near ventilation, were selected to ensure adequate representation of laboratory air quality. At each point, air samples were collected in duplicate using a microbiological air sampler equipped with sterile Petri dishes. Each sampling point drew air at a flow rate of 1 L/min for 5 minutes through a sterile filter. After sampling, the filters containing the collected

microorganisms were transported to the laboratory for further processing. The collected filters were then processed to identify trapped microorganisms using appropriate media, such as NA (Nutrient Agar), PDA (Potato Dextrose Agar), or PCA (Plate Count Agar), for microbial growth. Samples were incubated for 48 hours for bacteria and 7 days for fungi at 37°C in an incubator.



**Figure 1.** Air sampling points in the environmental laboratory

### Enumeration of Microorganisms

After incubation, the next step was to count the microbial colonies that had grown on the culture media. For each Petri dish, the number of colonies formed was counted manually. Only plates containing 30-100 colonies were included in the count, in accordance with established standards to ensure accurate and representative results. Plates with colony numbers outside this range were considered invalid. Colony counts were then expressed as Colony Forming Units per cubic meter of air (CFU/m<sup>3</sup>) to determine the concentration of airborne microorganisms.

The colony count calculation followed the equations below (SNI 9099:2024):

#### 1. Air volume (m<sup>3</sup>)

$$\text{Air volume (m}^3\text{)} = \frac{Q \times t}{1000} \quad (1)$$

Note:

Q = airflow rate (L/min)

t = sampling duration (min)

1000 = conversion factor from liters to cubic meters

#### 2. Number of colonies (CFU)

$$\text{Airborne microorganism concentration (CFU/m}^3\text{)} = \frac{\text{number of colonies on agar medium (CFU)}}{\text{air volume (m}^3\text{)}} \quad (2)$$

For statistical conversion of microorganism counts, the Feller table was used to convert colony counts based on the sampled air volume and the surface area of the sampling medium. The Feller table converts the number of colonies found on a Petri dish into the number of microorganisms per unit volume of sampled air, taking into account the dilution factor and sampling duration. Each sample was counted three times to ensure data accuracy and consistency, and the results were then converted into CFU/m<sup>3</sup> for further analysis (Cappucino & Sherman, 2016). In this study, the microorganism count was non-specific; therefore, it represented the combined growth of bacteria and fungi on the culture media.

### Statistical Analysis of Air Microbiological Counts

Statistical analysis was performed using a non-parametric approach. The Mann-Whitney U test was used to assess significant differences in microbial counts between

Room A and Room B, as well as between sampling times (morning and afternoon). This method was selected because the dataset contained fewer than 30 observations and was not normally distributed. Microbial data from each group (room and sampling time) were ranked, and hypothesis testing was then performed to determine whether there were statistically significant differences in median values.

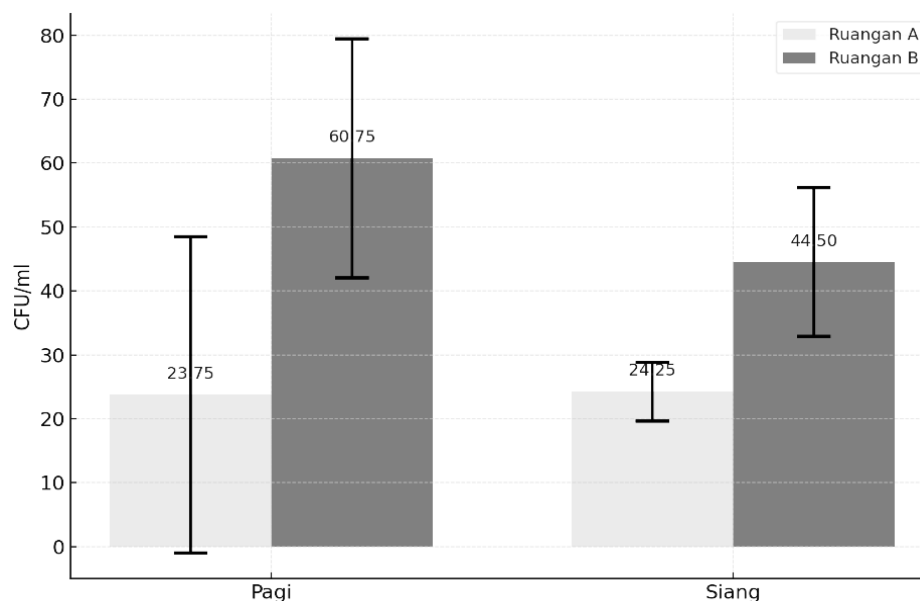
### Morphological Analysis Using Microscopy

After microbial growth on the media, morphological analysis was carried out to identify the types of microorganisms present. Microscope slides were prepared by taking a small portion of a colony grown on the medium and placing it on a glass slide. Gram staining was then performed, consisting of Gram A (crystal violet) as the primary stain, Gram B (iodine) as the mordant, Gram C (95% alcohol) for decolorization, and Gram D (safranin) as the counterstain. This staining procedure was used to differentiate Gram-positive and Gram-negative bacteria. In addition to Gram staining, fungal samples were stained using techniques such as lactophenol blue or India ink. The stained preparations were observed under a light microscope at 100× magnification. Each sample was analyzed for identification purposes. Bacteria were examined based on cell morphology, such as cocci, bacilli, and spirilla, while fungi were observed for structures such as hyphae and spores. The results of this morphological analysis were used to determine whether the microorganisms were potentially pathogenic and to support further classification using microscopic references (Cappucino & Sherman, 2016).

## RESULTS AND DISCUSSION

### Airborne Microorganism Counts

Air samples were collected and subsequently used to determine total bacterial counts in two laboratory rooms. Observations were conducted in the morning at 09:00 WIB and in the afternoon at 14:00 WIB. The morning sampling results showed a difference in airborne microorganism counts (CFU/m<sup>3</sup>) between Rooms A and B. Room A had a mean microorganism count of 23.75 CFU/m<sup>3</sup>, whereas Room B showed a substantially higher mean count of 60.75 CFU/m<sup>3</sup> (Figure 2). These findings indicate that Room B had a markedly higher level of airborne microbial contamination than Room A in the morning.



**Figure 2.** Comparison of airborne microorganism counts in rooms A and B

The non-parametric statistical analysis also demonstrated a significant difference in airborne microbiological presence between Rooms A and B across the morning and afternoon observations. The test results showed that the null hypothesis was rejected for the comparison between Rooms A and B, indicating a significant difference with a p-value < 0.05. This significant difference confirms that microbial presence was not homogeneously distributed between the two rooms. The difference may be attributed to variations in room conditions, particularly ventilation, light intensity, and air circulation rate, as discussed previously.

**Table 1.** Non-parametric analysis results for rooms A and B

Group	N	Mean Rank	H/U	df	Chi-square	p-value	Interpretation
Room A	8	5.625	9	1	5.592	0.018	U = 9.00; H0 rejected, significant difference between A and B
Room B	8	11.375					

In addition to the morning sampling, the afternoon sampling also showed slightly higher microbial counts in Room B than in Room A (Figure 2). In Room A, the bacterial count in the afternoon was 24.25 CFU/ml, while in Room B it was 44.5 CFU/ml. In Room A, the afternoon bacterial count increased slightly relative to the morning, whereas in Room B, the afternoon microbial count decreased compared with the morning. Sampling time, whether morning or afternoon, may influence microorganism abundance due to changes over time in air circulation, ambient temperature, and indoor light intensity. In Room B, the number of microorganisms was lower in the afternoon than in the morning; however, this difference was not statistically pronounced. This pattern was also supported by the non-parametric analysis comparing morning and afternoon measurements. The results showed that the null hypothesis was accepted for the comparison between morning and afternoon, indicating no significant difference at  $p > 0.05$  with a 95% confidence level (Table 2).

**Table 2.** Non-parametric analysis results for morning and afternoon sampling

Group	N	Mean Rank	H/U	df	Chi-square	p-value	Interpretation
Morning	8	9.125	37	1	0.224	0.636	U = 37.00; H0 accepted, no significant difference between morning and afternoon
Afternoon	8	7.875					

The research data indicate high variability in microbial distribution, which may be caused by human activity, ventilation systems, or differences in humidity between rooms. This finding is supported by the poorer air circulation system in Room B, which likely contributed to its higher microbial concentration (Yogeswaran et al., 2023). When ventilation is inadequate, the outgoing airflow rate decreases, promoting the accumulation of microbial pollutants because elevated CO<sub>2</sub> levels and humidity create favorable conditions for microbial growth (Hazrine et al., 2015). Room B was also more enclosed and darker, conditions that may further support microbial proliferation. This is consistent with the study of Maisuningtyas & Yuliawati (2021) in an educational institution, which found that indoor spaces with low light intensity and humidity reaching 80% exhibited relatively high microorganism counts of up to 200 colonies/m<sup>3</sup>.

In contrast, Room A had better ventilation and was located closer to the entrance. Light intensity in Room A was also higher than in Room B because more sunlight entered the room. Greater ventilation likely improved air circulation and reduced the density of bacterial distribution, particularly under favorable lighting conditions. Room A had more glass windows, allowing more sunlight to enter than Room B. High light

intensity with continuous exposure can damage the structure of non-autotrophic microorganisms (Islam et al., 2022).

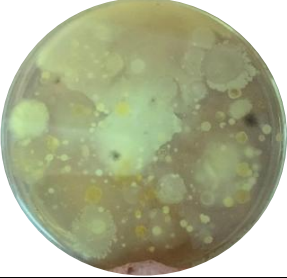
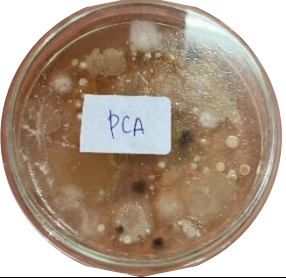


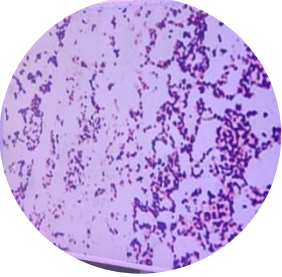

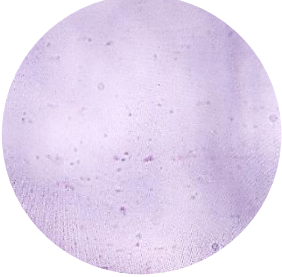

However, the sampling times in the morning and afternoon showed a relatively uniform distribution of microorganisms, with no significant temporal difference. Several factors may explain this pattern. One possible reason is that the exhaust fan was turned on in the afternoon, which may have improved air circulation and carried microorganisms out of the room along with dust particles, thereby increasing the airflow rate indoors (Hazrin et al., 2015). In addition to time, ventilation is not the only major determinant of microbial presence. Major emission sources are also strongly influenced by human activities and the lack of effective air filtration (Kwan et al., 2020). Human activity, especially student presence, was greater in the morning than in the afternoon, which may have contributed to increased microorganism distribution during the morning period.

Overall, the results from the morning and afternoon analyses indicate that indoor microbial levels in the laboratory remained below the regulatory threshold for airborne microbes in indoor environments established by the Indonesian Ministry of Health Regulation No. 50 of 2017, namely 200 CFU/m<sup>3</sup>. In addition, laboratory conditions recorded on 13 June 2025, the sampling day, were 26°C and 70% humidity. These temperature and humidity levels strongly support the presence of airborne microorganisms. According to the Technical Guidelines for the Implementation of Occupational Environmental Health and Safety, Supplementary Explanation to the Minister of Manpower Regulation No. 05 of 2018 concerning Occupational Safety and Health in the Work Environment, the standard indoor air quality values for workspaces are a temperature of <25.5°C and relative humidity of <70%. This is further supported by the positive correlation showing that humidity reaching 70% and temperatures above 25°C are associated with relatively high airborne microorganism concentrations (Purnomo et al., 2023). Therefore, indoor air quality risk analysis is essential because rooms with higher microbial loads, such as Room B, may potentially serve as sources of pathogen exposure (e.g., *Staphylococcus aureus*, *Aspergillus* spp.) that can trigger infection or allergic responses. Nevertheless, this study has several limitations, including the limited sampling period, which was conducted on only one day and at a single sampling event, thus not representing seasonal variation, as well as the absence of an outdoor control.

### **Bacterial Morphological Analysis on PCA Medium**

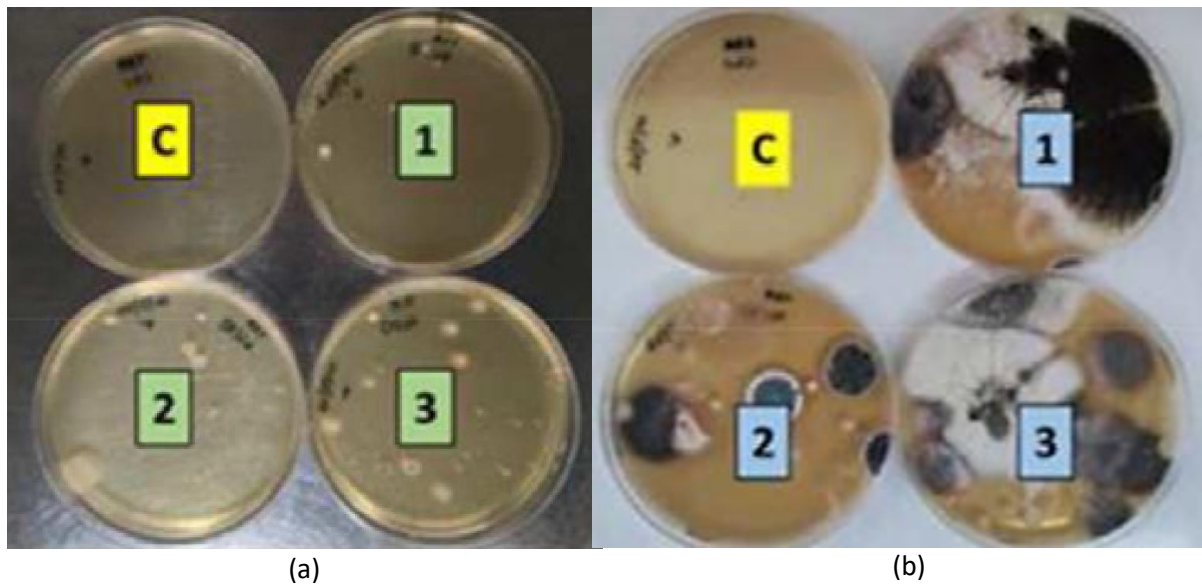
Airborne microbiological morphology was observed using samples grown on PDA and PCA media. Both morning and afternoon observations showed substantial bacterial colony growth. These colonies predominantly exhibited white colony morphology. The morphological characteristics of airborne bacterial colonies in the morning were more diverse (Table 3) than those observed in the afternoon. Growing colonies were examined and characterized based on several criteria, including colony shape, colony margin, and elevation. Based on these criteria, airborne bacteria in both the morning and afternoon were predominantly characterized by circular colonies, entire margins, and flat elevation. After macroscopic colony characterization, bacterial cell morphology was further examined using Gram staining. Gram staining of the morning isolates revealed Gram-positive cocci and bacilli, whereas the afternoon isolates were predominantly Gram-positive bacilli. As shown in Table 3, one of the morning bacterial isolates consisted of Gram-positive cocci belonging to the genus *Staphylococcus*.

**Table 3.** Macroscopic and microscopic morphology of airborne microorganisms on PCA medium

Morning		Afternoon	
			
			
Morphology: <ul style="list-style-type: none"> <li>▪ Shape: circular, irregular.</li> <li>▪ Margin: entire, undulate, lobate.</li> <li>▪ Elevation: flat.</li> <li>▪ Color: white, yellow.</li> <li>▪ Gram reaction: positive (coccus)</li> </ul>	Morphology: <ul style="list-style-type: none"> <li>▪ Shape: circular, irregular.</li> <li>▪ Margin: entire, undulate.</li> <li>▪ Elevation: flat.</li> <li>▪ Color: white.</li> <li>▪ Gram reaction: positive (bacillus)</li> </ul>	Morphology: <ul style="list-style-type: none"> <li>▪ Shape: circular.</li> <li>▪ Margin: entire.</li> <li>▪ Elevation: flat.</li> <li>▪ Color: white.</li> <li>▪ Gram reaction: positive (bacillus)</li> </ul>	Morphology: <ul style="list-style-type: none"> <li>▪ Shape: circular.</li> <li>▪ Margin: entire.</li> <li>▪ Elevation: flat.</li> <li>▪ Color: white.</li> <li>▪ Gram reaction: positive (bacillus)</li> </ul>

*Staphylococcus* is a coccus-shaped, aerobic bacterium capable of fermenting glucose, lactose, sucrose, and mannitol (Khairunnisa et al., 2018). Its ability to ferment sugars such as glucose, lactose, and sucrose is indicated by the development of a yellow color in both the butt and slant of TSIA medium. This is supported by Apriyanthi et al. (2022), who reported that *Staphylococcus* can ferment sugars present in TSIA medium, thereby increasing acidity and turning the medium yellow; this bacterium also possesses the catalase enzyme. In addition to *Staphylococcus*, morning bacterial isolates also included Gram-positive bacilli belonging to the genus *Bacillus*. *Bacillus* is a Gram-positive bacterium with short rod to single rod morphology arranged singly (Wiguna et al., 2019). This bacterium produces catalase, which decomposes toxic H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> under aerobic conditions (Khairunnisa et al., 2018).

*Bacillus* is a bacterial genus that includes obligate aerobic or facultative aerobic species and is positive for the catalase enzyme test (Puspita et al., 2017). When comparing the PCA medium used in this study (Table 2) with the findings of Yogeswaran et al. (2023) (Figure 3), the colonies formed were similar and suggested the presence of *Bacillus* on the medium. The genus *Bacillus* showed yellow coloration in the butt and slant regions on TSA medium, indicating that these bacteria are unable to ferment lactose and sucrose. *Bacillus* sp. is known to be unable to ferment lactose and sucrose. Both *Staphylococcus* and *Bacillus* are common airborne microorganisms found in various environments, including hospitals and other indoor spaces (Palawe et al., 2015).







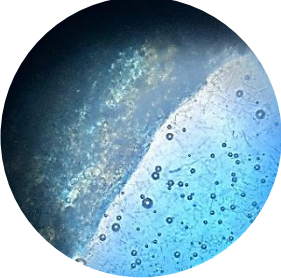
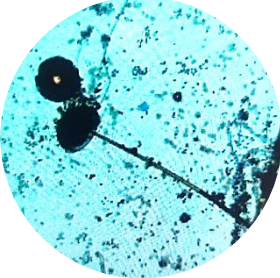
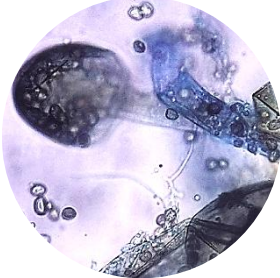
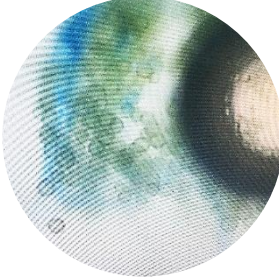
**Figure 3.** Morphology of Airborne Microorganisms in the Laboratory: (a) bacteria on TSA medium and (b) fungi on SDA medium (Source: Yogeswaran et al., 2023)

The presence of *Staphylococcus* in the laboratory is likely associated with student activities, which were more intense in the morning, during which microbes may be expelled in droplets from the mouth during sneezing, coughing, and conversation. Microorganisms suspended together with dust particles from surfaces can remain airborne throughout activities conducted in the room. The presence of *Staphylococcus* may also be influenced by temperature and humidity conditions. In the morning, the practicum room had a temperature of 26°C and humidity of 70%. Based on Paulutu et al. (2015), indoor humidity >45% is significantly associated with the presence of bacteria belonging to the genus *Staphylococcus*. The presence of *Bacillus* may be explained by its ability to adapt to environmental temperature changes and chemical stress by forming endospores, which are easily dispersed by wind and can migrate over long distances. *Bacillus* is part of the normal flora of soil, air, water, and composted soil (Rahmaningsih et al., 2012).

#### Fungal Morphological Analysis on PDA Medium

The fungi growing on PDA medium were macroscopically dominated by forms with white hyphae, black spores, and green spores (Table 4). Fungal colonies grew extensively and covered the entire medium. This indicates that fungal presence in the laboratory was highly diverse and not dominated by a single species. White hyphae with black spores showed characteristics similar to *Aspergillus niger* and *Trichoderma viridae*.

**Table 4.** Macroscopic and microscopic morphology of airborne microorganisms on PDA medium

Morning		Afternoon	
			
			
Morphology: <ul style="list-style-type: none"> <li>▪ Black unicellular spores</li> <li>▪ White hyphae</li> </ul>	Morphology: <ul style="list-style-type: none"> <li>▪ Black unicellular spores</li> <li>▪ Conidiophore</li> <li>▪ White hyphae</li> </ul>	Morphology: <ul style="list-style-type: none"> <li>▪ Black unicellular spores</li> <li>▪ Conidiophore</li> <li>▪ White hypha</li> </ul>	Morphology: <ul style="list-style-type: none"> <li>▪ Black unicellular spores</li> <li>▪ Green unicellular spores</li> <li>▪ Conidiophore</li> </ul>

Fungal morphology was then identified microscopically at 10× magnification to obtain more detailed fungal characteristics. Microscopic analysis showed that microorganisms detected in both morning and afternoon samples exhibited black unicellular spores and conidiophores resembling *Aspergillus* sp. (Table 4). The presence of this fungal type is also supported by the study of Yogeswaran et al. (2023) on laboratory air quality (Figure 3). The fungi reported in that study showed several morphological similarities to those observed in the present study. Likewise, microscopic observations from other samples revealed green unicellular spores characteristic of *Trichoderma* sp. (Table 4). White hyphae were also observed microscopically, suggesting the possible presence of certain species such as *Candida* sp. or *Phanerochaete* sp.

The presence of fungi such as *Aspergillus niger* is likely related to laboratory environmental conditions that strongly support fungal growth. The laboratory humidity of 70% and temperature of 26°C may facilitate the presence of microorganisms, including *Aspergillus niger*. In addition, the conidia of *Aspergillus niger* can easily be transported in airborne aerosols, and their highly hydrophobic nature allows them to disperse readily throughout a room under low airflow conditions (Mousavi et al., 2016). The presence of other fungal groups, such as *Trichoderma* sp., may be influenced by laboratory activities involving this species, allowing fungal spores to become airborne and escape into the surrounding environment. Therefore, follow-up evaluation of laminar air flow systems and incubation conditions during laboratory activities is necessary.

Overall, the results showed that airborne microorganism concentration (CFU/m<sup>3</sup>) differed significantly between the two laboratory rooms, with Room B having a higher concentration than Room A ( $p < 0.05$ ). This finding confirms that the physical

characteristics of the rooms, particularly ventilation, lighting, and air circulation, play an important role in determining the microbiological quality of indoor air. Morphological identification indicated the dominance of Gram-positive bacteria such as *Staphylococcus* and *Bacillus*, as well as fungi such as *Aspergillus* and *Trichoderma*, providing an initial overview of potential health risks in the laboratory environment. The most important finding of this study is that differences in room characteristics had a greater influence on microbial concentration variation than differences in sampling time. Although all values remained below the regulatory threshold, the presence of opportunistic microorganisms still warrants attention in the context of long-term risk prevention. These findings are relevant for laboratory management because they highlight the importance of optimizing ventilation, controlling humidity, and conducting regular indoor air quality monitoring as part of the laboratory occupational health and safety (K3) management system. Thus, the results provide a scientific basis for the formulation of technical policies to control bioaerosols in educational environments in environmental engineering.

### **Management Implications and Recommendations for Laboratory Air Quality Control**

The findings of this study indicate that variation in airborne microorganism concentrations is influenced more strongly by the physical characteristics of the room than by differences in sampling time. Therefore, the primary implication lies in laboratory air quality management strategies. Several international studies have confirmed that inadequate ventilation can increase bioaerosol accumulation due to low air exchange rates (AER) and elevated CO<sub>2</sub> concentrations and humidity (Kwan et al., 2020; Morawska et al., 2017). Accordingly, applicable technical recommendations include increasing mechanical or natural ventilation rates to ensure minimum air exchange in accordance with workspace standards, as well as conducting regular maintenance of exhaust fans and air filtration systems. In addition, adequate natural lighting has been shown to contribute to reduced viability of certain microorganisms because exposure to UV radiation and high light intensity can damage microbial cell structures (Madsen et al., 2023). Humidity control is also a critical factor, as relative humidity above 60% increases the survival of airborne bacteria and fungi (Li et al., 2021). Given that the laboratory was recorded at approximately 26°C with 70% humidity, the installation of dehumidifiers or adjustment of the HVAC system is necessary to maintain relative humidity below 60%, thereby inhibiting the proliferation of opportunistic microorganisms such as *Aspergillus* spp. and *Staphylococcus* spp. The implementation of air quality management based on periodic CFU/m<sup>3</sup> monitoring is also recommended as part of the laboratory K3 system, as suggested in indoor bioaerosol studies conducted in educational and research facilities (Yogeswaran et al., 2023; Janczarek et al., 2024).

Although this study provides an initial empirical contribution to understanding bioaerosol quality in a tropical educational laboratory, several limitations should be noted. First, sampling was conducted over a limited time period and does not represent seasonal variation, which may affect bioaerosol dynamics, as reported by Adams et al. (2015), who showed that indoor microbiome composition can change significantly across seasons. Second, the culture-based approach (CFU/m<sup>3</sup>) identifies only microorganisms capable of growing on specific media, meaning that non-culturable microorganisms may have remained undetected. Future studies could integrate molecular approaches such as metagenomic analysis to provide a more comprehensive characterization of the microbial community (Chen et al., 2020). In addition, further research should evaluate the quantitative relationship between

physical parameters (temperature, humidity, CO<sub>2</sub>, and AER) and microbial concentration using a longitudinal design in order to model exposure risk more precisely. By expanding both the temporal and methodological scope, future studies could generate more robust predictive models of laboratory air quality and provide a stronger basis for developing technical standards for bioaerosol control in educational facilities located in tropical regions.

## CONCLUSION

This study quantified airborne microorganism concentrations (CFU/m<sup>3</sup>) and identified their morphological characteristics in an environmental engineering laboratory under tropical climatic conditions. The results revealed significant differences between rooms ( $p = 0.018$ ), with Room B exhibiting higher mean concentrations (60.75 CFU/m<sup>3</sup> in the morning and 44.50 CFU/m<sup>3</sup> at midday) than Room A (23.75 CFU/m<sup>3</sup> and 24.25 CFU/m<sup>3</sup>, respectively). In contrast, temporal variation between morning and midday sampling was not statistically significant ( $p = 0.636$ ), indicating that room-specific environmental characteristics exerted a stronger influence on bioaerosol concentrations than short-term variation within a single day of observation. Morphological identification indicated the dominance of Gram-positive cocci and bacilli, morphologically suggestive of the genera *Staphylococcus* and *Bacillus*, as well as fungi with characteristics resembling *Aspergillus* and *Trichoderma*. Although all measured concentrations remained below the national threshold of 200 CFU/m<sup>3</sup>, the presence of opportunistic genera highlights the importance of systematic airborne microbiological monitoring in laboratory environments. Overall, the findings indicate the presence of bioaerosol dynamics in environmental engineering laboratories located in tropical regions, despite certain limitations in the sampling approach. These results further emphasize the importance of environmental control as a mitigation strategy to prevent microbial accumulation and to strengthen indoor air quality management in environmental engineering laboratories.

## RECOMMENDATION

Future studies are recommended to expand the sampling period and increase the number of sampling locations to obtain a more representative profile of bioaerosol dynamics in laboratory environments. Further research should also incorporate molecular identification methods and environmental parameter measurements, such as temperature, humidity, and ventilation rate, to better explain the factors influencing airborne microbial concentrations.

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