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Sample collection of airborne microbial diversity and analysis of air quality in a brazilian school environment

Coleta de amostras da diversidade microbiana do ar e análise da qualidade do ar em um ambiente escolar brasileiro

Recolección de muestras de diversidad microbiana en el aire y análisis de la calidad del aire en un entorno escolar brasileño

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Abstract
Microorganisms are essential components of ecosystems, playing crucial roles in maintaining environmental, human, and animal health. In the context of public health and One Health, understanding the dynamics of these organisms in the environment is key to disease prevention and control. The present study aims to investigate the diversity of airborne bacteria in a school environment in Cuiabá, a city in the Midwest region of Brazil, using the impingement on solid technique. Air samples were collected from indoor and outdoor locations, including a bathroom, cafeteria, teachers' lounge, reception area, classroom, and an outdoor sports court. At each site, two Petri dishes were exposed for 15 minutes. The samples were then incubated for 48 hours at a controlled temperature of 37°C, followed by morphological analysis and microscopic examination. Unexpectedly, outdoor areas, particularly the sports court, showed higher bacterial concentrations, to the common assumption of higher microbial loads in enclosed spaces due to limited ventilation. This finding suggests that even naturally ventilated areas require more careful analysis to ensure air quality in public spaces. Additionally, fungal growth was observed in two samples, further highlighting potential environmental risks. These results underscore the importance of improving hygiene strategies, and management in school settings to mitigate health risks associated with airborne microorganism exposure.
Keywords: Air pollution; School environment; Microbial sampling; Impingement on solid; Indoor and outdoor areas; Microbial analysis.

Resumo
Os microrganismos são componentes essenciais dos ecossistemas, desempenhando papéis cruciais na manutenção da saúde ambiental, humana e animal. No contexto de saúde pública e One Health, a compreensão da dinâmica desses organismos no ambiente é fundamental para a prevenção e controle de doenças. O presente estudo visa investigar a diversidade de bactérias transportadas pelo ar em um ambiente escolar em Cuiabá, uma cidade da região Centro-Oeste

do Brasil, utilizando a técnica de sedimentação simples. Amostras de ar foram coletadas em locais internos e externos, incluindo um banheiro, refeitório, sala dos professores, área de recepção, sala de aula e uma quadra esportiva ao ar livre. Em cada local, duas placas de Petri foram expostas por 15 minutos. As amostras foram então incubadas por 48 horas a uma temperatura controlada de 37°C, seguido de análise morfológica e exame microscópico. De forma inesperada, as áreas externas, particularmente a quadra esportiva, apresentaram concentrações mais elevadas de bactérias, uma tendência que contrasta com a suposição comum de maior carga microbiana em ambientes fechados devido à ventilação limitada, demonstrando que ainda que haja uma ventilação natural, estes ambientes requererem uma análise mais cuidadosa nos esforços para garantir a qualidade do ar em espaços públicos. Além disso, foi observado crescimento de fungos em duas amostras, destacando ainda mais os potenciais riscos ambientais. Esses resultados enfatizam a importância de melhorias nas estratégias de higiene e gestão ambiental em ambientes escolares para mitigar os riscos à saúde associados à exposição a microrganismos transportados pelo ar.
Palavras-chave: Contaminação do ar; Ambiente escolar; Amostragem microbiana; Sedimentação simples; Áreas internas e externas; Análise microbiana.

Resumen
Los microorganismos son esenciales para los ecosistemas, desempeñando roles cruciales en la salud ambiental, humana y animal. En salud pública y One Health, comprender su dinámica en el ambiente es clave para prevenir y controlar enfermedades. Este estudio investiga la diversidad de bacterias transportadas por el aire en un ambiente escolar en Cuiabá, Brasil, mediante la técnica de sedimentación pasiva. Se recolectaron muestras de aire en zonas internas y externas, como baño, cafetería, sala de profesores, aula y una cancha deportiva. En cada sitio, dos placas de Petri se expusieron por 15 minutos. Las muestras fueron incubadas por 48 horas a 37°C, seguidas de análisis morfológico y examen microscópico. Sorprendentemente, las áreas externas, especialmente la cancha, mostraron mayores concentraciones de bacterias, lo que contrasta con la creencia de una mayor carga microbiana en espacios cerrados. Esto resalta que incluso áreas ventiladas naturalmente requieren atención para garantizar la calidad del aire en espacios públicos. Además, se observó crecimiento de hongos en dos muestras, destacando los riesgos ambientales. Los resultados subrayan la importancia de mejorar las estrategias de higiene y gestión ambiental en escuelas para mitigar los riesgos de exposición a microorganismos transportados por el aire.
Palabras clave: Contaminación del aire; Ambiente escolar; Muestreo microbiano; Sedimentación pasiva; Áreas interiores y exteriores; Análisis microbiano.

1. Introduction

The analysis of air contamination has gained significant attention due to its critical importance for human health. Not only that, recent global health crises, including the COVID-19 pandemic, mpox, Ebola outbreaks, and ongoing threats from zoonotic diseases, food safety issues, antimicrobial resistance (AMR), along with environmental degradation and climate change, highlight the urgent need for robust health systems and faster global responses. The One Health approach is key to addressing these interconnected and complex challenges facing society (WOAH, 2023). Moreover, air serves as a primary medium for the transport and dispersion of various microorganisms, including vegetative cells, spores of bacteria, fungi, algae, viruses, and protozoan cysts. These organisms have the potential to travel long distances and infect multiple individuals. The microflora present in the air is classified into two main categories: outdoor and indoor microflora (Pepper & Gerba, 2015; Ruiz-Gil et al., 2020). This study aims to analyze samples from both environments, focusing on airborne bacteria; nevertheless, some fungal residues were also found in the experiment.

In this context, it is important to recognize that each environment harbors a distinct microflora, with significant variations in both composition and abundance influenced by many factors. Nonetheless, certain microorganisms are more prevalent in specific locations. For instance, the outdoor microflora is typically dominated by fungi, with *Cladosporium* and *Sporobolomyces* being common genera. Among bacteria, the most frequently encountered genera in outdoor environments include *Bacillus*, *Clostridium*, *Sarcina*, *Micrococcus*, *Corynebacterium*, and *Achromobacter*. In contrast, indoor microflora tends to exhibit a different microbial profile, with *Penicillium* and *Aspergillus* being the most common fungal genera, while the predominant bacterial genera include *Staphylococcus*, *Bacillus*, and *Clostridium*. Moreover, the composition of microflora is known to vary based on factors such as latitude and altitude (Pepper & Gerba, 2015; Ruiz-Gil et al., 2020).

Humans are the primary source of airborne microorganisms, which are disseminated through activities such as coughing, shedding skin, sneezing, talking, and laughing, all of which release significant quantities of pathogenic microorganisms from the respiratory tract. For example, a single sneeze can release between 10,000 and 100,000 microorganisms (Pepper & Gerba, 2015; Ruiz-Gil et al., 2020). These microorganisms are carried in droplets of saliva and mucus, and the size of these droplets determines how long they remain airborne. Larger droplets tend to settle quickly, while smaller droplets, particularly in warm and dry conditions, can form droplet nuclei that remain suspended in the air for extended periods and travel long distances. When large aerosol droplets dry out completely, they become infectious dust particles that settle rapidly (Pepper & Gerba, 2015; Ruiz-Gil et al., 2020).

Air pollution has been exacerbated by the growth of large urban centers, which has led to an increased concentration of people in cities and a demand for the construction of larger buildings (Giyasov, 2023). This has had a significant impact on the concentration of microorganisms in the air. Indoor air quality has become a particular concern, as people now spend more than 90% of their time indoors (USEPA, 1996). Poor ventilation is a major contributing factor, as indoor air is often isolated from outdoor air to reduce energy costs associated with air conditioning (Wu & Liu, 2023). However, air conditioning systems frequently lack proper maintenance, often failing to meet maintenance and control standards (PMOC). Additionally, synthetic materials used in construction, interior decoration, lighting, electrical energy sources, and air diffusers further contribute to the proliferation of pathogens by creating stagnant air pockets that are not properly ventilated (Mannan & Al-Ghamdi, 2021; Asim et al., 2022; Zuo et al., 2024).

Human activities are also a key factor in the dissemination of microorganisms in the air. High population densities in confined spaces, where the number of people exceeds recommended limits, can significantly increase the microbial load in the air (Xie et al., 2020; Zuo et al., 2024).

The industrialization and expansion of urban centers have also intensified hypersensitivity reactions in city populations due to poor air quality (Liang & Gong, 2020; Chukwu et al., 2022; Gupta & Kumar, 2023). Airborne pathogens contribute to the development of diseases, with common symptoms including skin and respiratory tract irritations, dermatitis, rhinitis, conjunctivitis, asthma, and other conditions such as coughing, headaches, diarrhea, nausea, dizziness, and general malaise (Liang & Gong, 2020; Chukwu et al., 2022; Gupta & Kumar, 2023). In response to the growing prevalence of these cases, the World Health Organization (WHO) introduced the concept of "Sick Building Syndrome" (SBS). This condition is diagnosed when 20% or more of a building's occupants exhibit one or more of the mentioned symptoms for at least two weeks, and the symptoms are directly linked to the individual's interaction with the building. If the person leaves the building for a period, the symptoms tend to subside (Nakayama et al., 2019; Nag, 2019; Kalender-Smajlović et al., 2021).

The implications of poor indoor air quality extend beyond health concerns, as they also negatively impact businesses and educational institutions. In work environments, poor air quality leads to decreased productivity due to absenteeism or employees working while unwell (Kaushik et al., 2022). In the United States, it is estimated that approximately $64 billion is spent annually on absenteeism and respiratory illness treatment, with potential savings of $6 to $19 billion if respiratory illness cases were reduced by just 10% to 30% (Fisk & Rosenfeld, 1997).

This situation in schools is particularly concerning, as studies indicate both short- and long-term negative impacts on health, cognitive development, and learning. Indoor air pollution in classrooms increases health risks, may reduce teacher productivity, and hinder students' learning outcomes (Brink et al., 2021; Wargocki, 2022).

Currently, there is a noticeable gap in research on this topic, particularly concerning the investigation of the origin, sources, and extent of contamination in locations suspected of poor air quality, especially those exhibiting symptoms of Sick Building Syndrome (Nakayama et al., 2019; Nag, 2019; Kalender-Smajlović et al., 2021)). Additionally, there is a significant

lack of bacteriological parameters for air quality analysis compared to other chemical, physical, and biological contaminants (Basińska et al., 2019). This gap is compounded by the absence of standardized methods for analysis, with available techniques being highly varied, which complicates the comparison of research findings. Commonly employed methods include passive sedimentation (also known as impingement on solids), filtration, electrostatic precipitation, centrifugal impaction, liquid impingement, and solid impingement (Palacios et al., 2022; Galičič et al., 2024; Langiano et al., 2024). In this study, due to limitations related to the availability of materials, reagents, and equipment, priority was given to the passive sedimentation method. This approach involves exposing Petri dishes containing culture media to the study environment, allowing airborne particles to settle by gravity (Palacios et al., 2022).

Furthermore, the lack of national and international health authority guidelines regarding ideal levels of bacterial concentrations in the air is a critical issue in bacterial microflora analysis. While Brazil has legislation addressing air quality in indoor environments, these regulations primarily focus on polluting gases concentration, and fungi, with no direct reference to bacterial contamination levels. This regulatory gap discourages routine laboratory testing of air quality due to the absence of established standards (Moldoveanu, 2015). As a result, the current approach is to compare findings with existing studies and interpret the data in that context.

Similarly, it is important to consider the influence of physicochemical factors on the growth of these colonies, particularly highlighting the impact of temperature and humidity on the survival of airborne microorganisms. In this context, there is a logarithmic rate of bacterial survival decline with decreasing humidity and temperature, which can reduce survival by up to 70% at very low temperature and humidity conditions (Igo & Schaffner, 2019; Qiu et al., 2022).

The present study aims to investigate the diversity of airborne bacteria in a school environment in Cuiabá, a city in the Midwest region of Brazil using the impingement on solid technique.

2. Methodology

Field research of a qualitative and quantitative nature was done (Pereira et al., 2018). This research was carried out at a K-12 school, located in the capital city of the state of Mato Grosso, Cuiabá in September 2024, which is one of the hottest and driest periods of the year, with temperatures going up to 40°C and air humidity levels as low as 10%. The school comprises both external and internal areas (the internal ones are climate-controlled using air conditioning systems). Thus, the investigation was developed in six places chosen to include various environments with a large flow of people such as the reception (a service area for parents and students), the court, restrooms, classrooms, the teachers´ lounge, and the school´s cafeteria.

2.1 Impingement on Solids

Sample collection was made by the simple sedimentation method on solid culture media. Petri dishes with blood agar (5% sheep's blood) were used for sampling and cultivation of the microorganisms. Two open Petri plates were placed, one in the corner area and the other in the middle part of each environment, and they were left exposed for 15 minutes. Since the process relies solely on impingement, it is classified as passive sampling (Pasquarella et al., 2007). Blood agar plates were incubated at 35-37°C for 48 hours to allow bacterial growth. Additionally, the average of colony forming units (CFU) was calculated using the equation:

$$R = \frac{\text{Number of colonies} \times 2.880}{\text{Plate area} \times \text{Time}}$$

Where:

- 2.880: number of minutes in 48 hours
- Area: area of the culture medium in the plate in cm^2
- Time: plate exposure time in minutes (15 minutes)

The results were expressed in CFUs/cm^2/week.

2.2 Colony Morphology Analysis

After counting the CFUs, the morphology of bacterial colonies were analysed comparing to image in Figure 2. It was considered if the colonies were hemolytic or non-hemolytic, the form, the size, elevation, the margin, surface, opacity and colour.

2.3 Smearing and Gram Staining

A smearing technique was used to transfer the bacteria of two colonies per area into the slide so that, they could be analyzed under the microscope. The first step was to obtain a sample using a sterile inoculating loop to collect a small amount of the selected bacterial colony from an agar plate placed on a clean and dry glass slide.

To analyze the bacteria under the microscope and, be able to differentiate them between gram-positive and gram-negative, the gram staining method was applied to all slides. The first step in the smearing process was to prepare the glass slide, cleaning it with absolute alcohol to ensure that it was free from any kind of contamination. Subsequently, a drop of distilled water was poured into the glass slide, so that the spreading of the culture could be performed. After that, a small amount of the bacterial colony was collected from an agar plate by using a sterile loop, and it was spread as a monolayer across the surface of the slide. Then, the slide was passed through a flame from an alcohol burner for three times, to allow the smear to heat-fix. This step is crucial to guarantee the adherence of the bacteria to the slide, and the colonies are also killed during this stage, afterwards it was left to air dry. This method can be observed in Figures 1, 2 and 3.

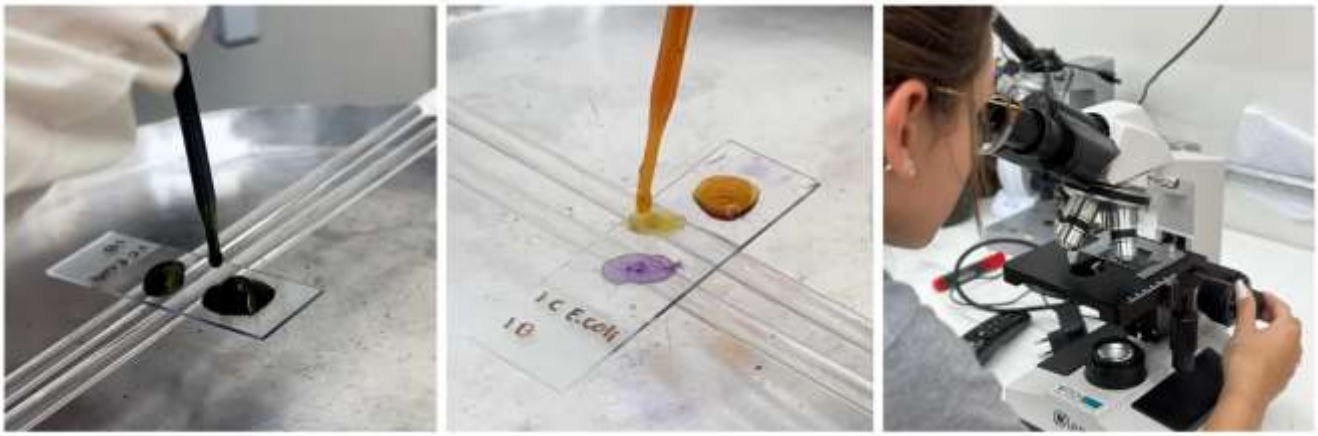
Figures 1, 2 and 3 - Diagram showing how to identify a colony morphology.

Source: Research files.

The Gram staining technique was subsequently applied to determine whether the bacterial colonies were Gram-positive or Gram-negative. Initially, the bacterial smear was stained with crystal violet dye, imparting a purple hue to the cells. This stain was left for one minute before being rinsed with distilled water. Following this, an iodine solution was introduced to enhance the dye's adherence to the bacterial cell wall by forming a complex with the crystal violet. Like the previous step, the iodine

solution was left for one minute and then rinsed with distilled water. The decolorization step was performed using an acetone-alcohol mixture to wash the slide, a crucial step for distinguishing bacterial types. Gram-positive bacteria retain the purple color, while Gram-negative bacteria lose it during this phase. The counterstaining step was then conducted using fuchsin, which stained the Gram-negative bacteria pink. This step lasted approximately 30 seconds, after which the slide was rinsed with water and left to air-dry. Finally, the colonies were examined under an optical microscope to identify the bacterial categories, shapes, and structures.

Figures 4, 5 and 6 - Gram staining method and observation.



Source: Research files.

2.4 Catalase Reaction

Even after microscopic examination, two colonies remained suspected of being either *Staphylococcus* spp. Or *Streptococcus* spp. One of the bacterial colonies had already been identified as *Staphylococcus* sp., allowing for comparison with the uncertain colonies using the catalase test. This method, which catalyzes the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen, is instrumental in differentiating between catalase-positive organisms, such as *Staphylococcus* sp., and catalase-negative organisms, such as *Streptococcus* sp. A positive catalase reaction is indicated by the formation of bubbles, upon the addition of hydrogen peroxide to the bacterial culture.

The procedure began with the preparation phase, ensuring that the bacterial colonies were fresh (48 hours old). A sterile loop was then used to collect a small amount of the bacterial colony from the agar plate, which was transferred to a clean glass slide and mixed with a drop of distilled water. Subsequently, 1-2 drops of 3% hydrogen peroxide were added directly onto the bacterial sample using a pipette. The final step involved observing the reaction to determine whether it was catalase-positive or catalase-negative. Figures 7, 8 and 9 illustrate the catalase reaction process.

Figures 7, 8 and 9 - Catalase reaction technique.

Source: Research files.

3. Results

In the conducted experiment, the number of colonies (Figura 10) ranged from 4 to 42, varying based on the location of each Petri dish and the level of human activity. Sites with a high flow of people exhibited a higher concentration of airborne microorganisms when compared to those with less activity. Air contamination levels were measured through colony counting and expressed in CFU/cm²/week (Figure 11) and CFU/m³ (Figure 12). The findings indicated that the most contaminated environment was the middle of the school's courtyard, an outdoor area with a high density of people. Conversely, the location with the lowest level of contamination was the school's reception, an indoor area with fewer individuals, and regular cleaning.

Figure 10 - Number of colonies in the Petri dishes placed around a school

Number of microbial colonies from Petri dishes placed around the school

	*A	**B
Classroom	35	28
Cafeteria	42	19
Teachers' lounge	4	7
Toilet	24	25
Court	98	46
Reception	3	5

*A - Petri dishes placed in the middle of each room and inside the toilet cabin.
**B - Petri dishes placed in the corner of each room and on the toilet's sink.
Source: Research files.

Figure 11 - Level of microbial air contamination in locations around a school in CFU/cm²/week.

Air contamination in CFU/cm²/week.

	*A	**B
Classroom	105.69	84.55
Cafeteria	126.83	57.37
Teachers' lounge	12.07	21.13
Toilet	72.47	75.49
Court	295.94	138.91
Reception	09.05	15.09

*A - Petri dishes placed in the middle of each room and inside the toilet cabin
**B - Petri dishes placed in the corner of each room and on the toilet's sink
Source: Research files.

Figure 12 - Level of microbial air contamination in locations around a school in CFU/m³.

Air contamination in CFU/m³.

	*A	**B
Classroom	1,834.95	1,467.96
Cafeteria	2,201.95	996.12
Teachers' lounge	209.7	366.99
Toilet	1,258.25	1,310.68
Court	5,137.88	2,411.65
Reception	157.28	262.13

*A - Petri dishes placed in the middle of each room and inside the toilet cabin
**B - Petri dishes placed in the corner of each room and on the toilet's sink
Source: Research files.

The bacteria found in the school environment exhibited diverse morphologies, with the majority being circular and raised. Following smearing and gram staining, the slides were examined under a 100X microscope lens. The analysis showed that the most prevalent bacteria were gram-negative bacilli (33.3%) and gram-positive cocci, likely staphylococcus (33.3%). Gram-positive bacilli accounted for 16.6% of all the bacteria analyzed.

3.1 Classroom

The Petri dishes were set in a second-floor classroom with two air conditioners adjusted at 20°C. The windows and doors were closed, and 12 people were present (11 students and 1 teacher). One Petri dish was placed in the middle of the room, while the other was positioned in the corner. The morphology of the bacteria found in each sample from the classroom was similar. However, when comparing the colonies within the same Petri dish its morphology was divergent. The colonies included smooth, raised, circular colonies, as well as dark, irregular, flat, undulated colonies that formed veils (found only in the middle sample). Some colonies were wrinkled and opaque, and most were white and glistening. All colonies analyzed in the blood agar Petri dishes (both in the corner and in the middle) were non-hemolytic. In the Petri dish placed in the middle of the room, 35

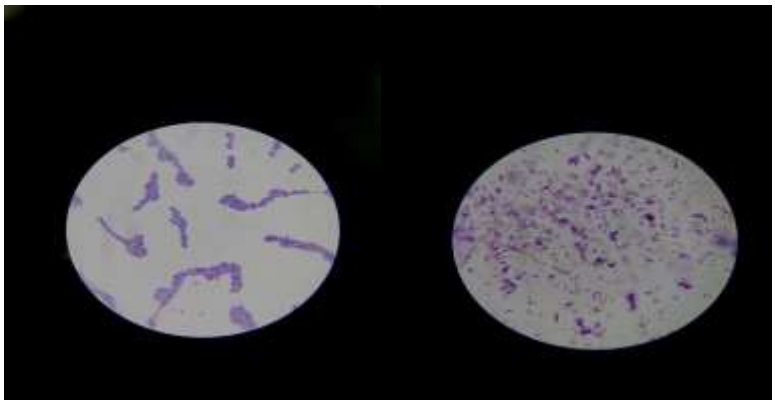
colonies grew in 48 hours, and the concentration of airborne bacteria was 1827.19 CFU/m³. The Petri dish in the corner had 28 colonies after 48 hours, and the air contamination level was 1461.75 CFU/m³. All colonies analyzed in the blood agar Petri dishes (both in the corner and in the middle) were non-hemolytic (Figures 13 and 14).

Figures 13 and 14 - Microbial colonies in Petri dishes after 48 hours of exposure in a classroom at Colegio Ibero Americano.

Source: Research files.

After smearing and gram staining two colonies from the same Petri dish that were exposed in the corner of the classroom, the slides were analyzed under a 100X magnification optical microscope. One of the slides showed both purple and pink cocci (Figure 15), which later tested positive in the catalase test. The other slide showed gram-negative rod-shaped bacteria (Figure 16).

Figures 15 and 16 - Optical microscopic analysis of bacteria from a classroom at Colegio Ibero Americano.



Source: Research files.

3.2 Cafeteria

The Petri dishes were positioned in the cafeteria, which is a central hub where approximately 585 students gather daily for lunch. The cafeteria features fans, open windows and doors, spacious tables with benches, a self-service buffet, and windows connecting it to the kitchen. When the dishes were set out, the cafeteria was empty of people, and the food was already stored in the kitchen. One Petri dish was placed on the self-service buffet area, specifically where the plates are located, while the other was positioned on the window in the corner where the kitchen-prepared meals are brought to the cafeteria. The colonies' morphology ranged from small, white, circular, raised, and complete bacterial formations to filamentous, hemolytic fungal

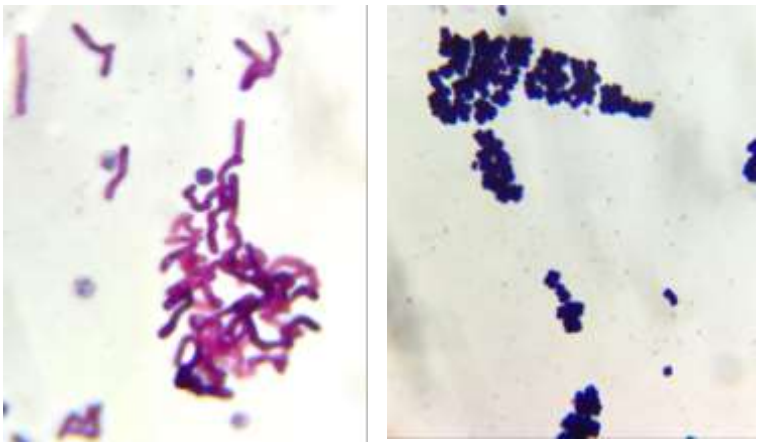
colonies (Figures 17 and 18). The samples from both locations in the cafeteria were similar, displaying smooth bacterial colonies and fungi, except for a specific filamentous fungal colony that developed in the Petri dish placed in the middle of the cafeteria that is likely Aspergillus sp. In this area, the fungal colony and one smooth gray bacterial colony were hemolytic, most likely Bacillus sp., while the rest were non-hemolytic. In the Petri dish placed in the center, 42 colonies had grown after 48 hours, and the level of air contamination was 126.83 CFU/cm²/week or 2201.95 CFU/m³. Meanwhile, the Petri dish from the corner had 19 colonies, and the concentration of airborne microbes was 57.37 CFU/cm²/week or 996.12 CFU/m³.

Figures 17 and 18 - Microbial colonies in Petri dishes after 48 hours of exposure in the cafeteria at Colegio Ibero Americano.

Source: Research files.

In the cafeteria study, a single colony from each Petri dish was selected for analysis using smearing and gram staining techniques. Subsequently, the slides were scrutinized under a 100X lens optical microscope, revealing the presence of either gram-negative bacilli or possibly fungi hyphae (Figure 19) and gram-positive cocci (Figure 20).

Figures 19 and 20 - Optical microscopic analysis of samples from the cafeteria at Colegio Ibero Americano.



Source: Research files.

3.3 Teachers' room

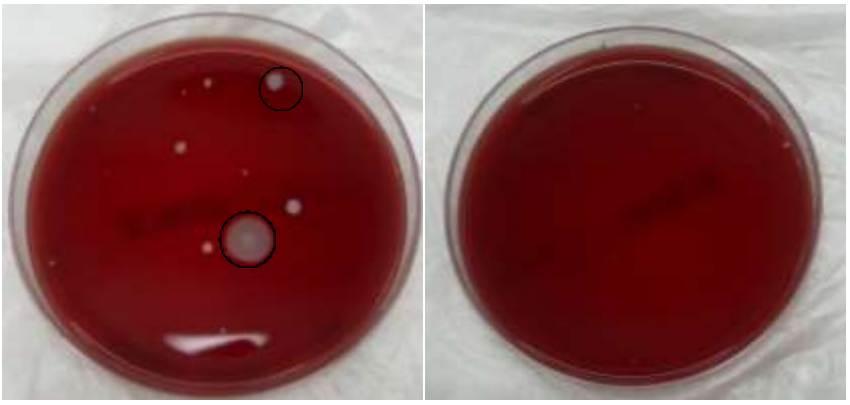
Two Petri dishes were placed in the teachers' lounge, a room located on the first floor, with one air conditioner, windows and doors closed, a large flow of people at specific times of the day, a coffee corner with a coffee machine, sandwich maker,

refrigerator and kitchen supplies. At the time the Petri dishes were exposed, the room had 3 people sitting and one used the supplies from the coffee corner immediately next to one of the dishes. One Petri dish was placed in the middle of the table and the other on the coffee corner's counter.

The morphology of the colonies in the middle of the teachers' lounge was circular, raised, smooth, and punctiform. The phenotype of the colonies found in the corner Petri dish was similar to the colonies found in the middle sample, except for one colony that was white, irregular, wrinkled, opaque, and flat. All the colonies in that location (middle and corner) were non-hemolytic (Figures 21 and 22).

In the Petri dish located in the middle, 4 colonies grew and the CFU counting was 208.8 CFU/m³ and the one in the corner had 7 bacterial colonies and an air contamination level of 365.43 CFU/m³.

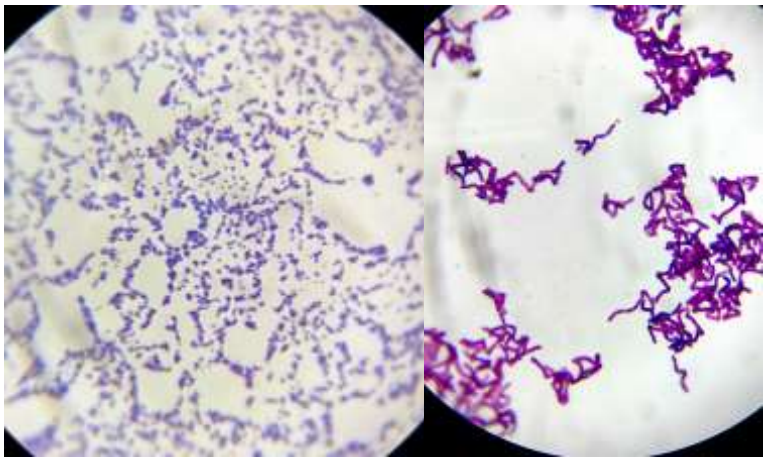
Figures 21 and 22 - Microbial colonies in Petri dishes after 48 hours of exposure in the teacher's room at Colegio Ibero Americano.



Source: Research files.

After smearing and staining two colonies selected from the corner sample, the slides were examined under a 100X magnification optical microscope. One colony was identified as Gram-positive cocci and tested positive in the catalase test, indicating that it is likely *Micrococcus sp.*(Figure 23). The second colony was identified as a Gram-negative rod-shaped bacteria (Figure 24), but could not be further classified due to resource limitations in the school's laboratory.

Figures 23 and 24 - Optical microscopic analysis of bacteria from the teacher's room at the school.



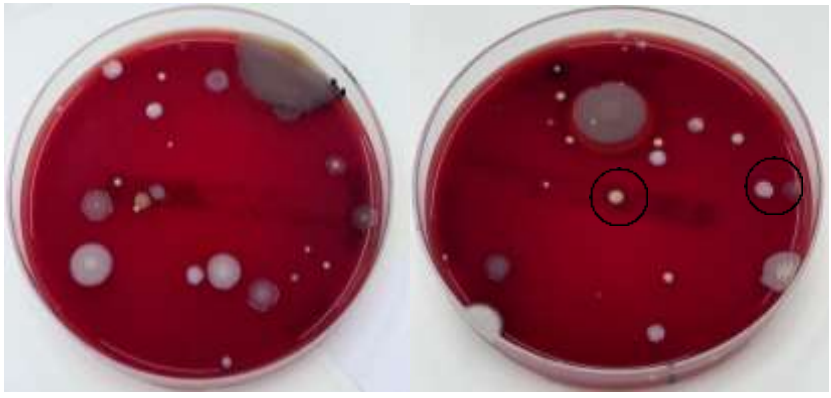
Source: Research files.

3.4 Toilet

The Petri dishes were positioned in the women's restroom, which is used by middle and high school female students, as well as some female staff members throughout the day. The restroom consists of 4 stalls, 4 sinks on a single counter, a large window, and a door, and it undergoes regular cleaning multiple times a day. The Petri dishes were exposed during lunchtime, when the restroom was unoccupied after the peak usage.

The colonies in the Petri dishes appeared mostly smooth, white, and glistening, with a few exceptions. Notably, the central dish contained a yellow, circular, opaque bacterial colony with a dark halo, alongside a white, circular, convex, filiform fungal colony (Figures 25 and 26).

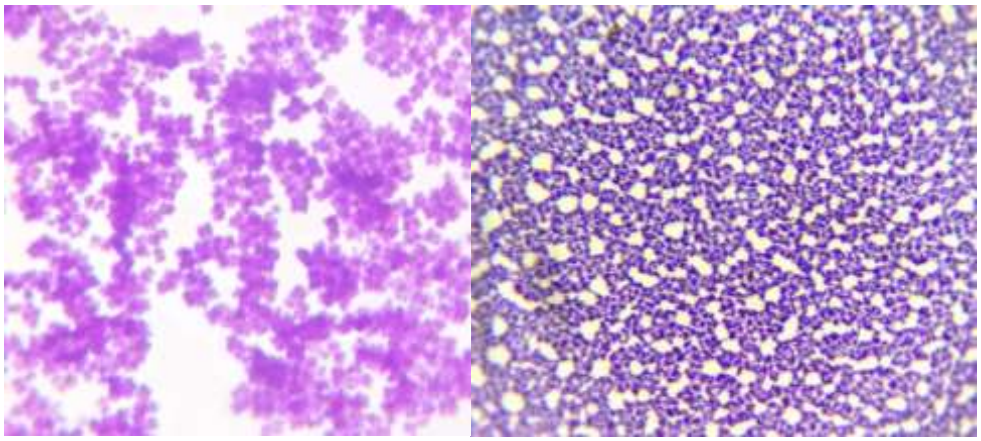
Figures 25 and 26 - Microbial colonies in Petri dishes after 48 hours of exposure in a toilet at the school.



Source: Research files.

Following the smearing and gram staining of 2 samples from the Petri dish located on the sink, in the restroom, the results were examined under a 100X lens optical microscope. The analysis revealed that one of the colonies was a gram-negative cocci (Figure 27), while the second was a gram-positive cocci (Figure 28). Subsequently, the catalase test was conducted on the samples, and both yielded positive results, indicating that the bacteria could possibly be micrococcus or staphylococcus.

Figures 27 and 28 - Optical microscopic analysis of bacteria from a women 's toilet at the school.



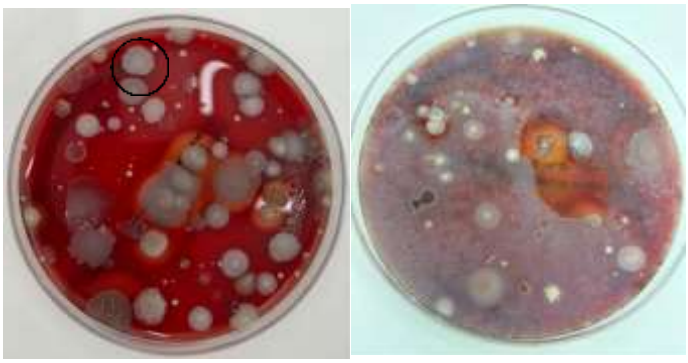
Source: Research files.

3.5 Court

Two Petri dishes were opened and exposed to the air in the school's multi-sports court, an outdoor area surrounded by trees and adjacent to the cafeteria. This space is frequented by students throughout the day, particularly during the lunch break. At the time the dishes were exposed, there were no students present, and the area had not been cleaned after lunch.

After 48 hours, a variety of colonies were found in the Petri dishes. One dish contained a bacterial colony with a white veil that spread across the entire surface, making it impossible to isolate individual colonies. The other colonies were mostly white, circular, and smooth, or gray, translucent, and glistening. Two samples were chosen for further analysis under the optical microscope: the first sample was irregular, smooth, translucent, and entire, while the second sample was opaque, irregular, entire, gray, and wrinkled (Figures 29 and 30). Some colonies from both sites were hemolytic, but most were non-hemolytic.

Figures 29 and 30 - Microbial colonies in Petri dishes after 48 hours of exposure in the court at the school.



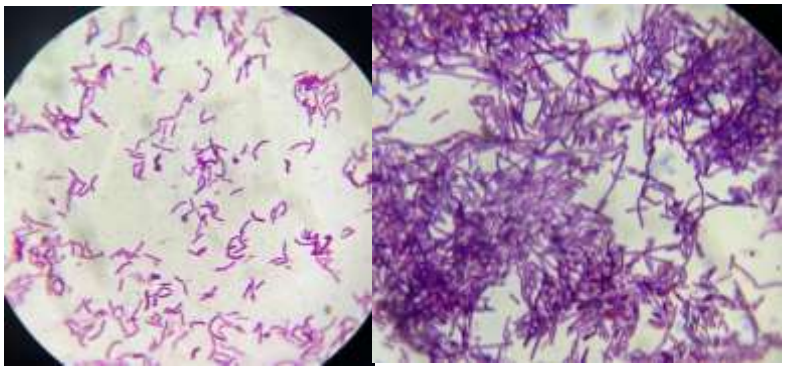
Source: Research files.

After the smearing and gram staining of 2 colonies from the court, the slides were analyzed under the 100X lens optical microscope. Both samples were Bacillus, however, one sample was a gram-negative species, probably *Actinomyces sp.* (Figure 29), and the other was a gram-positive colony (Figure 30), most likely *Streptomyces sp*.

3.6 Reception

The school's reception is equipped with an air conditioner, doors leading to other rooms, and secretaries working, with regular cleaning and a modest flow of people throughout the day. The two Petri dishes were exposed, and there were just three professionals present, without students in the vicinity.

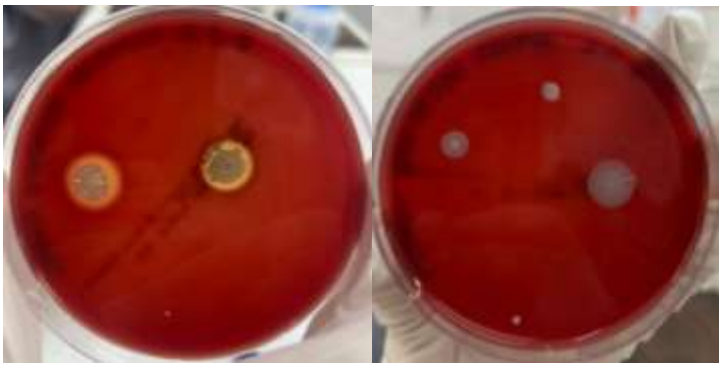
Figures 31 and 32 - Optical microscopic analysis of samples from the reception at the school.



Source: Research files.

The colonies exhibited various morphologies, including white, circular, smooth, punctiform, irregular, wrinkled, and flat (Figures 33 and 34). Two out of the three colonies from the Petri dish exposed to the middle of the reception were hemolytic, while all colonies from the corner's Petri dish were non-hemolytic.

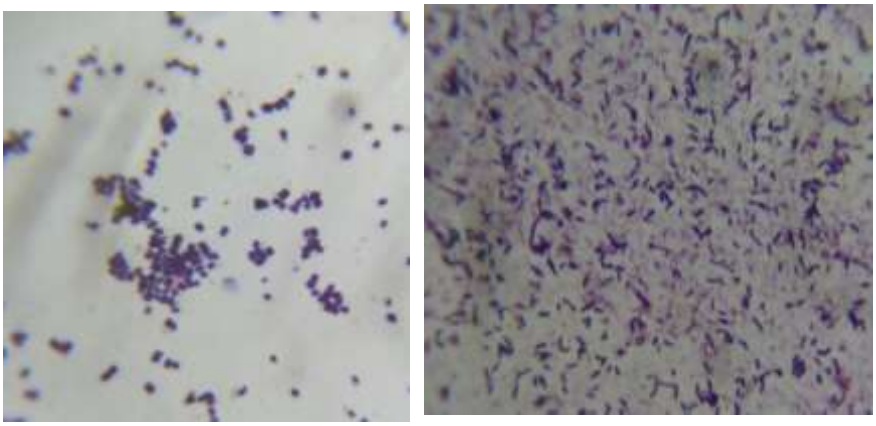
Figures 33 and 34 - Microbial colonies in Petri dishes after 48 hours of exposure in the reception at the school.



Source: Research files.

Two samples were selected for microscopic analysis. Following smearing and gram staining, the slides were examined using the 100X lens of an optical microscope. The first sample exhibited the presence of gram-positive cocci (Figure 35), while the other displayed gram-positive rod-shaped bacteria (Figure 36).

Figures 35 and 36 - Optical microscopic analysis of bacteria from the reception at the school.



Source: Research files.

4 Discussion

4.1 Microbial Sampling Results

The primary microorganisms identified in the study were *Staphylococcus* sp. and *Bacillus* sp., determined through morphological analysis of the colonies and Gram staining observed under an optical microscope. Notably, the presence of two distinct genera of fungi was also detected, which was unexpected. Fungal growth typically occurs between 96 and 168 hours at 25°C (Palaz et al., 2023), yet fungal colonies were observed in two of the Petri dishes within a much shorter time frame. Based on morphological comparison with photographic evidence and scientific literature, the fungi were tentatively identified as belonging to the *Aspergillus* genus (Kashefi et al., 2021). Microscopic examination further supported this hypothesis. It is important to note that our study was conducted over a 48-hour period, during which the presence of *Bacillus* sp. and *Staphylococcus* sp. was expected, as both genera are known to thrive under the temperature and temporal conditions of the experiment. The unexpected fungal growth, however, deviates from the typical growth timeline for fungi in such conditions.

4.1.1 Courtyard

Most of the samples found were *Staphylococcus* sp. and *Bacillus* sp. The highest concentration of microbial load both quantitatively and qualitatively was found in the court, that is due to the high temperature and low air humidity levels of the period between June and September in the Midwest region of Brazil, added to the intense flow of people and animals in the court which, as previously mentioned is located outdoors (Qiu et al. 2022), are all possible contributing factors to the high level of air contamination and microbial growth in the plates sampled in the school court. The lack of humidity helps because the nuclei are dispersed and can remain suspended in the air for hours or days, traveling long distances (Aithal, 2010) The high numbers of microorganisms might have also been present because of the high traffic of students who play sports right after lunchtime and are a source of air microbial contamination through skin shedding.

4.1.2 Classroom

An analysis of the results, showing a mean of 746.6 CFU/cm²/week in the central area of the classroom and 597.33 CFU/cm²/week in the corners, indicates a higher concentration of colony-forming units (CFU) in the center of the room where students are seated. This disparity is primarily attributed to skin shedding and saliva contamination, as students occupy the room throughout the day, from 8 AM to 12 PM and from 1 PM to 8 PM. According to research by Dr. Shiva (2010), human activity

is a significant source of airborne microorganisms, with actions such as laughing, coughing, sneezing, and talking contributing to the emission of droplets and droplet nuclei. The more individuals present and participate in communication within the room, the higher the concentration of airborne bacteria. Additionally, the use of an air conditioning system that recirculates indoor air without introducing fresh air from outside further exacerbates the accumulation of microorganisms within the classroom environment.

4.1.3 Toilet

When comparing the microbial contamination of the toilet area to other locations studied, the following results were observed: the medium had an average of 512 CFU/cm²/week, and the corner exhibited a slightly higher contamination level of 533.33 CFU/cm²/week. It is important to note that the Petri dish was placed on the sink, a region typically associated with a higher level of contamination. This elevated contamination level can be attributed to several factors, including the effectiveness of cleaning and hygiene practices, the high foot traffic, and the poor ventilation of the area, with only a relatively small window providing airflow.

Additionally, personal hygiene habits of students and staff who use the restroom must be taken into account. The restroom is accessible from 7 am to 6 pm and is cleaned three times daily—at 7 am, 12 pm, and 4 pm. However, students often engage in personal hygiene activities, such as brushing their teeth, which can contribute to contamination. Other important factors include the presence of paper towels discarded on the floor, unclean sinks, and droplets dispersed when users flush toilets without closing the lid. Temperature and foot traffic further exacerbate the situation. Given these conditions, it is clear that the observed contamination levels are consistent with known patterns of microbial growth, particularly in areas where water accumulates and creates favorable environments for microorganisms, as noted in studies by the University of Arizona in Tucson.

4.1.4 Teachers lounge

The teachers' lounge experiences peak activity primarily in the morning prior to the commencement of classes and during the afternoon. At the time of the experiment, three educators were present in the room, which significantly influenced the resultant calculations: the plate placed in the middle of the room concentration was measured at 85.33 CFU/cm² per week, while the corner concentration reached 149.33 CFU/cm² per week. The dynamics of staff movement and the room's ventilation, facilitated by air conditioning, are crucial factors in these findings. Notably, environmental conditions such as humidity and temperature appear to be less significant than the presence and movement of individuals, including their personal belongings. For the same causes, previously mentioned when discussing humans as sources of contamination, periods of heightened occupancy, contributes to the dispersal of respiratory droplets and aerosolized nuclei within the space.

4.1.5 Cafeteria

Recent measurements indicate that the middle area of the school cafeteria exhibits a microbial contamination level of 896 CFU/cm²/week, while the corners show a significantly lower count of 405.33 CFU/cm²/week. This disparity suggests that the central areas, likely subjected to higher traffic, are more prone to microbial proliferation.

The school cafeteria serves a substantial daily population, comprising approximately 685,585 students and 100 staff members during its operational hours from 10 AM to 1 PM. The absence of air conditioning, coupled with the presence of windows and fans, may exacerbate humidity and temperature fluctuations, further contributing to an increased incidence of microbial contamination.

Research conducted on microbiological contamination in commercial restaurant environments, notably by (Coelho et al., 2010), highlights the presumptive counts of aerobic mesophiles on various surfaces, including countertops, equipment, and utensils. While air quality may not significantly impact the microbiological safety of low-perishable foods, it poses a heightened risk for more perishable items, which are particularly vulnerable to contamination from airborne microorganisms. Given the identified risk factors and the high foot traffic in the cafeteria, it is imperative to implement rigorous cleaning and sanitation protocols. Additionally, enhancing ventilation and air filtration may mitigate the microbial load, thereby promoting food safety and overall health in this communal dining environment. The school cafeteria welcomes all students, staff members, and parents. Occupied from 10 am until 1 pm, and everyone has lunch there. Approximately 685,585 students and 100 employees visit the site, so the flow of people is very large. Analyzing the place, it is noticed the absence of air conditioning and the presence of windows and fans, therefore, along with the flow of people, humidity, and temperature can be considered as aggravating the incidence of batteries. Along with these factors, the study on Microbiological contamination of environments and surfaces at commercial restaurants (Coelho et al., 2010) shows the presumptive count of aerobic mesophiles on surfaces of countertops, equipment, and utensils and, air quality about food may not directly affect microbiological safety, or the maintenance of quality, in the case of low-perishable foods. However, foods that are more susceptible to spoilage are particularly sensitive to contamination by airborne microorganisms.

4.2 Comparison to Previous Studies, Standards and Guidelines

Currently, Brazil lacks specific standards regarding bacterial concentration and total microbial load in air contamination analyses. Consequently, it is necessary to refer to international standards and legislation from other countries to assess and compare contamination levels. Legislation from Portugal, Singapore, and Sweden, as well as guidelines from the American Conference of Governmental Industrial Hygienists (ACGIH), stipulate that bioaerosol contamination in indoor environments should not exceed 500 CFU/m³ to maintain safe conditions (Pegas et al., 2011; Abel et al., 2002; Obbard & Fanf, 2003; Jensen & Schafer 1998; Katiyar, 2013). Less strict standards, such as those set by the Hong Kong Indoor Air Quality (HKIAQ) guidelines and the World Health Organization (WHO), consider a limit of 1,000 CFU/m³ for microbial concentration in indoor spaces, with HKIAQ classifying locations with fewer than 500 CFU/m³ as excellent (WHO, 2009; IAQ, 2019). In contrast, the European Commission Report adopts a different classification: 0 CFU/m³ as undetectable, 1-499 CFU/m³ as low, 500-999 CFU/m³ as medium, and levels exceeding 1,000 CFU/m³ as high (CEC, 1994).

These data highlight concerning levels of contamination in the school areas analyzed. When applying the 500 CFU/m³ standard for indoor environments, only four out of ten locations (teachers´ lounge A and B, and receptions A and B) fall within safe limits. This finding remains largely unchanged when considering the broader threshold of 1,000 CFU/m³, as only five locations (teacher's lounges A and B, receptions A and B, and cafeteria B) exhibit acceptable contamination levels.

While these results are alarming, they align with findings from similar studies conducted in schools and universities, suggesting that this type of environment tends to exhibit elevated contamination levels. In this study, the average contamination rate in classrooms was 1,651.455 CFU/m³, lower than the 1,709 CFU/m³ recorded in Raisa Jabeen's study at a college in India, but higher than the results from studies conducted by M. Stryjakowska-Sekulska at a university in Poland (605 CFU/m³) and by S.C. Lee and M. Chang at a school in Hong Kong (800 CFU/m³) (Lee, S. C., & Chang, M., 2000; Stryjakowska-Sekulska et al., 2007; Jabeen et al., 2023). Variations in occupancy rates during each study likely contributed to the differences observed.

Similarly, the contamination rate in bathrooms, measured at 1,599.035 CFU/m³, was considerably lower than the averages reported in Raisa Jabeen's (2,647 CFU/m³) and M. Stryjakowska-Sekulska's (2,100 CFU/m³) experiments. The discrepancy may be attributed to the fact that the bathroom in this study had been recently cleaned and experienced low foot

traffic at the time of sampling. Lastly, the cafeteria contamination rate averaged 1,284.46 CFU/m³, representing a moderate level. This result is higher than that reported in the Polish study (855 CFU/m³), but significantly lower than the 2,750 CFU/m³ recorded in the Indian study (Stryjakowska-Sekulska et al., 2007; Jabeen et al., 2023).

Nevertheless, it is important to consider that due to certain limitations, these experiments did not strictly follow the standard Index of Microbial Air Contamination (IMA), which requires exposing the Petri dish for 1 hour, placed 1 meter from the floor, and at least 1 meter away from walls or any obstacles (Pasquarella et al., 2000). This could have caused some discrepancies in the results, making it challenging to compare with other studies.

Despite these difficulties, it is evident that the contamination level in this type of environment is extremely high due to the large number of people and continuous human activity. In this regard, it can be observed that the average contamination level of the analyzed environments exceeds the contamination levels typically found in hospitals. The average result of this study was 1,467.96 CFU/m³, while a study conducted by researcher Predrag Ilić in Bosnia and Herzegovina demonstrated an average of 1,414 CFU/m³. This highlights the need for increased attention to contamination in schools (Ilić et al., 2018).

4.3 Impact of Building Design and Ventilation

One of the primary factors contributing to elevated microbial contamination in indoor environments is the physical structure of the building, particularly its ventilation systems (Hays et al., 1995). The widespread use of air conditioning and other climate control methods aimed at enhancing thermal comfort can inadvertently exacerbate microbial proliferation. This occurs because many air conditioning systems are isolated from outdoor air, leading to the recirculation of indoor air, which distributes microorganisms throughout occupied spaces via mechanical ventilation. These systems also promote microbial growth through the accumulation of particles and moisture in air conditioning coils, humidification systems, and other components. Moreover, improper maintenance of air conditioning systems is a common issue, often resulting in non-compliance with maintenance and control standards (PMOC) (Nunes, 2005; Gupta et al., 1982; Santos et al., National Academies of Sciences, Engineering, and Medicine. 2017, Daisey et al., 2003).

In this context, it is critical to reference the ASHRAE Standard 62-1999, which recommends a minimum ventilation rate of 8 l/s per person (15 cfm/person) for classrooms (ASHRAE, 1999; Daisey et al., 2003). Ventilation rates below 10 l/s per person pose significant health risks, as studies have shown a statistically significant increase in health complaints and reduced air quality in such conditions. It has been demonstrated that increasing ventilation rates to between 10 and 20 l/s per person results in a substantial reduction in symptoms associated with Sick Building Syndrome (SBS) (Seppänen, 1999). Nevertheless, it is evident that many schools worldwide fail to meet these ventilation standards. A comparative analysis of four studies revealed that fourteen out of twenty-one classrooms had ventilation rates below the 10 l/s per person benchmark set by ASHRAE (Daisey et al., 2003, Casey et al., 1995; Turk et al., 1987; Turk et al., 1989; Turk et al., 1993; Nielsen, 1984) .

Further supporting this, studies on carbon dioxide (CO2) levels have demonstrated that reducing indoor CO2 concentrations below 800 ppm can significantly lower the risk of Sick Building Syndrome (SBS) symptoms (Seppänen et al., 1999). This underscores the crucial importance of adequate ventilation in maintaining healthy air quality in schools. In this context, it is possible to compare these findings with the results obtained in this study, suggesting a high likelihood that inadequate ventilation contributed to the elevated contamination levels observed in the classrooms. In contrast, more efficient ventilation systems likely played a role in the relatively lower CFU/m³ levels recorded in areas such as the teachers' lounge and reception.

In addition to ventilation, other factors such as the materials used in construction, interior decoration, lighting, electrical sources, and air diffusers can also contribute to pathogen proliferation. These elements often create stagnant air pockets that are

inadequately ventilated, leading to a greater accumulation of microorganisms in the air and on surfaces (Nunes, 2005; Hays et al., 1995; National Academies of Sciences, Engineering, and Medicine, 2017) . In schools, this is particularly concerning due to the high density of occupants and the frequent contact with shared surfaces, such as doorknobs, handrails, faucets, remotes, keyboards, counters, light switches, and elevator buttons, which facilitate microbial transfer (National Academies of Sciences, Engineering, and Medicine, 2017).

The type of construction material used also significantly impacts microbial growth (Verdier et al., 2014). Research indicates that cellulose-based materials are more susceptible to contamination than inorganic materials such as gypsum, mortar, and concrete, as cellulose serves as a nutrient source for microorganisms (Hoang et al., 2010; Gutarowska, 2010). Additionally, porosity and surface roughness are critical factors; highly porous and rough materials provide reservoirs for nutrients and water, both of which are essential for microbial proliferation (Martinez et al., 2014). Dust infiltration and organic compounds from the external environment further contribute to nutrient availability, promoting microbial growth on surfaces such as walls (Hoang et al., 2010). Carbon-based materials, like carboxymethyl cellulose or emulsion paint, also provide an excellent nutrient source (Ayerst, 1969; Grant et al., 1989).

However, another important factor in microbial proliferation is pH. Most bacteria thrive in neutral pH environments, so construction materials with a pH between 6 and 8 are more prone to microbial colonization. Using more alkaline materials, such as cementitious substances, can inhibit microbial growth, especially when the materials are new. Over time, the carbonation process lowers the pH of these materials to around 9, creating conditions more favorable for microbial growth (Wiktor et al., 2009; Shirakawa et al., 2001; Wilimzig & Bock, 1994; Verdier et al., 2014).

In conclusion, addressing air quality and microbial contamination in indoor environments, particularly in schools, requires a comprehensive approach that includes a whole analysis of the building.

4.4 Health Implications

The health implications of microbial presence in school environments warrant careful consideration, as both pathogenic and opportunistic microorganisms pose risks to students and staff. Major routes of human exposure to airborne microorganisms and their metabolites include inhalation, ingestion, and dermal contact (Srikanth et al., 2008).

The current study, utilizing phenotypical and microscopic analysis, five distinct types of microorganisms were identified by genus, including *Staphylococcus* sp., *Micrococcus* sp., and three suspected species of *Bacillus*: *Streptomyces* sp., *Actinomyces* sp., and *Bacillus cereus.*

Staphylococcus species exhibit a varied pathogenic potential; some are considered non-pathogenic or of low virulence, while others can cause significant disease in immunocompromised individuals (Otto, 2020). These bacteria typically colonize the skin without causing harm; however, when introduced to the bloodstream or other tissues through breaks in the skin, they can lead to infections. Common manifestations include skin infections, respiratory tract infections, and foodborne illnesses. In the school setting, close physical contact among students can facilitate the transmission of skin infections such as boils or impetigo (CDC, 2024).

Micrococcus strains are widely distributed across various terrestrial and aquatic ecosystems, including soil, freshwater, marine environments, sand, and various vegetative surfaces. Notably, the skin of warm-blooded animals, including humans, serves as a primary reservoir for these strains, which frequently contaminate foods of animal origin (Nuñez, 2014). While *Micrococcus* is not typically classified as a pathogenic genus, there have been documented instances of these strains causing infections, primarily as opportunistic pathogens. They are generally regarded as contaminants derived from skin and mucous

membranes. However, clinical literature indicates that *Micrococcus* has been implicated in a range of infections, including bacteremia, endocarditis, ventriculitis, peritonitis, pneumonia, endophthalmitis, keratolysis, and septic arthritis (Von et al., 1996).

Numerous species within the *Bacillus* genus, particularly *Bacillus cereu*s, are acknowledged as opportunistic pathogens affecting both humans and livestock, while the majority are benign saprophytes (Kotiranta, 2000; Turnbull, 1996). While *Bacillus anthracis* is predominantly associated with anthrax, an expanding number of *Bacillus* species have been implicated in a range of infections, including abscesses, bacteremia, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmitis, osteomyelitis, peritonitis, as well as respiratory and urinary tract infections. These infections frequently present as secondary or mixed infections in immunocompromised individuals, such as those suffering from diabetes or alcoholism, though a significant proportion may also arise as primary infections in otherwise healthy individuals. The severity of these infections varies considerably, ranging from mild to potentially life-threatening (Turnbull, 1996).

According to the World Health Organization (WHO), indoor air pollution (IAP) accounts for approximately 3.8 million premature deaths annually (WHO, 2023). While contemporary environmental concerns predominantly focus on outdoor air quality, energy consumption, and sustainable infrastructure, indoor air quality often remains overlooked. Despite this, the majority of health conditions resulting from environmental exposures are linked to indoor environments (Sundell, 2004). Therefore, addressing the health implications of indoor air pollution is critical, especially in controlled environments such as schools, where proper maintenance and monitoring of air quality are essential for ensuring the well-being of occupants.

4.5 Limitations of the Study

Although the study was carefully conducted, some limitations were found among the many topics of the project. To analyze daily and seasonal variations in microbial populations, the guidelines suggest collecting samples from different periods so that the data can be compared and interpreted, and considering the temporal limitations, the current study was carried out only once. Because of the short-term data not all kinds of variation could be registered and captured. (Patoja et al., 2007).

While useful, passive sampling may not capture all types of airborne microbes or peak concentrations during high activity periods. It's important to mention that these kinds of samples do not produce precisely quantitative and qualitative results making it harder to analyze the concentration and variety of organisms.

Even though the Petri dishes capture the microorganisms in the air, other kinds of bacteria might be present in the environment but are not detected due to the limitations of passive sampling. (Nunes et al., 2005).

The analysis method was developed in two colonies from each place; however, many other colonies could be included in the study. Due to the lack of time, the smearing and the gram staining techniques could not be done in all the bacterial colonies, so not all microorganisms could be differentiated between gram-negative and gram-positive species.

Another limitation of the study was that our laboratory was not equipped with a DNA sequencing machine, which prevented the study from identifying the collected samples on a species level, thus narrowing the possibility of identification to solely rely on morphological analysis followed by differentiation between gram-positive or negative of certain samples. However, comparative gene sequence analysis of ribosomal DNA (rDNA) is now considered the most accurate method for identifying microorganisms. Research has indicated that sequencing the 16S rRNA gene is valuable for both discovering and identifying pathogens (Petrosino et al., 2009).

4.6 Strengths of the Study

Numerous positive aspects of the study contributed to the development of consistent results. Firstly, sample collection from six different areas of the school enhanced the accuracy, and consistency of the results by providing an overview of the

microbial distribution and enabling a comparison of microbial air quality across locations (Cunha et al., 2013). Besides that, the presence of a well-equipped laboratory at the school facilitated the application of smearing and gram-staining methods for sample analysis under the microscope and the phenotypical characterization of bacteria (Smith & Hussey, 2005). Furthermore, the involvement of various professionals in the field supported the researchers in identifying different bacteria, conducting colony-forming unit (CFU) counts, and addressing any uncertainties encountered during the experiment.

4.7 Recommendations for Improving Air Quality and Hygiene

The school's climate-control system, which is designed to cool the environment, is also characterized by the circulation of untreated indoor air along the external air entering through windows and doors (Morais et al., 2010). This system creates conditions conducive to microbial growth (Lacerda et al., 2003). Considering this, certain improper practices should be avoided to prevent air contamination. The recommended protocol involves activating the air-conditioning system several hours before occupants arrive and deactivating it immediately after their departure, to minimize the deterioration of air quality (Seelig et al., 2004). It is also important to note that regular maintenance of the air-conditioning equipment is essential for minimizing the risks associated with air contamination (Cartaxo et al., 2007).

4.8 Future Research Directions

To guide future research, it is recommended that sampling be conducted according to the IMA protocol, which specifies placing Petri dishes 1 meter from walls and the floor and exposing them to the environment for 1 hour. Additionally, sampling should occur at various times throughout the day, both in the presence and absence of students and staff, and before and after cleaning procedures.

Another valuable approach would involve collecting samples at different times of the year, encompassing both dry and rainy seasons, to observe how fluctuations in temperature and humidity influence the indoor and outdoor microbiomes of schools. Ideally, all colony-forming units (CFUs) observed on the plates should be transferred to slides, subjected to Gram staining, and examined microscopically. Subsequent DNA sequencing would enable precise species identification.

Given that air quality assessments also consider factors such as fungal spores and other pollutants, protocols addressing these contaminants should be incorporated to provide a more comprehensive air quality analysis. Furthermore, comparative studies across different schools and educational institutions could examine the influence of building structures and ages on air quality outcomes.

5. Final Considerations

In conclusion, it is critical to recognize that air quality significantly influences hygiene and public health. Notably, within educational settings such as schools, an increasing body of research suggests that indoor air quality may also affect teacher productivity and student learning outcomes (National Academies of Sciences, Engineering, and Medicine, 2017). Consequently, indoor and outdoor air quality in schools and other educational institutions must be consistently monitored, with the establishment of clear regulatory standards to ensure healthy environments (Naruka & Gaur, 2013). The current study revealed microbial concentrations exceeding the expected averages in prior research. Thus, implementing measures to reduce microbial air contamination is essential, including regular maintenance of climate-control systems. The adoption of more effective cleaning products, and the promotion of hygiene awareness among individuals frequenting school facilities. Ultimately, safeguarding environmental air quality by preventing microbial pollution is crucial for the well-being of all occupants in educational settings.

It is crucial to conduct further studies on microbial contamination in the air to raise awareness of this important issue and to disseminate the knowledge, techniques, and standards necessary for air quality analysis to become more accessible and reliable. Moreover, investigating air quality not only helps assess indoor contamination levels but also aids in the implementation of corrective measures to improve it. This research is important for environments, such as schools, which cater to younger individuals in critical stages of, physical, immunological, and psychological development. Given the long-term health implications, schools should be a primary focus of air quality research.

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