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# Characterization of fungal bioaerosols in indoor spaces: in a Higher Education Institution (HES) in Cartagena de Indias, Colombian Caribbean

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***Abstract*– Bioaerosols are airborne particles or microfragments that contain a variety of microorganisms. In closed environments these can affect the health of humans, especially, fungal bioaerosols can generate allergies, infections or respiratory issues. In this study, airborne fungi were identified and quantified in closed environments inside a Higher Education Institution (IES) in the city of Cartagena de Indias in the Colombian Caribbean during 2023. 452 samples were collected using the gravitational deposition technique. The results showed that the predominant genera and species were Mucor sp (40%), Cladosporium sp (25%) and Penicillum sp (19%); followed by Asperillus niger (7%), Microsporum sp (5%), finally, with a percentage of occurrence equal to or less than 1%, Alternaria sp, Aspergillus flavus, Aspergillus fumigatus, Paecilomyces sp and Rhodotorula sp were identified. It is concluded that it is important to carry out an evaluation of the microbiological quality of indoor air, especially the characterization of potentially pathogenic fungi, which can affect the health of workers; who spend a large part of their day exposed to the air in the indoor environments of the higher education institution under study.***

***Keywords*-- Air quality, Closed environments, microorganisms.**

## I. INTRODUCTION

Bioaerosols are particles of biological origin and large molecules (0.1 to 100 µm) that carry live or dead micro-organisms (bacteria, viruses, fungi, and pollen) [1] These can be of natural origin in soil, water, plants, and animals, among others, or anthropogenic, such as waste management, industries, food processing, agriculture, and livestock [1], [2] Fungal spores represent a frequent component among these aerosols, being able to disperse in the surrounding environment due to the aerosolization process and being able to enter the human respiratory tract due to their size of between 2 and 10 µm [3], [4].

Therefore, the presence of fungal bioaerosols in indoor environments is of particular interest due to their significant impacts on public health, such as asthma, rhinitis, infectious diseases, acute toxic reactions, allergies, and cancer [5]. Its main emission sources include the transport of outdoor bioaerosols indoors, building materials, furniture, animals, plants, organic waste, and human occupants (sneezing, coughing, talking, among others) [6]. Indoors, their presence and development are conditioned by temperature, moisture content, as well as the presence of suitable growing surfaces [4].

Through the numerous studies that have been carried out worldwide to determine the sporulated fungi that could be a source of allergens in the environment, about 180 fungal allergens that induce IgE-mediated hypersensitivity in atopic patients have been identified. Similarly, between 20-30% of allergies are attributed to fungal spores, of which 44% are atopic and 80% are asthmatic [4]. Several institutions at the international level have suggested standards for acceptable concentration of indoor bioaerosols, the World Health Organization (WHO) recommended a maximum of 500 colony-forming units (CFU)/$m^3$ , to minimize health damage caused by exposure to these [5], [7].

At the national level, different studies have been conducted on the presence of various bioaerosols in indoor spaces in places such as administrative buildings where the predominant species was *Aspergillus* sp detected in 77.2% of the samples in building one, 91% in building two and 100% in building three [8]. The presence of the genera *Candida* sp and *Aspergillus* sp, known to cause serious infections especially in patients with weakened immune systems, was observed in the neonatal intensive care units [9] and in Higher Education Institutions with concentrations of up to 1197 CFU/$m^3$ of *Aspergillus* sp and *Curvularia* sp [10].

In Cartagena de Indias, limited studies are conducted to determine the influence of fungal bioaerosols on air quality and public health [11] Studies on indoor PBAs in schools, and universities are important because of the hours of exposure of students, teachers and administrative staff (children spend 25% of their time in school, and university students can spend more than 12 h per day) [2].

To date, studies on the incidence of indoor air quality of fungal bioaerosols in indoor environments and their significant effects on human health and well-being are limited [12]. This study aims to identify and quantify airborne fungi in indoor

environments within a higher education institution in Cartagena de Indias, Colombia. Sampling was carried out using the gravitational deposition technique during 2023, covering the main climatic epochs. It identified 11 species of sporulated fungi present in the indoor environments of the institution and their effects on air quality and local public health.

## II. MATERIALS AND METHODS

### a. Sampling site

Cartagena de Indias is the tourist district and capital of the department of Bolivar, Colombia. It is in the southwest of the Colombian Caribbean, with an average humidity of 90% and an average temperature of 31.5 °C [1], [2]. It has a semi-arid tropical climate regime and is under the influence of the meteorological phenomena of trade winds, easterly waves, and cold fronts tributary to the northern hemisphere and intertropical convergence zone [13]. Based on these main phenomena, the climate in Cartagena can be divided into (4) main stages: major dry season (December/April); minor rainy season (May/June); minor dry season, also known as the summer of San Juan (July/August), and the major rainy season (September-November) [13].

The sampling was carried out inside an HEI located in the south of the city (10° 22′ 13.6″ N-75° 27′ 55.5″ W), which can be detailed in Fig. 1. In this industrial sector, activities count with the presence of metallurgical companies and the storage of different materials and supplies.

For this study, nine (9) internal points in the HEI were monitored (See Table 1); these correspond to closed places such as the institutional library and classrooms; in addition to offices with individual cubicles. Samples were taken in triplicate for each sampling site during the year 2023.

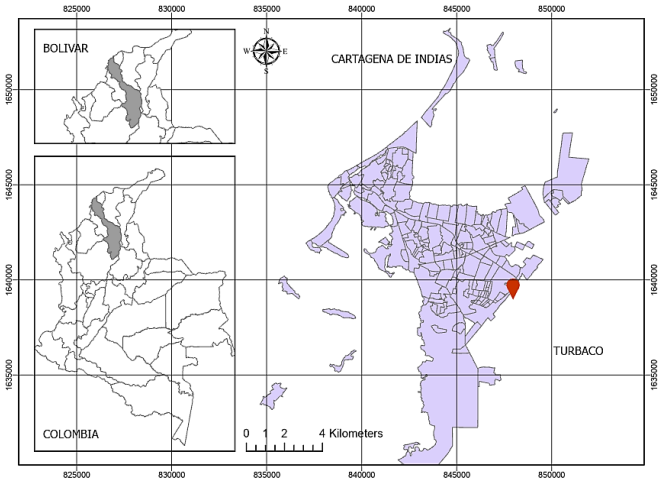



Fig. 1 Location of the sampling site in a HEI in Cartagena de Indias, Colombian Caribbean.

Table 1. Indoor sampling points in the HEI under study.

| Sampling point | Offices | # of replicas |
|---|---|---|
| A | Basic Sciences | 4 |
| B | Library | 3 |
| C | UTB Lab | 1 |
| D | Engineering | 1 |
| E | Human resources | 2 |
| F | Rectory | 1 |
| G | Budget | 1 |
| H | EXDA | 1 |
| I | Planning | 1 |
| | Total | 15 |

### b. Method

Sampling was performed using the gravitational deposition technique, in this technique, airborne particles are deposited on a flat surface or a collection medium due to the action of gravity, as the surrounding air flow rate decreases and the particle size increases, gravitational deposition increases [14].

These airborne particles manage to adhere to any surface they come in contact with thanks to adhesive forces such as Van der Waals, electrostatic or surface tension [15]. For aerosol collection, sterile petri dishes with fungal growth medium are opened at a height of 1.5 m above the ground, which is the representative measurement of the human breathing zone, and are kept away from walls and obstacles [16].

Samples (452) were taken during seven months and strategically selected to cover the climatic regime of the city. Samples were collected in triplicate, at each sampling point (see Table 2); during 12 hours from the evening to the following morning, starting the sampling at the end of the standard working day (17h00) and ending the following day (7h00). Petri dishes with Sabouraud dextrose agar, which is specific for fungal growth, were used [17]. The fungi were grown at room temperature (25°C) in a closed environment with little incidence of sunlight, for a period of 5 to 7 days [16].

### c. Quantification and identification of bio-aerosols

The identification of the genus for each isolated fungus was performed based on its macroscopic characteristics (Color, texture, and shape) [1], [2].

### d. Statistical analysis of samples

For the statistical analysis of the data, the 2016 version of the Microsoft Excel tool was used [16]. These were complemented with graphs elaborated with Statgraphics software in its 2019 version [2].

## III. RESULTS

### a. Identification of fungi

Ten (10) different species of fungi were identified using the description of their morphology (texture, shape, and color), as well as their family, phylum, genus, and species presented by Huertas et al. 2018 & Rubiano-Labrador et al. 2022 (Table 2). Table 3 shows the fungi isolated from the sampling points of the HEI and their possible effects on public health.

**Table 2.** Phenotypic characterization of the isolated fungi.

| Cd | Filo | Family | Genre | Related species |
|---|---|---|---|---|
| **F1** | *Ascomycota* | *Trichocomaceae* | *Aspergillus* | *Aspergillus niger* sp |
| **F2** | *Ascomycota* | *Pleosporaceae* | *Alternaria* | *Alternaria* sp |
| **F3** | *Ascomycota* | *Trichocomaceae* | *Penicillum* | *Penicillum* sp |
| **F4** | *Ascomycota* | *Trichocomaceae* | *Aspergillus* | *Aspergillus flavus* |
| **F5** | *Ascomycota* | *Trichocomaceae* | *Aspergillus* | *Aspergillus fumigatus* |
| **F6** | *Mucoromycota* | *Mucoraceae* | *Mucor* | *Mucor* sp |
| ***F7*** | *Ascomycota* | *Arthrodermataceae* | *Microsporum* | *Microsporum* sp |
| **F8** | *Ascomycota* | *Davidiellaceae* | *Cladosporium* | *Cladosporium* sp |
| **F9** | *Ascomycota* | *Eurotiomycetes* | *Paecilomyces* | *Paecilomyces* sp |
| **F10** | *Basidiomycota* | *Sporidiobolaceae* | *Rhodotorula* | *Rhodotorula* sp |

### b. Quantification of the identified bioaerosols

The frequency of appearance of colonies throughout the study was considerably variable (See Fig. 2), the month with the highest number of fungal bioaerosols with 178 colonies was August, while November was the month with the lowest number of colonies found with a total of 36. *Mucor* sp stood out as the predominant species with 40% of total occurrence, followed by *Cladosporium* sp, with 25%, and *Penicillium* sp with 19%, while less common species included *Aspergillus flavus* sp, *Alternaria* sp, *Paecilomyces* sp, *Rhodotorula* sp *and Aspergillus fumigatus*, with about 1%. The colony-forming units are shown in Fig. 3.

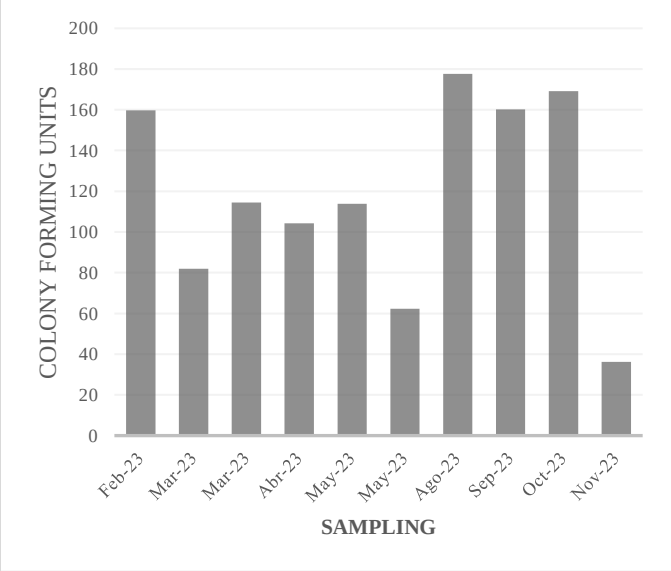



**Fig. 2.** Total colony forming units identified during the sampling time.

*Mucor sp* and *Microsporium* sp, were the most frequent species in the different sampling points of the HEI indoor environments, followed by *Aspergillus Niger* sp and *Cladosporium sp* are shown in Fig. 4.

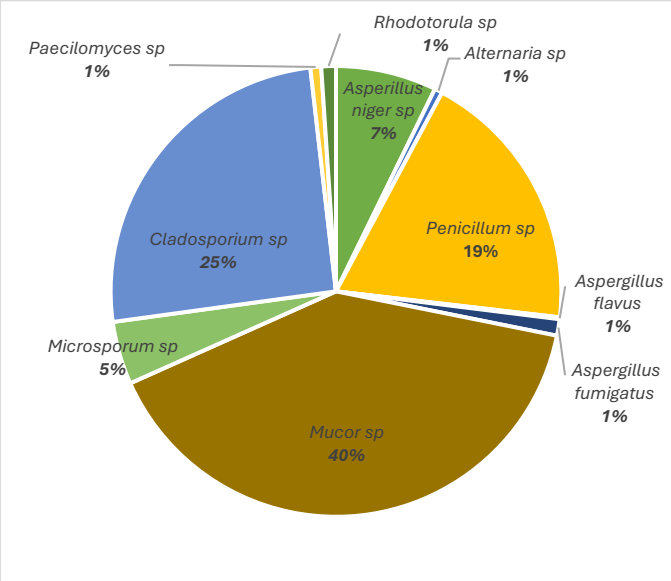



**Fig. 3** Percentage of genera and species of fungi in the different sampling points of the HEI.

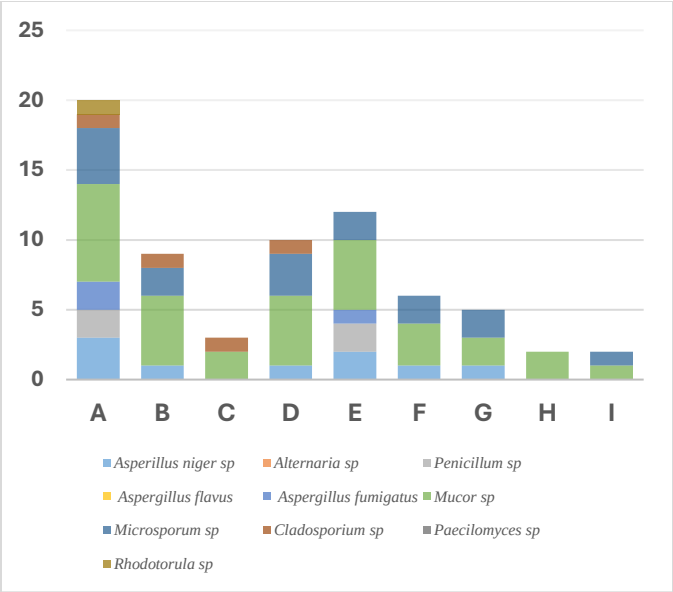



**Fig. 4** Frequency of fungal species identified in the sampling sites.

### c. Number of fungal sporulates identified by time of year

In the major dry season, there was a greater presence of fungal bioaerosol colonies, followed by the major rainy season, while in the minor dry and major rainy seasons only half of the colonies appeared (Fig. 5). *Mucor sp*, the predominant species of the study, appears mostly in the major rainy season and disappears in the minor rainy season, *Cladosporium* sp, predominates in the major dry season as well as *Penicillium* sp, are shown in Fig. 6.

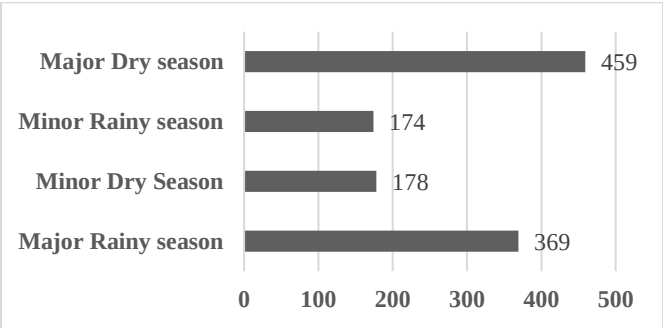



**Fig. 5** Total number of colony-forming units at different times of the year.

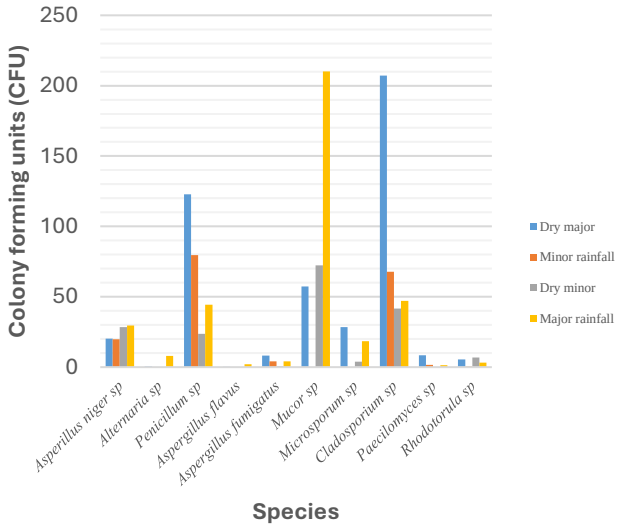



**Fig. 6** Frequency of mushroom species and genera at different times of the year.

### d. Comparison of the presence of bioaerosols between different offices at the same sampling point in the HEI.

A comparison of the frequency of appearance of the colonies in different offices at the same sampling point was made to indicate the predominance of the species, and to determine if there are significant differences between them.

In the offices of the Basic Sciences faculty of the HEI, the results do not show significant differences, since a distribution of 49% versus 51% was obtained in the two offices analyzed (Fig. 7). *Penicillium* sp is the predominant species in almost all points, followed by *Cladosporium* sp, *Mucor* sp is positioned as the third most abundant species in these offices, despite being the predominant in the overall analysis are shown in Fig. 8.

The situation was different in the library floors, the second library floor obtained the highest number of colonies in the whole sampling, while the first one had the lowest, but no considerable differences are observed, Fig. 9. In contrast to the previous one, *Cladosporium* sp predominates in the library, followed by *Penicillium sp.* But *Mucor* sp also occupies third place, are shown in Fig. 10.

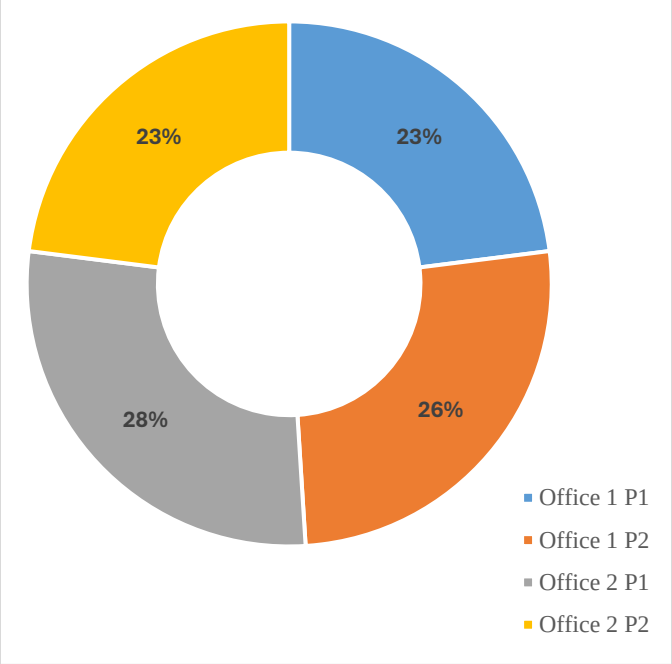



**Fig. 7** Percentage of species occurrence in offices of the Basic Sciences faculty of the HEI studied.

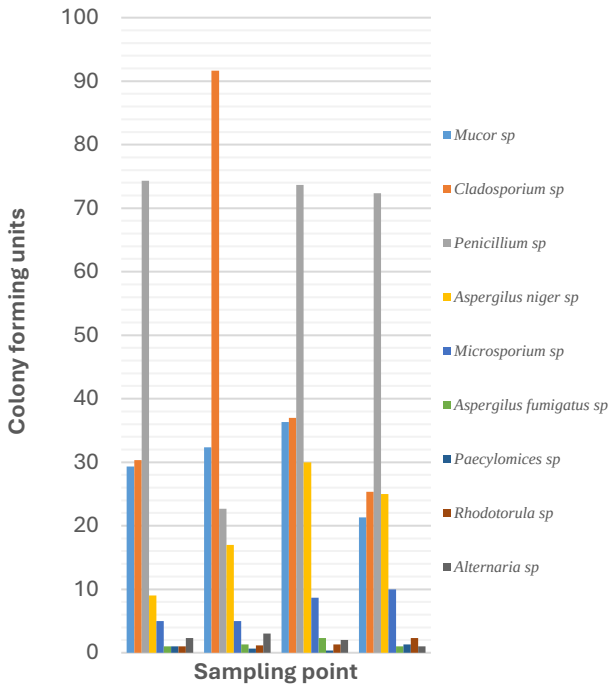



**Fig. 8** Species identified in the different sampling points, from the offices of the Faculty of Basic Sciences.

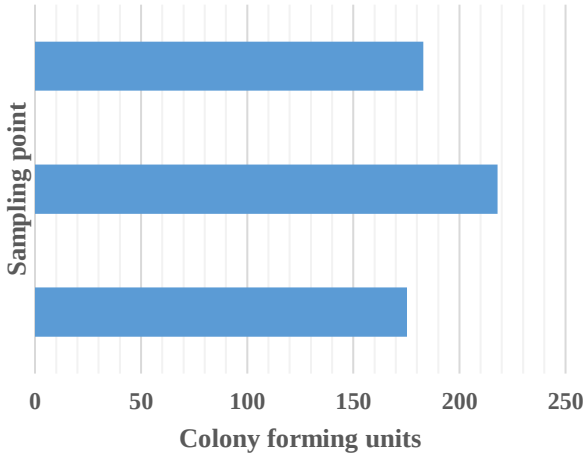



**Fig. 9** Total fungi isolated in the different floors of the library at HEI

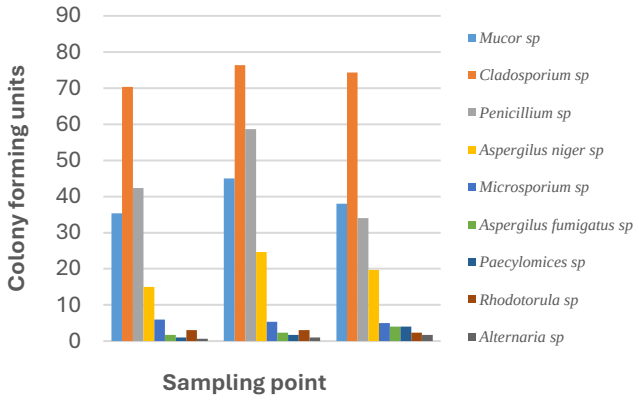


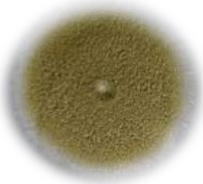

**Fig. 10** Frequency of species and genera of fungi isolated in the different floors of the HEI Library.

e. **Potential public health effects of identified fungal aerosols.**

The fungi species identified have public health effects after prolonged exposure to their spores, ranging from mild reactions such as sneezing and allergies to serious diseases such as dermatitis and allergic rhinitis. Table 3 shows the characterization of the identified species, the potential health effects, and possible mitigation strategies that HEI could apply to minimize the effects after prolonged exposure.

**Table 3** Characterization of the genera and species identified in the different sampling points and possible effects on human health.

| Species | % of occurrence | Photo colonies | Associated diseases | Mitigation strategy |
|---|---|---|---|---|
| *Aspergillus niger* | High |  | Aspergillosis: allergic reaction, allergic rhinitis, possible pneumonias. | 1. Weekly cleaning of floors, walls, and ceilings with a solution of hypochlorite, vinegar, and quaternary ammonium.<br><br>2. Weekend cleaning of surfaces with quaternary ammonium (without the presence of employees).<br><br>3. Identification and remediation of leaks in the building structure to prevent fungus from growing again.<br><br>4. Moisture control associated with roof or façade leaks, plumbing leaks, flooding, condensation, and high relative humidity.<br><br>5. Cleaning of ventilation ducts and air conditioners on a quarterly basis to lower the fungal load. Use of humidifiers.<br><br>**Recommendations to cleaning and general services personnel:**<br><br>1. Use of respiratory protection (disposable respirator N95).<br><br>2. Use of gloves and safety glasses for eye protection.<br><br>3. The work area must be unoccupied when cleaning is performed.<br><br>4. Cover the floor, exit ways and items in the work area with plastic sheeting, and seal them before starting the repair.<br><br>5. You should try to reduce dust generation.<br>6. Improve ventilation of closed environments.<br><br>7. Improve ventilation of closed environments- (h) Improve |
| *Alternaria* sp | Moderate |  | Chronic rhinosinusitis is associated with | |
| *Penicillum* sp | Moderate |  | Asthma, allergies: nasal congestion<br>Runny nose<br>Sneezing<br>Irritated and watery eyes<br>Cough<br>Itchy eyes, nose and throat<br>Dry and scaly skin | |
| *Aspergillus flavus* | High |  | Aspergillosis: allergic reaction, allergic rhinitis. | |
| *Aspergillus fumigatus* | High |  | Aspergillosis: allergic reaction, allergic rhinitis. | |
| *Mucor* sp | High |  | Allergic rhinitis | |
| *Microsporum* sp | Moderate |  | Dermatitis | |

| | | | | |
|---|---|---|---|---|
| *Cladosporium* sp | Moderate |  | Skin and respiratory infections. | ventilation of closed environments.<br><br>8. Some methods to consider for daily cleaning of floors and surfaces include a solution of soap or detergent, with diluted vinegar for the removal of fungal spores.<br><br>9. Use of vacuum cleaners or humidifiers to improve indoor air quality.<br><br>10. All areas should be dry and visibly free of mold, dust, and debris. |
| *Paecilomyces* sp | Moderate |  | Skin infections, eyeball infections, sinusitis. | |
| *Rhodotorula* sp | Moderate |  | Allergies and dermatitis. | |

## IV. DISCUSSION

Studies conducted on the presence of fungal bioaerosols outdoors in Cartagena de Indias presented the phyla *Ascomycota (*83%) and *Basidiomycota* (17%) as the predominant ones in marine environments, with the dominant fungal species being *Aspergillus* sp [1] .These findings were corroborated by the study conducted in the exteriors of an HEI in Cartagena, where the dominant species were *Aspergillus* sp (60.8%) and *Penicillium* sp (24.5%) [2].

The presence of fungal bio-aerosols in interiors of coastal areas does not seem to differ from the exterior, an investigation carried out in the interiors of a university library in Santa Marta found seven (7) genera of fungi *Aspergillus* and *Curvularia the* most abundant ones [10]. It is important to remember that the variation in the presence of bio-aerosols in indoor areas is due to the exchange between indoor and outdoor air since fungi have as a common access route to the buildings the outdoor air that is extracted by ventilation and air conditioning systems, and doors or windows [18].

Bioaerosols inside an insulated room enter mainly from outside air [19]. Some components of air conditioning and ventilation systems become ideal substrates.

for the growth of fungal colonies, whose spores spread indoors with the airflow causing indoor pollution [2].

The species *Mucor sp*, has great colonization potential due to its ease of transport in humid air and high capacity to adhere to surfaces, making it a common species in environments with temperatures of 20 to 25°C and relative humidity greater than 90%, in addition some of its species are thermotolerant, which makes it more abundant [20].

On the other hand, *Cladosporium* sp, *Aspergillus* sp and *Penicillium* sp are considered predominant and common genera in indoor and outdoor environmental conditions [19], [21].

*Cladosporium* sp is known as a cellulose degrading fungus, therefore, it could feed on paper and other materials [4] thus increasing its reproduction in the library and offices. Its spores are abundant and easily transported through the air in temperate zones [2].

Although several studies have found a greater presence of *Aspergillus* sp than *Penicillium* sp [22], in this research *Penicillium* sp, a genus quite common in indoor air and is deposited on food, wood, paper and cardboard materials, predominated [23].

## V. CONCLUSION

This study characterized fungal bioaerosols in the interiors of a HEI in Cartagena de Indias, Colombian Caribbean. The results show that the predominant species during the sampling period was *Mucor* sp, being the most frequent in the different sampling points with 40% of total occurrence, followed by *Cladosporium* sp, with 25%, and *Penicillium* sp, with 19%. These differ from the results obtained in previous studies where isolates belonging to the *Ascomycota* phyla were more frequent.

The presence of sporulated fungi increased during the dry season from December to April, which is characterized by strong northeast trade winds from the northeast, little or no rainfall, and high temperatures. Comparing the results obtained between the offices of the same sampling point, no significant differences were found.

The public health effects of these fungi can vary, having mild effects such as allergies and evolving into epidermal and respiratory tract diseases. This study considers the presence of bioaerosols in enclosed spaces as an important measure in monitoring air quality and the health and well-being of people exposed to their spores for prolonged periods.

## ACKNOWLEDGMENT

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