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# Concentrations of Airborne Cultivable Fungi at a Higher Education Institution in Cartagena de Indias, Colombian Caribbean

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## Abstract

Bioaerosols are as small particles suspended in air that contain several microorganisms (bacteria, fungi, and viruses). These particles are studied because of the possible impacts that they have on the health of humans and animals in open and closed spaces. In this study, the presence of airborne fungi presents in a Higher Education Institution (HEI) of the city of Cartagena de Indias, Colombian Caribbean was evaluated. Over 4 months (June–October 2018), 128 fungal samples were collected using a two-stage cascade impactor, and the fungi present in these samples were counted, characterized, and identified. Bioaerosol concentrations were correlated with meteorological data from the Rafael Núñez Airport meteorological station to establish the possible influence of these factors on the presence of bioaerosols. The results obtained showed an average concentration of fungal aerosols of  $123.71 \pm 17.97$  UFC/m<sup>3</sup>, with the highest proportion occurring in the morning. The predominant genera were *Aspergillus* (60.8%), followed by *Penicillium* (24.5%) and *Fusarium* (9.3%). According to the correlations obtained from a multivariate analysis, the meteorological parameters did not influence the presence of microorganisms at the HEI studied; however, a possible influence of emission sources such as vegetation and industry is suggested.

**Keywords** Air quality · Bioaerosols · External environments · Meteorological factors · Microorganisms

## 1 Introduction

Bioaerosols are defined as very small particles of biological origin that are found in the air, with sizes ranging from 0.001 to 100  $\mu\text{m}$  (Kim et al. 2018). These particles can be made up of live or dead microorganisms (Douwes et al. 2017), such as bacteria, viruses, fungi, and, pollen, and their metabolic products, and even insect remains (Hurtado et al. 2014). Bioaerosols are derived from natural sources such as plants, animals, people, soil, and water (Heo et al. 2014) and from anthropogenic sources such as industries, solid and liquid waste management, agriculture, livestock, and food processing (Humbal et al. 2018).

In particular, fungi are abundant and ubiquitous in nature, representing 30% of all atmospheric aerosols (El-Morsy 2006; Fröhlich-Nowoisky et al. 2016). In some cases, they can be found in high concentrations due to particular

characteristics of the atmosphere (Douwes et al. 2017), such as meteorological conditions, because they influence the processes of aerosolization, transport, and deposition of aerosols (Velez-Pereira 2017). Particularly, temperature and humidity can have the greatest influence on the presence of fungal bioaerosols because they intervene in the biological activity and emission of these microorganisms (Almaguer et al. 2014; Dedesko et al. 2015; Fröhlich-Nowoisky et al. 2016). In addition, these variables can affect hydrological processes because they contribute significantly to the formation of ice cores and the condensation of clouds (Huertas et al. 2018; Hurtado et al. 2014).

The concentrations of fungal bioaerosols in the air can affect human, plant, and animal health because they cause diseases and other adverse effects (Fayad et al. 2021; Fröhlich-Nowoisky et al. 2016; Nazaroff 2016). Some species of bioaerosols are considered pathogens, as they are associated with infectious, allergic, and respiratory diseases, in addition to causing the generation of acute toxic, neurological and even cancer effects (Douwes et al. 2003). Therefore, in recent years, research has been conducted to identify fungal bioaerosols and their potential impact on biota (Douwes et al. 2017; Fröhlich-Nowoisky et al. 2016). Most of these

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studies have been conducted in indoor environments such as offices (Bręgoszewska et al. 2018; Gołofit-Szymczak and Górny 2018; Yu et al. 2018), schools (Bręgoszewska et al. 2018; Yen et al. 2020), and hospitals (Mousavi et al. 2019; Tolabi et al. 2019). Likewise, studies have been carried out in outdoor environments such as coastal areas (Huertas et al. 2018), urban centers (Liu et al. 2019; Ruíz-Fonseca and Rubiano-Labrador 2021) and open spaces in educational centers (Rodríguez-Gomez et al. 2020).

In Colombia, studies have been carried out in intramural environments such as hospitals and universities in Santa Marta and Barranquilla (Caicedo et al. 2015; Medina and De la Hoz 2018; Suarez et al. 2017; Velez-Pereira and Caicedo 2014). In the case of fungal aerosols in extramural environments, despite the relevance of their study for public health and agriculture (Ruiz-Gil et al. 2020), there have been few studies in the country, and to date, most studies have focused on landfills (Morgado 2017). In the city of Cartagena de Indias only two studies have been reported; the first sought to determine the sensitization to allergens in a local emergency room (Caraballo et al. 1998), while the second quantified and characterized the biological particulate material at the Blas El Teso beach (Huertas et al. 2018). In the case of extramural environments with a high influx of people such as universities or colleges, to date there are no studies in the city of Cartagena de Indias on their exposure to bioaerosols. Studies in this type of environment are necessary because they are important facilities associated with the environmental exposure of students, teachers and administrative staff, and where they spend a large part of their time during the week (children spend 25% of their time in school, and university students can spend more than 12 h a day) (Jo and Seo 2005; Oliveira et al. 2009). Considering the limited information that exists on fungal bioaerosols in outdoor environments in the city of Cartagena de Indias, the objectives of our study were: (1) to determine the concentration of airborne fungi present in a higher education institution (HEI), (2) to characterize the airborne cultivable fungi present in the HEI, and (3) to determine the influence of meteorological conditions on the concentrations of isolated airborne fungi.

## 2 Materials and Methods

### 2.1 Sampling Site

This study was conducted in the city of Cartagena de Indias, Colombia. This city is located in the center of the Colombian Caribbean coast, and is characterized by a semi-arid tropical climate with an average humidity of 90% and an average temperature of 31.5 °C. The climate changes several times during the year: a dry season or summer (August–November),

a rainy or winter season (December–March) and a transition season (April–July) (CIOH 2010).

The sampling site was located on the roof of an academic building on the campus of a HEI (10° 22' 13.6" N–75° 27' 55.5" W), which is located south of the city in the Carlos Vélez Pombo Industrial and Technological Park. In this industrial park, there are storage warehouses and companies related to the supply of materials and services related to the energy industry. In addition, it is characterized by a high presence of vegetation and green areas. The building selected as the sampling point was located in the most vegetated area of the institution. The locations of the sampling sites are shown in Fig. 1. This location was selected because of its accessibility and favorable wind current conditions.

The sampling instruments were located at an approximate height of 1.5 m, which corresponds to the average height of human respiration (Camargo et al. 2011). In this study, a systematic demonstration was used, since the samples were taken in the same period of time during the 16 sampling campaigns. The bioaerosol sampling was carried out in duplicate once a week between June and October 2018 (16 sampling campaigns), and the samples were collected in the morning (9h00–10h00) and afternoon (13h00–16h00). During the sampling period, 128 samples were collected. The methodology proposed by Huertas et al. (2018), who have carried out studies in outdoor environments such as coastal areas. The meteorological data (temperature, precipitation, wind speed and humidity) were obtained from the platform of the Institute of Hydrology, Meteorology and Environmental Studies (IDEAM), which correspond to the main synoptic station located at the Rafael Núñez Airport.

### 2.2 Sampling Methods

Bioaerosol sampling was performed using a two-stage viable Andersen cascade impactor with a flow rate of 28.3 L/min. This device has a design that allows it to simulate the functioning of the human respiratory system, sucking in the air from the environment, and allowing the impaction of the particles in each of stage. Each stage was designed to capture a range of particles with a defined aerodynamic size range. In stage 1, particles with a diameter greater than 7 µm were captured, representing the area of the nasal cavity of the human respiratory system; in stage 6, particles with a diameter of approximately 2 µm were captured, which could enter the area of the primary bronchi or alveoli of the respiratory system (Huertas et al. 2018).

Previously, a pre-sampling test was carried out to determine the appropriate time to collect the samples, considering: (i) the growth of microorganisms without overlapping colonies and (ii) the appropriate time to identify the growth of microorganisms in the laboratory. The optimal sampling time was 8 min. Cultivable fungi samples were collected

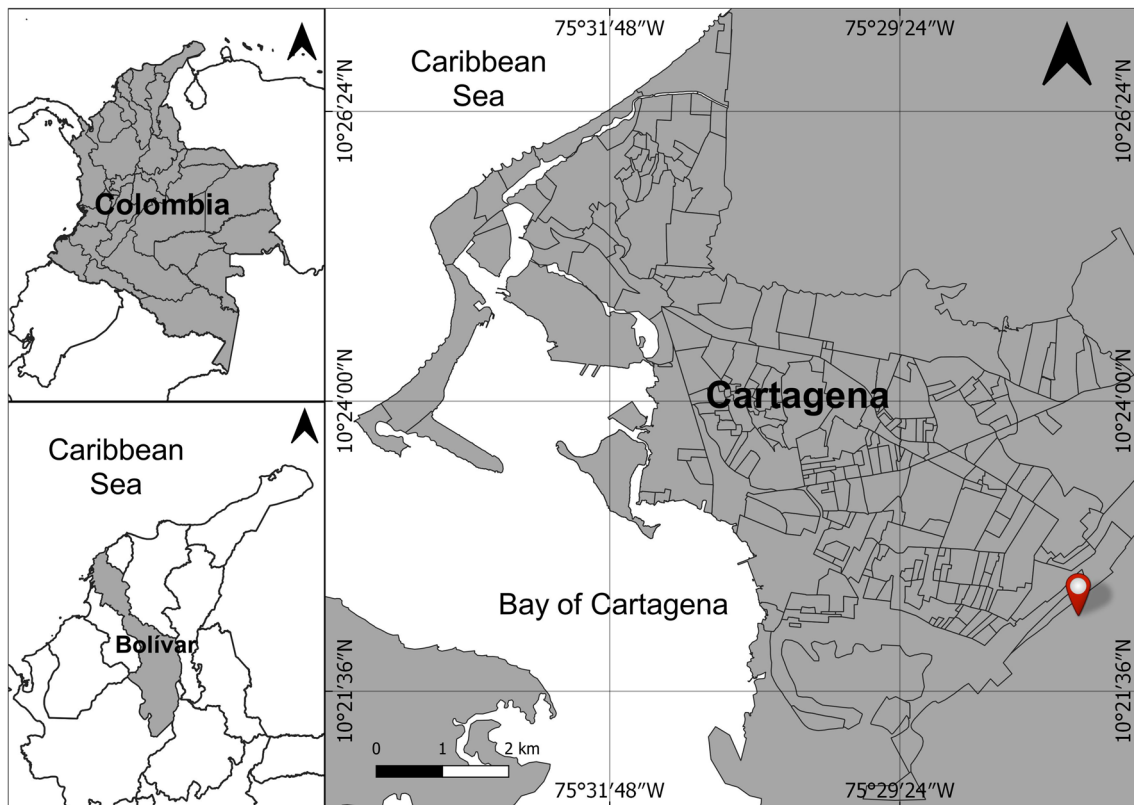


Fig. 1 Location of the sampling site (HEI) in Cartagena de Indias

in 9.0 cm Petri dishes containing Sabouraud agar and were incubated at 26 °C for 8 days.

### 2.3 Counting and Identifying Fungal Bioaerosols

The fungal genera were identified based on their macro (color, texture and shape) and microscopic (hyphae, conidophores and ascospores) characteristics using standard taxonomic keys (Koneman 2008; Vos et al. 2009). The colony-forming units (CFU) were counted according to the formula proposed by Caicedo et al. (2015):

$$\frac{\text{CFU}}{\text{m}^3} = \frac{\text{Colony forming units}}{\text{Air volume}},$$

where the air volume is calculated as the product of the pumping flow rate and sampling duration.

### 2.4 Statistical Analyses

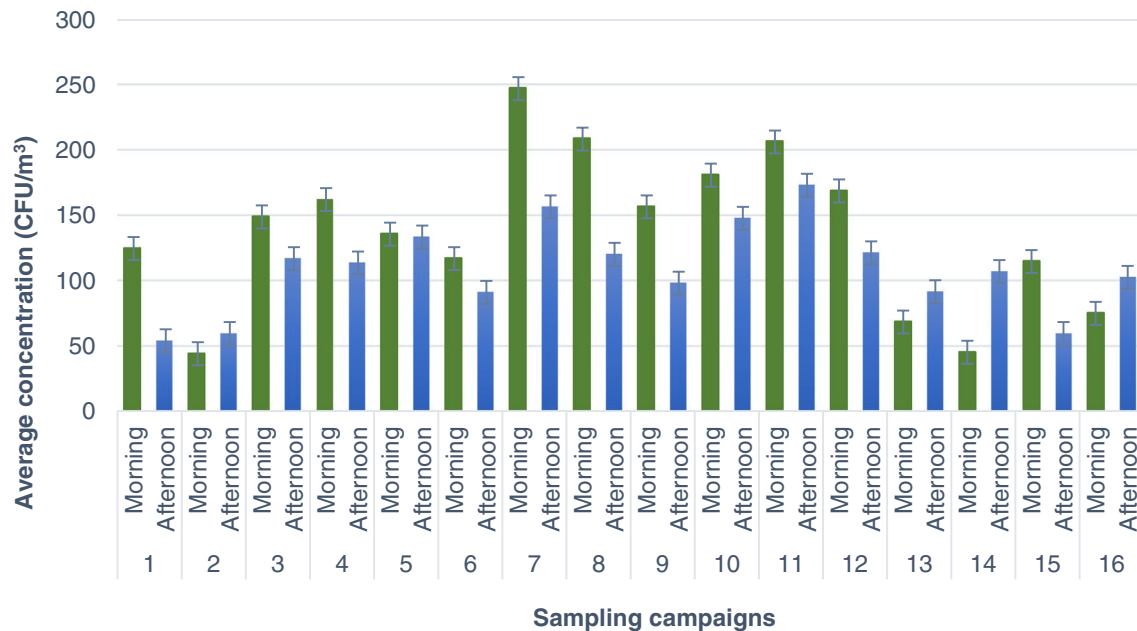
In the present work, statistical analyses were performed using the Statgraphics software (version 16). The fungal concentrations were evaluated at 95% confidence intervals. Comparisons were made between samples collected in the morning and afternoon during each week (average

concentration obtained for each campaign). Pearson's correlation coefficient ( $r$ ) was used to determine the correlation between the concentrations of bioaerosols and meteorological variables.

## 3 Results and Discussion

### 3.1 Airborne Culturable Fungi Concentration

The average concentration of fungi was  $123.71 \pm 17.97$  CFU/m<sup>3</sup> during the 4 months of sampling. The minimum value was presented in week 2 of sampling with 44.17 CFU/m<sup>3</sup>, in the morning, and the maximum value in week 7 with 247.35 CFU/m<sup>3</sup>, at the same time. Figure 2 shows the results of the concentration (CFU/m<sup>3</sup>) in the HEI and its area of influence during the 16 monitoring campaigns. There have been few studies on bioaerosols carried out in the same sampling area as in this study (coastal zone). To date, studies have been conducted on the beaches of the city of Cartagena de Indias (Huertas et al. 2018), in a sanitary landfill in the city of Santa Marta (Velez-Pereira and Camargo 2011), and in the coastal city of Mérida, Mexico (Rodriguez-Gomez et al. 2020). The concentrations found in our study were lower than those reported in these previous reports.



**Fig. 2** Average concentration of fungi by times and sampling campaigns at a HEI in Cartagena de Indias, Colombia

However, it is important to note that the sampling location was different in each study; therefore, this may be a factor that can influence the differences in the concentrations. In addition, there are other factors that may influence the results, such as the various natural or anthropogenic emission sources and the different meteorological variables or climatic conditions of each place.

In the case of the values registered by campaign and sampling times, a higher concentration was reported in the morning in 11 of the 16 campaigns. This trend may be related to wind speed since a lower average speed was recorded in the morning (8.75 km/h) than in the afternoon (16.18 km/h). These results coincide with reported by Huertas et al. (2018) who quantified the concentrations of fungi on a beach in Cartagena de Indias and can be explained by the influence of sea and land breezes on the wind patterns in coastal areas. Moreover, high wind can act as a spore dilution factor, and could reduce the concentration of fungal spores (Fayad et al. 2021).

### 3.2 Characterization and Identification of Fungi Bioaerosols

During the 16 sampling campaigns, 128 samples were collected, from which 11 fungi were identified and characterized (Table 1). According to the results obtained, it was determined that the isolated fungal strains belonged mainly to the *Ascomycota* phylum (82%), followed by *Mucoromycota* (9%) and *Basidiomycota* (9%). The dominance of the phylum *Ascomycota* coincides with that reported by Shelton

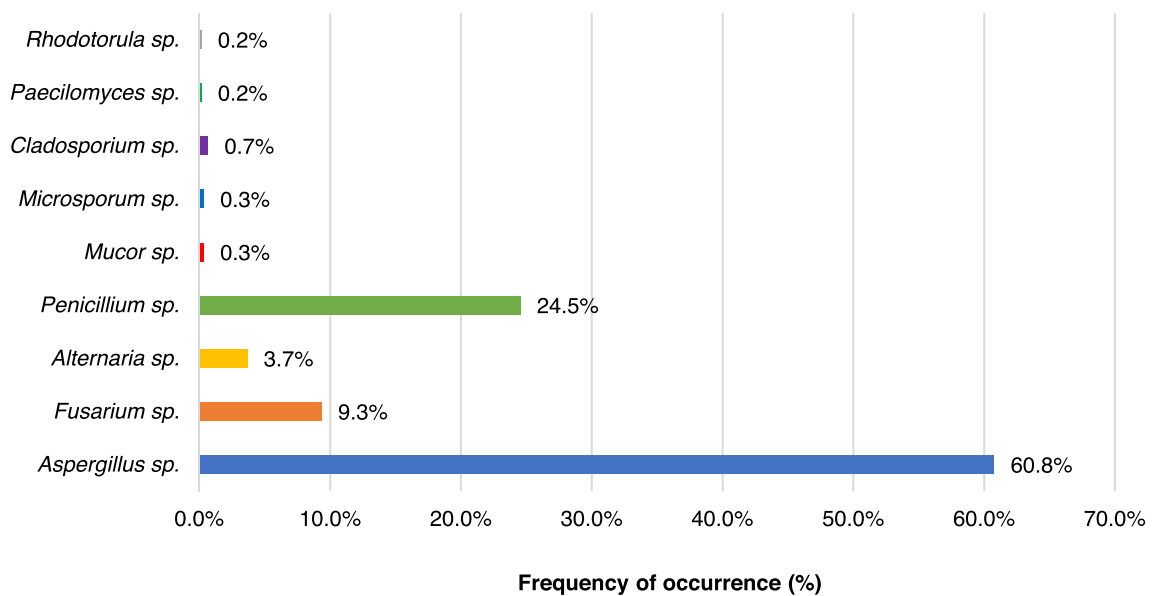
et al. (2002), Shin et al. (2015) and Valsan et al. (2015), and may be related to rapid growth and intense sporulation on standard agar media (Pashley et al. 2012).

The main genera of airborne fungi present in the air of the HEI were *Aspergillus* (1607.8 CFU/m<sup>3</sup>), *Penicillium* (649.3 CFU/m<sup>3</sup>), *Fusarium* (247.3 CFU/m<sup>3</sup>), *Alternaria* (97.2 CFU/m<sup>3</sup>), and *Mucor* (8.8 CFU/m<sup>3</sup>) (Fig. 3). Regarding the frequency of appearance of the fungi according to time, it was determined that all the identified genera appeared at both times (morning and afternoon) (Fig. 4) Araujo et al. (2013). *Aspergillus* sp. was the most common fungal genus represented by two species: *Aspergillus niger* and *Aspergillus flavus*. This genus reached its maximum concentration in the afternoon. *Aspergillus* is a saprophytic filamentous fungus widely distributed worldwide, and some of the species of this genus are infectious agents that cause different syndromes that affect the respiratory tract (Belizario et al. 2021; Navale et al. 2021). The presence of *Aspergillus* sp. is very common in the air because of its versatility to adapt to environmental changes (Fayad et al. 2021), and it has been previously reported in studies on bioaerosols (Fayad et al. 2021; Huang et al. 2002; Huertas et al. 2018; Madhwal et al. 2020). In addition, this genus has been reported to be highly abundant in tropical and subtropical regions (Fayad et al. 2021).

*Penicillium* sp. was the second most predominant genus, and its frequency was similar in the two sampling campaigns evaluated. This genus is abundant and frequent in nature, and some species are associated with keratitis, otomycosis, pneumonia, and endocarditis (Egbuta et al. 2017). Previous

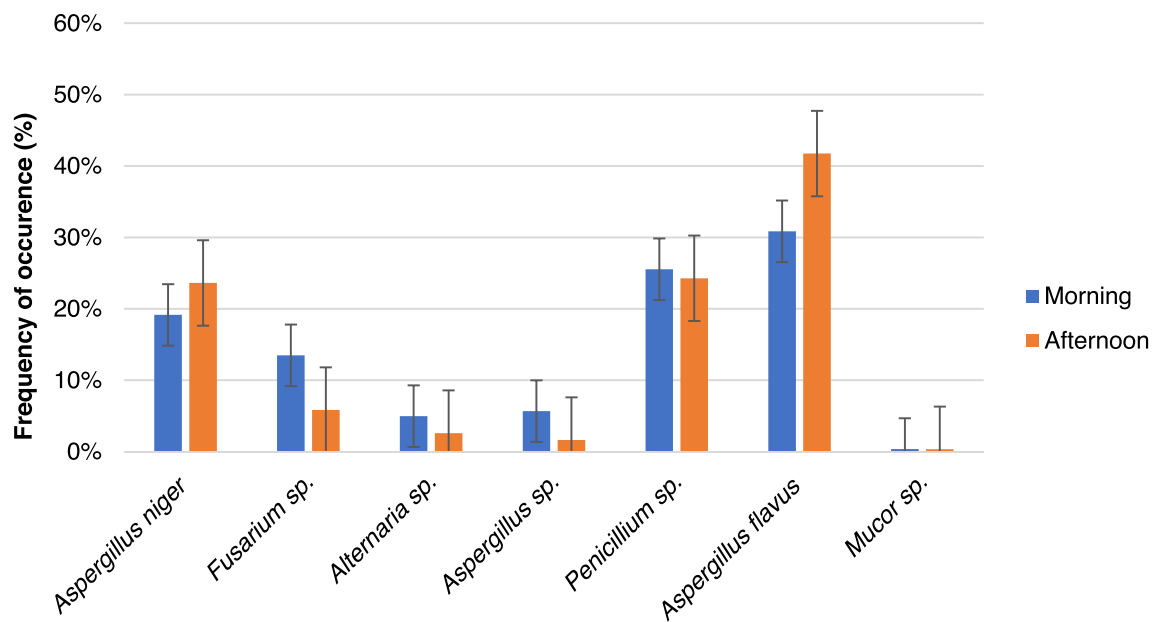
**Table 1** Phenotypic characterization of isolated fungi

Code	Morphology	Macroscopic description	Phylum	Family	Genus	Related species
H1	Septate hypha, profusely branched, globose and wrinkled conidia	Colony with cottony texture and black aerial conidia	<i>Ascomycota</i>	<i>Trichocomaceae</i>	<i>Aspergillus</i>	<i>Aspergillus niger</i>
H2	Septate hypha with simple conidiophores	Yellow colony with white center and cottony texture	<i>Ascomycota</i>	<i>Nectriaceae</i>	<i>Fusarium</i>	<i>Fusarium</i> sp.
H3	Septate hypha, young conidia rounded at the base	Dark green cottony colony	<i>Ascomycota</i>	<i>Pleosporaceae</i>	<i>Alternaria</i>	<i>Alternaria</i> sp.
H4	Non-septate hypha, conidia formed from conidiophore	Colony with black filaments with white edges, and with a cottony texture	<i>Ascomycota</i>	<i>Trichocomaceae</i>	<i>Aspergillus</i>	<i>Aspergillus</i> sp.
H5	Hyphae not septate, hyaline, thin, conidia round	Green colony with white edges and cottony texture	<i>Ascomycota</i>	<i>Trichocomaceae</i>	<i>Penicillium</i>	<i>Penicillium</i> sp.
H6	Septate hypha, globose conidia, bluish green	Green colony with white edges and cottony texture	<i>Ascomycota</i>	<i>Trichocomaceae</i>	<i>Aspergillus</i>	<i>Aspergillus flavus</i>
H7	Hypha septate, hyaline, broad	Brown cologne and cottony texture	<i>Mucoromycota</i>	<i>Mucoraceae</i>	<i>Mucor</i>	<i>Mucor</i> sp.
H8	Septate hypha, slender conidia, and micro conidia on the sides of the hyphae	White colony with beige center and cottony texture	<i>Ascomycota</i>	<i>Arthrodermataceae</i>	<i>Microsporium</i>	<i>Microsporium</i> sp.
H9	Septate hypha, slender conidia, and micro conidia on the sides of the hyphae	Green color cologne and black reverse, with velvety texture	<i>Ascomycota</i>	<i>Davidiellaceae</i>	<i>Cladosporium</i>	<i>Cladosporium</i> sp.
H10	Slender septate hypha, hyaline conidia	Yellow colony with cottony texture	<i>Ascomycota</i>	<i>Eurotiomycetes</i>	<i>Paecilomyces</i>	<i>Paecilomyces</i> sp.
H11	Septate hypha, presence of blastoconidia	Branched salmon colony	<i>Basidiomycota</i>	<i>Sporidiobolaceae</i>	<i>Rhodotorula</i>	<i>Rhodotorula</i> sp.

**Fig. 3** General frequency of fungal genera identified in the air of the higher education institution (HEI)

studies have reported *Penicillium* sp. as a fungal aerosol abundant in outdoor environments both in winter and summer, as its spores can grow under different meteorological

conditions (El-Morsy 2006; Faridi et al. 2015; Lee and Chang 2000; Pashley et al. 2012; Rodriguez-Gomez et al. 2020; Spicer and Gangloff 2005). *Fusarium* sp., which was



**Fig. 4** Frequency of fungal bioaerosols occurrence in the morning and afternoon

the third most frequent genus in this study, is a group of filamentous fungi widely distributed in soil and plants; they are considered opportunistic due to their ability to grow at high temperatures, and can cause systematic infections in immunocompromised patients, and some produce toxins that affect humans and animals (Georgiadou et al. 2014). These results coincide with those of previous studies in which the genus *Fusarium* has been considered as an outdoor-relevant group (Huertas et al. 2018; Nageen et al. 2021; Oliveira et al. 2009; Rodriguez-Gomez et al. 2020).

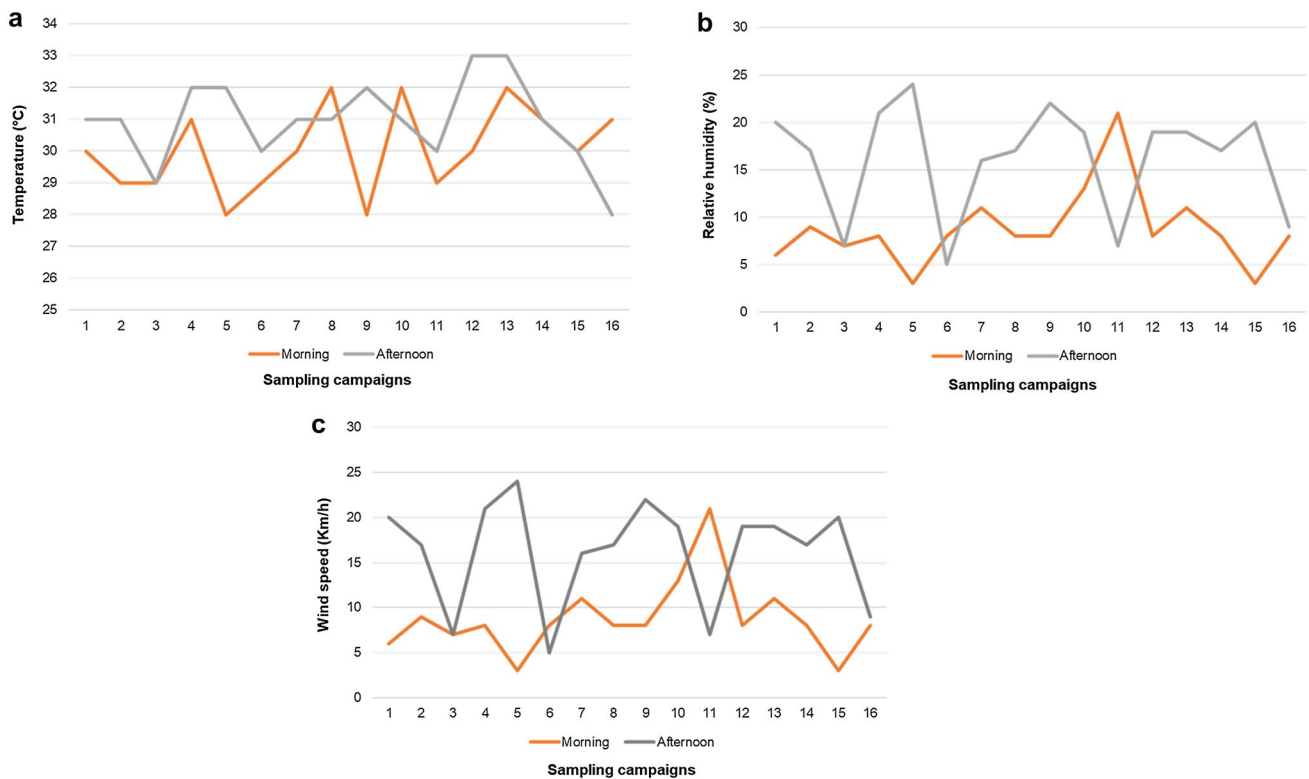
The genus *Alternaria* is a ubiquitous genus of fungi, which is associated with a wide variety of substrates, including seeds, plants, agricultural products, animals, soil, and the atmosphere (Woudenberg et al. 2013). Some species of this genus can be plant pathogens, causing great losses in a wide range of crops, and can also have negative health effects in humans (Woudenberg et al. 2013). *Alternaria* spp. are commonly found in the air, and their presence in outdoor environments has previously been reported (El-Morsy 2006; Faridi et al. 2015; Fayad et al. 2021; Hosseini et al. 2021; Nageen et al. 2021; Rodriguez-Gomez et al. 2020). On the other hand, the species of the genus *Mucor* are very frequent in nature, they are found in a great variety of substrates, but more frequently they are associated with humid environments. The spores of this genus, which are emitted in large quantities, have a strong moisture absorption capacity, and a high level of adherence to various surfaces, and can be dispersed in humid air (Morin-Sardin et al. 2017). This genus has been reported in previous studies of airborne fungi (El-Morsy 2006; Fayad et al. 2021; Huertas et al. 2018; Niazi et al. 2015).

### 3.3 Analysis of Meteorological Conditions and Bioaerosols Concentrations

In this study, three meteorological variables were monitored in parallel: temperature (°C), relative humidity (%) and wind speed (km/h). The behavior of these variables during the four months of sampling is shown in Fig. 5 and Table 2 presents the results of the statistical analysis performed.

The relative humidity values ranged between 80 and 90%, while the temperature remained in the range of 28–33 °C, and the speed of the wind, ranged between 5 and 20 km/h, the meteorological variable being more fluctuation. Based on the information in Table 2, it can be inferred that the meteorological variables, temperature, and relative humidity maintain a high consistency in their data because the standard deviation and coefficient of variation values are low, indicating that there is little dispersion in the data of these variables. Likewise, it is observed that there are no significant differences between the minimum and maximum values of these variables. Regarding the linear correlation coefficients of the analyzed variables, a low negative relationship was determined between temperature and the concentration of fungal bioaerosols ( $r = -0.1$ ). Similarly, a low correlation between wind speed and fungal concentration was observed ( $r = -0.11$ ). In addition, a low positive correlation was found between relative humidity and fungal concentration ( $r = 0.18$ ).

The low correlation between relative humidity and bioaerosol concentrations determined here coincides with the studies carried out by Fayad et al. (2021), Jianhua et al. (2015) and Zhong et al. (2016), who reported that relative



**Fig. 5** Behavior of meteorological variables during the sampling period. Bars represent a 95% confidence interval. **a** Temperature (°C), **b** relative humidity and **c** wind speed

**Table 2** Statistical features of the analyzed variables

Variable	Average	Standard deviation	Variation coefficient (%)	Minimum	Maximum	Pearson's coefficient	P value
Average concentration of fungi (CFU/m <sup>3</sup> )	123.71	49.84	40.29	42	247.4	NA	NA
Temperature (°C)	30.5	1.39	4.56	28	33	- 0.10	0.68
Relative humidity (%)	74.5	5.95	7.98	63	85	0.18	0.48
Wind speed (km/h)	12.47	6.27	50.31	3	24	- 0.11	0.3

humidity and the microbiological activity of bioaerosols were not significantly correlated. Therefore, the results obtained in our study suggest that fungal activity was not affected by relative humidity in the range of 66–85%. Wind is a factor that can influence bioaerosols, depending on the speed and direction. Wind speed is associated with the release and dispersal of spores; however, this effect varies with intensity and height (Li et al. 2011). In this study, a negative correlation between wind speed and concentration was found because fungal concentrations were higher in the morning when wind speeds were lower. These results coincide with those reported by Jones and Harrison (2004), who indicated that fungal concentrations increase when wind speed increases from 0.5 to 1 m/s but decrease when the wind speed increases to 5 m/s, probably as a result of

the depletion of the source. A low negative correlation was found between fungal concentration and temperature. These results have been reported in previous studies, in which no correlation was found between the concentration of fungi and temperature (Huertas et al. 2018; Rodriguez-Gomez et al. 2020). According to Camargo et al. (2011), this behavior may be associated with the stress processes to which fungi aerosols are subjected at high temperatures and low water content, which reduces their viability and subsequent recovery.

In this study, no correlations were observed between the meteorological variables and the concentration of bioaerosols during the sampling period, as there were no statistically significant differences between them, which indicates that the meteorological parameters had no influence



on the presence of fungal bioaerosols in the air of the HEI analyzed. Fungal concentration can also be influenced by emission sources close to the test site because the natural environment provides the substrates and habitats for their survival (Kummer and Thiel 2008; Ruzer and Harley 2004). In our study, the HEI sampling site was located within the urban settlement between the cities of Cartagena and Turbaco, which is characterized by vegetation in the tropical dry forest ecosystem (FCDS 2013; Ulloa-Delgado 2016). This vegetation, represented by the presence of vascular plants, can influence the presence, composition, and quantity of bioaerosols present in the area (Fröhlich-Nowoisky et al. 2016). On the other hand, close to the institution there are industries related to the activities of handling iron and steel, according to Gilbert and Duchaine (2009), industries related to the handling of metals can be sources of bioaerosols because these activities can favor the aerosolization of these microorganisms and guarantee their transport in the air. However, to determine the influence of the mentioned emission sources on the concentration of fungal bioaerosols, future studies based on a large-scale test are required.

## 4 Conclusions

In this study, the concentration of culturable fungal bioaerosols at a higher education institution in a tropical city (Cartagena de Indias, Colombia) was evaluated. The highest concentrations of fungi occurred in the morning, and the airborne fungi that occurred most frequently were related to the genera *Aspergillus*, *Penicillium* and *Alternaria*. The outdoor fungal bioaerosol concentrations in the sampling area were not influenced by meteorological conditions. Other emission sources close to the sampling point may influence the presence and concentration of fungal bioaerosols, however, future studies with broader monitoring are required, and it is suggested that their presence and concentration were due to various emission sources. This is the first study to identify airborne fungi in an HEI in the city of Cartagena de Indias. Therefore, this research constitutes a baseline for future research related to the measurement of bioaerosols in outdoor environments in the city of Cartagena de Indias, allowing the identification of microorganisms present in the area, their concentration, and their possible impact on the population.

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**Author contributions** CRL designed the experiments, analyzed the data and wrote the paper. LBA and DDP performed the experiments. RAB contributed to the design of the experiments, analyzed the data, and critically read the manuscript. All authors read and approved the final manuscript.

## Declarations

**Conflict of Interest** The authors declare no conflict of interest.

**Ethics Approval and Consent to Participate** This article does not contain any studies involving human participants or animals performed by any of the authors.

**Consent for Publication** All the authors have consented to publish this research.

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