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Study of the fungal microbiota from the air in three environments of a University in Santa Catarina

Estudo da microbiota fúngica do ar em três ambientes de uma Universidade em Santa Catarina

Estudio de la microbiota fúngica del aire en tres ambientes de una universidad en Santa Catarina

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ABSTRACT

Introduction: Although respiratory diseases have different etiologies, many of them are related to ambient air pollution. Breathing polluted air, fine particles can be inhaled, which contain pathogens that penetrate the lungs and the cardiovascular system causing infections. The objective of this study was to quantify and identify fungal bioaerosols in three different environments of a private university in Santa Catarina state. **Outline**: The samples were collected at October 7, 2016, in the canteen, library and classroom, with two periods of exposure: 30 minutes and two hours, in three periods of the day (morning, afternoon and evening). Colony forming units counting was performed, as well as observation of micro and macromorphological characteristics for identification of genera of fungi. **Results**: It was found that microbiological contamination is directly related to the flow of air and people circulating in closed environments. The genera of fungi most prevalent were *Gliocladium*, *Fusarium*, *Penicillium* and *Cladosporium*. **Implications**: The fungi identified in the study can cause problems for human health, requiring special attention to the microbiological control of the air, especially in indoor environments where the flow of people is high and there is not adequate air exchange.

DESCRIPTORS

Air Microbiology; Fungi; Air Conditioning.

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INTRODUCTION

Respiratory diseases are a serious public health problem in Brazil and worldwide. They have different etiologies, being among the 10 main causes of death in the world. Many of these diseases are related to ambient air pollution, which kills about 7 million people each year, mainly in low-income countries.[1-2]

Breathing polluted air, fine particles can be inhaled, which contain pathogens that penetrate the lungs and the cardiovascular system causing infections, such as pneumonia, responsible for about 1.5 million deaths annually.[2-3] The fungi, eukaryotic organisms that obtain energy through the absorption of nutrients are among these pathogens. Fungi have a variety of morphological types, are structurally unicellular or multicellular, are made up of filaments called hyphae and the entire mass of hyphae is collectively called mycelium.[4] The capacity of infection of these microorganisms is related to several factors. Among them stand out genetic predisposition to infections, impaired immune system, and resistance to drugs routinely used in treatments, e.g. *Candida albicans* resistant to fluconazole.[5-6]

In addition, the scarce renewal of indoor air due to the use of air-conditioning systems is another related problem linked to fungal infection. Air conditioners devices enable the dispersion of pathogens, even though after sterilization, disinfection and environment washing.[7-8]

For the aforementioned reasons the objective of this study was to evaluate the presence of fungi in indoor and outdoor environments of a private university in Santa Catarina state. In addition, both diversity and quantity of these microorganisms were assessed at different times of the day, providing data for creating strategies for infection control in the community.

METHOD

SAMPLE COLLECTION

The indoor and outdoor air of three environments of a private university in Santa Catarina state was verified. The environments were analyzed in three periods of the day, which were represented by morning, afternoon, and evening. The canteen was considered an outdoor environment, as it is an open place. The library and a classroom with a capacity of approximately 25 students in the morning and evening, but empty in the afternoon, were considered indoor environments. Samples were collected in different places, during a period of intense flow of people.

The samples were collected at October 7, 2016, at the spring season. The meteorology reported a minimum of 12ºC and a maximum of 26ºC. Indoor and outdoor ambient air were evaluated using the passive gravitational settling on surface of Petri dish containing the growth medium.[9] The study was conducted in two exposure times: 30 minutes and 2 hours.

COLLECTION METHODS

Potato Dextrose Agar culture medium – PDA (potatoes infusion 200 g, dextrose 20 g, agar 15 g, distilled water 1000 mL), appropriated for fungi cultivation,[10] was prepared following the manufacturer's instructions and enriched with streptomycin to inhibit bacterial proliferation. All procedures for sterilizing the culture medium were followed using autoclave at a pressure of 15 psi and 120ºC for 15 minutes. The dispensing of the culture medium into the Petri dishes occurred aseptically in horizontal laminar flow cabinet, which was cleaned and sterilized previously with 70% alcohol and 8W UV light for 30 minutes.

Exposure for 30 minutes: Samples were collected, in triplicate, considering both location and

period of the day. Thus, Petri dishes (90×15mm) containing PDA were placed 1 meter above the floor, and about 1 meter away from walls or any major obstacles, for the exact time of 30 minutes. After exposure, the plates were incubated aerobically at 25 ± 2ºC and 12 hours photoperiod for seven days.

Exposure for 2 hours: For this approach, the samples were also collected, in triplicate, considering both location and period of the day. As related to exposure for 30 minutes, Petri dishes (90×15mm) containing PDA were placed 1 meter above the floor, and about 1 meter away from walls or any major obstacles, exactly for 2 hours. After exposure, under aseptic conditions, the plates were incubated aerobically at 25 ± 2ºC and 12 hours photoperiod for seven days.

Colony forming units count (CFU) and identification of genera of fungi

After the incubation period, the number of colony forming units (CFU) for each Petri dish was registered, considering location, time of day and time of exposure.

After counting, all different types of colonies were carefully screened by inspection of macro and micro morphological characteristics. Also, microscopic characteristics of spores were evaluated. The structures were analyzed through a stereoscopic (magnification 10 and 40×) and optical microscope (magnification 100 and 400×). For identification of genera of fungi, the following bibliographies were consulted: Illustrated Genera of Imperfect Fungi,[11] Atlas of Clinical Fungi[12] and Medical Mycology.[13]

Method for CFU estimation

Colonies were counted individually through a marker pen and results were expressed as the number of CFU / unit of area, being the number in m^3 calculated according to the equation described by Friberg et al.[14]:

$$CFU/m^3 = \frac{Number\ of\ colonies\ on\ the\ plate}{Plate\ area} x \frac{1}{23}$$

For this, it was necessary to know the area of the Petri dish exposed to the air and the ratio between the number of cells on the medium surface and the number of cells in the air (SAR). The SAR for environments with spontaneous sedimentation, without devices that force the air is 23:1.[15] The Petri dishes used in the assays have 8 cm-diameter and area of approximately 50 cm^2, i.e., 0.0050 m^2.

Statistical analysis

The experimental design used was completely randomized with three treatments and three repetitions, being each Petri dish a different repetition. Firstly, data were tested to adhere to the normal distribution by Shapiro Wilk test, through BioEstat 5.0 software. Comparisons among means were tested by Tukey test, considering $p<0.05$, through SISVAR 5.3 software.[9]

RESULTS

CFU quantification

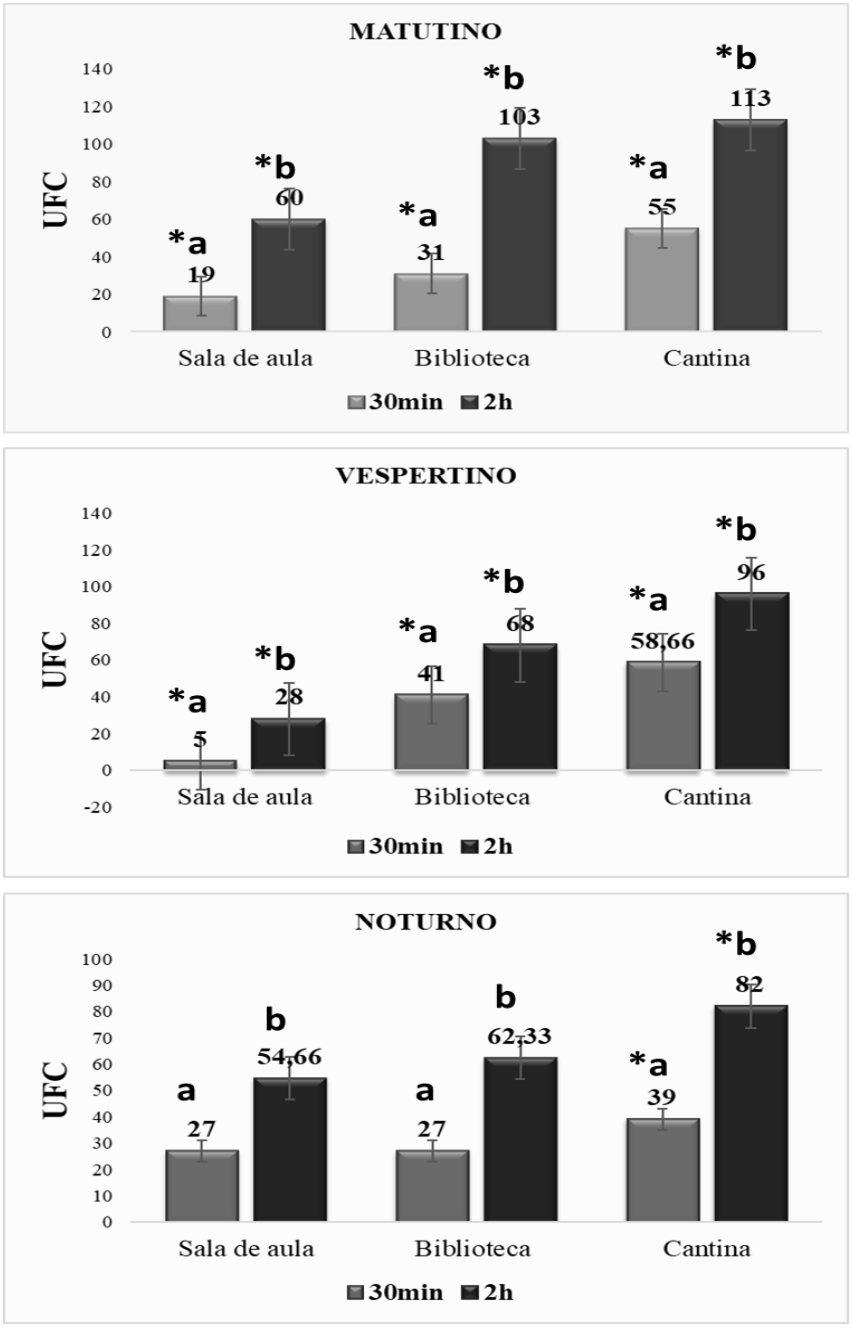
Five hundred and fifty-two CFU were quantified in the classroom, 1062 in the library and 1250 in the canteen. Exposure of the plates for two hours, regardless of the periods of the day and location, showed higher CFU number. Since, both indoor and outdoor environments did not have temperature and humidity controlled, it was admitted the temperature cited previously. The amount and diversity of microorganisms was obtained from indoor and outdoor flow of people in different periods of the day, and in the different exposure times.

Figure 1 shows the CFU values of air contamination in the classroom, canteen and library, in the morning, afternoon and evening after exposure for 30 minutes and two hours.

Outdoor ambient air in the canteen showed the highest CFU values, and all fungal genera were found there.

In the afternoon, statistical difference among the evaluated places was detected. In the morning, no significant difference was observed. It is important to considerer that in the morning, the flow of students and collaborators is relatively low. At this time, small changes in the flow of people were observed, with fewer people being found in the classroom and more people in the canteen. In the evening, there was no statistical difference in the CFU values in the different evaluated places.

Figure 1 – CFU values of air contamination in the classroom, canteen and library, in the morning, afternoon and evening after exposure for 30 minutes and two hours.



Tukey test, $p<0.05$. a*, statistical significance between the evaluated places with exposure for 30 min; b*, statistical significance between the evaluated places with exposure for 2 hours.

Source: Authors.

DIVERSITY OF GENERA OF FUNGI

Eight genera of fungi were identified, being *Gliocladium* sp., *Fusarium* sp., *Penicillium* sp. and *Cladosporium* sp. the most frequent and present in all evaluated places. Data containing all genera of fungi identified in different environments of the university are shown in the Table 1.

Table 1 – Diversity of genera of fungi identified in different environments of the university.

GENERA OF FUNGI	EVALUATED PLACES
Gliocladium sp.	Classroom, canteen and library
Fusarium sp.	Classroom, canteen and library
Aspergillus sp.	Canteen and library
Penicillium sp.	Classroom, canteen and library
Phoma sp.	Canteen
Trichoderma sp.	Classroom and canteen
Rhizopus sp.	Canteen
Cladosporium sp.	Classroom, canteen and library

Source: Authors.

DISCUSSION

Microbiological contamination is directly related to the flow of air and people circulating in closed environments. In this research, different exposure times were applied to screen fungal contamination in 3 different environments: classroom, canteen and library.

In the morning, the classroom had the lowest contamination, in both evaluated times. This fact can be explained by few changes in the flow of people and air during exposure for 30 minutes and 2 hours. In the library, the second location screened in the study, the values increased around three times when compared to those in the classroom. The high prevalence of microorganisms in the library ambient air can be related to the greater number of students who were at the place during the interval between classes, in order to carry out some research in the collection.

In the canteen, similar to the library, only the triplicates exposed for 2 hour were currently being evaluated during the break time. It is known that the canteen receives a large number of people to snacking, thus generating greater air flow and contamination.

In the morning, comparing the three evaluated places, the CFU values in canteen environmental air were higher after exposure for 30 min, differing statistically from the other environments

Regarding the afternoon period, it was not observed significant difference in the CFU values in the canteen and library comparing to the values obtained in the morning period. This result can be explained by the university does not have classes in the afternoon, which greatly reduces the flow of people. Nonetheless, considering indoor environments, the microorganisms acquired during the morning could continue circulating in the air.

In the evening, in view of the highest flow of students and collaborators at the university, it was found statistical difference in ambient air contamination for the evaluated environments regarding exposure times. After both exposition times, it was noticed a significant increase in CFU values in the canteen environmental air in comparison to the library and the classroom. This is possibly because the canteen is the first place where people go when they arrive at the university in the evening, as many are coming from their workplaces, and want to have a snack before going to the classroom or the library.

In agreement to the Maximum Recommended Value (VMR) for microbiological contamination, which

must be up to 750 CFU/m^3 of fungi, according ANVISA Resolution nº 09, January 16, 2003, any place evaluated in this study presented contamination exceeding the limit in both exposure times.[16]

Due to the high incidence of respiratory diseases, research aimed at assessing air quality is becoming increasingly necessary. The literature has reported the predominance of these studies in hospitals, a place where people tend to be more susceptible, since several invasive procedures are performed there.[17-19]

There are also reports about contamination of indoor and outdoor ambient air in universities. In accordance with the study conducted by Sobral et al,[20] the classroom was considered the least contaminated site, since reduced amount of fungi was observed in this space (14 CFU/m^3) when compared to different environments in the campus.

The ambient library air was considered as intermediate in the degree of contamination. Research carried out in libraries has explored ways to conserve the collection, with the maintenance of the temperature (below 20°C). Furthermore, mechanical cleaning of the materials has shown to be promising and necessary measures. In the present study, however, it should be emphasized that the greatest importance given to the results is related to the health of the academic community and not to the preservation of the collection itself. Other studies have already reported the presence of *Eurotium halophilicum*,[21-22] which was not found here. However, we identified *Aspergillus*, *Gliocladium*, *Fusarium*. *Penicillium* e *Cladosporium*.

The canteen proved to be the location with the highest CFU values, and consequently where all genera of fungi were present. This fact can be explained by the highest flow of people and air, during the three evaluated periods. In addition, the presence of food contributes to the development of fungi, even in the devices used to prepare and maintain food and drinks.[23-24] From the perspective of public health, fungi contamination is a serious concern, since the fungi identified are pathogenic. For instance, aflatoxins produced by *Aspergillus*, has toxic potential and its cumulative and lethal effect on the human organism is known. *Gliocladium* and *Fusarium* also present an eminent risk to food safety, since they produce mycotoxins, designed as toxic chemical substances that can cause damage to the humans and animals health.[25-29]

The literature has reported in studies that assessed indoor and outdoor air contamination, that genera of fungi are widely variable. Comparing to the genera of fungi presented previously, it is possible to notice that most of our findings are in agreement with scientific literature.[30-31]

In addition, the literature has shown that *Aspergillus*, *Penicillium* and *Cladosporium* are the genera of fungi most frequently described in indoor and outdoor environments of different places, such as houses, mosques, parks, public restrooms, grocery stores, laboratories and hospitals. All of these fungi were also identified in this study and were present in at least two of the three evaluated places.[32-33]

This issue brings worries, because into contact with human, mainly in large quantities, some fungi have the ability to cause several diseases. For instance, *Aspergillus* genus, which does not belong to human microbiota, is considered an opportunistic and may cause several clinical conditions with different forms, such as Aspergillosis.[4]

Fusarium was one of the four genera of fungi found in all evaluated places. Searching databases, we realized the need for more studies involving this genus, as they are versatile pathogens that causes different diseases, which after being established are difficult to treat, being prevention the best way to deal with them.[34]

The genus *Cladosporium*, known as dematiaceous, have great microbiological relevance, as they are capable of causing various respiratory allergies and infections; for instance, a rare case of

hemorrhagic pneumonia was previously reported.[35-37] It is observed that even fungi considered to be poorly pathogenic, such as *Gliocladium*, can affect humans, as demonstrated by Venkatesh et al., who related the occurrence of ocular infection caused by *Gliocladium* species.[38]

The genus *Penicillium* was related previously to cause infections in people living with HIV; nonetheless, it is now indiscriminately affecting individuals without the virus.[39-41]

Patel et al. demonstrated that the seasons of the year have great influence on the predominance of spores. Basidiomycetes are presented in greater number and frequency during the spring, while higher concentrations of *Cladosporium* occur during the summer and autumn months.[42] This study is relevant, as it demonstrated that the climate has interference in the reproduction of fungi.

Therefore, since the presence of fungi, as the genera found in the research, can cause not only severe respiratory damage, but dermatological and ophthalmological infection and even food contamination due to mycotoxins, our findings are extensively important from the perspective of public health. It is necessary that health professionals be aware of strategies involving prevention and control of infections by these microorganisms in the community. The initial action for this discussion is to elucidate the etiological factors of these diseases and the need for constant cleaning of the air conditioner units, especially in environments with high flow of people.

CONCLUSION

Present in practically all environments, the genera of fungi identified in this research can cause problems for human health, such as allergies in the respiratory tract and/or major illnesses in immunocompromised individuals. Therefore, it is necessary special attention to the microbiological control of the air, mainly in indoor environments where the flow of people is high and there is not adequate air exchange.

RESUMO

Introdução: Doenças respiratórias possuem diversas etiologias, mas muitas destas são relacionadas a poluição do ar ambiente. Ao respirar ar poluído, pode ocorrer a inalação de partículas finas contendo patógenos que penetram os pulmões e o sistema cardiovascular causando infeções. Objetiva-se quantificar e identificar os bioaerossóis fúngicos de três ambientes de uma universidade privada no estado de Santa Catarina. **Delineamento**: A coleta ocorreu no período de 07 de outubro de 2016 na cantina, na biblioteca e em sala de aula, com dois períodos de exposição: 30 minutos e duas horas, e nos três turnos do dia (matutino, vespertino e noturno). Foi realizada a contagem de unidade formadora de colônias bem como foi feita a observação de características micro e macromorfológicas para a identificação dos gêneros fúngicos. **Resultados**: Constatou-se que a contaminação microbiológica está diretamente relacionada com o fluxo de ar e de pessoas circulando nos ambientes fechados. Os gêneros fúngicos que se destacaram foram *Gliocladium*, *Fusarium*, *Penicillium* e *Cladosporium*. **Implicações**: Os fungos identificados podem ocasionar problemas para a saúde humana, sendo necessária a devida atenção ao controle microbiológico do ar, principalmente em ambientes internos onde o fluxo de pessoas é elevado e não há grande circulação do ar.

DESCRITORES

Microbiologia do Ar; Fungos; Ar Condicionado.

RESUMEN

Introducción: Las enfermedades respiratorias tienen diferentes etiologías, pero muchas de ellas están relacionadas con la contaminación del aire ambiente. Al respirar aire contaminado, se pueden inhalar partículas finas que contienen agentes patógenos que penetran los pulmones y el sistema cardiovascular que causa infecciones. El objetivo es cuantificar e identificar los bioaerosol fúngicos de tres ambientes de una universidad privada en el estado de Santa Catarina. **Delineación**: La colección tuvo lugar en el período del 7 de octubre de 2016 en el comedor, en la biblioteca y en el aula, con dos períodos de exposición: 30 minutos y dos horas, y en los tres turnos del día (mañana, tarde y noche). Se realizó el recuento de unidades formadoras de colonias, así como la observación de características micro y macromorfológicas para la identificación de géneros fúngicos. **Resultados**: Se descubrió que la contaminación microbiológica está directamente relacionada con el flujo de aire y las personas que circulan en ambientes cerrados. Los géneros fúngicos que se destacaron fueron *Gliocladium*, *Fusarium*, *Penicillium* y *Cladosporium*. **Implicaciones**: Los hongos identificados pueden causar problemas para la salud humana, lo que requiere la debida atención al control microbiológico del aire, especialmente en ambientes interiores donde el flujo de personas es alto y no hay una gran circulación de aire.

DESCRIPTORES
Microbiología del Aire; Hongos; Aire Acondicionado.

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COLLABORATIONS

BBB: Substantial contributions to work conception or design. BBB and ARSN: contributions to data collecting, analysis and interpretation. CGM and DRJF: Substantial contributions to writing the article or to its critical review. DRJF: Evaluation of the final version to be published. All the authors agree and take responsibility for the content of this manuscript version to be published.

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AVAILABILITY OF DATA

Does not apply.

FUDING SOURCE

Does not apply.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.