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Major Article

# Diversity and dynamics of airborne fungi in São Luis, State of Maranhão, Brazil

***Geusa Felipa de Barros Bezerra*[1], *Silvio Monteiro Gomes*[2], *Marcos Antonio Custódio Neto da Silva*[3], *Ramon Moura dos Santos*[1], *Walbert Edson Muniz Filho*[1], *Graça Maria de Castro Viana*[1] *and Maria do Desterro Soares Brandão Nascimento*[1],[4]**

[1]. Departamento de Patologia, Núcleo de Imunologia Básica e Aplicada, Universidade Federal do Maranhão, São Luis, MA. [2]. Departamento de Biologia, Universidade Federal do Maranhão, São Luis, MA. [3]. Curso de Medicina, Universidade Federal do Maranhão, São Luis, MA. [4]. Centro de Estudos Superiores de Caxias, Universidade Estadual do Maranhão, Caxias, MA.

**ABSTRACT**

**Introduction:** This study aimed to identify airborne fungi in São Luis, Maranhão, Brazil, to determine the prevalent genera and to correlate these genera with the area and season. **Methods:** In total, 1,510 colony-forming units (CFUs) of airborne fungi were isolated from the north, south, east and west sides and from the center of the city from January to December 2007. The samples were collected on Petri dishes that were exposed to the fungi by the gravitational method. **Results:** Twenty genera of fungi were isolated; the most common were *Aspergillus* (33.5%), *Penicillium* (18.8%), *Cladosporium* (14.2%), *Curvularia* (10.6%) and *Fusarium* (7.6%). The CFUs of the fungi were statistically significant ($p < 0.0001$). Fungal biological diversity was present all year, without any large seasonal variations but with slight increases in May, August and September. **Conclusions:** The fungal genera identified in this study were correlated with natural systems and could be useful when evaluating the impact of environmental changes on the region.

**Keywords:** Fungi. Environment. Biodiversity.

## INTRODUCTION

Fungi, especially filamentous fungi, which are common aeroallergens, form a major part of bioaerosols[1]. Fungi are ubiquitous in outdoor air, and their concentrations, aerodynamic diameters and taxonomic compositions have potentially important implications for human health[2].

The diversity and abundance of anemophilous microorganisms can be influenced by and can interfere with environmental conditions. These microorganisms are influenced by factors such as season, temperature, the relative humidity of the air and other parameters that exhibit seasonal variation[3,4].

The relationships among allergic exposure, the fungal presence in indoor and outdoor environments and consequent allergic diseases are not fully understood[5,6]. Therefore, it is important to know both the frequency of certain airborne fungi and their distributions according to the season and the main environment (i.e., indoor or outdoor) in order to evaluate their correlations with respiratory symptoms related to allergic processes[7-9].

These fungi can be used to assess effects on the environment and could contribute to determining the principal changes. The spores of fungi can be present in air particles and can potentially influence the hydrological cycle and climatic changes. In addition, humans are exposed daily to bioaerosols in their personal and professional lives, and these airborne particles represent a potential biological occupational hazard[10]. Biological particles in the air are approximately 40% organic carbon by mass and can be an important source of bioaerosols in the atmosphere above continents[4]. Despite the importance of airborne fungi, very little is known about their diversity[11], especially of the fungi in São Luis, Maranhão.

For this reason, it was important to perform further observations to determine and characterize the frequencies of the main airborne fungi in outdoor environments and to identify possible correlations with seasonality and the possibility of monitoring allergic respiratory diseases.

## METHODS

Airborne fungi were collected between January and December 2007 in five outdoor areas of the City of São Luis (northern, southern, eastern, western and central), located in the State of Maranhão, Brazil. São Luis is located at a latitude of 2º31'47" S and a longitude of 44º18'10"W, and it is 24m above sea level. This city covers an area of approximately 830km$^2$ and has a population of 1,017,772 inhabitants[12].

***Address to:*** Drª Maria do Desterro Soares Brandão Nascimento. Deptº de Patologia/NIBA/UFMA. Av. dos Portugueses 1966, Prédio do CCBS, Bloco 3, Sala 3A, Cidade Universitária do Bacanga, 65080-040 São Luis, MA, Brasil.
**Phone**: 55 98 3272-8535; **Fax:** 55 98 3272-8535
**e-mail:** cnsd_ma@uol.com.br
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An analysis of the mycobiota was performed using Petri dishes (10cm by 2cm) containing 10mL of Sabouraud agar medium. The dishes were exposed to open air, in selected districts and in a predetermined region, for 15min every month, while placed at a height of 1.5m from the ground to collect fungal spores by the action of gravity[5,10]. Three dishes were exposed each month in each area, resulting in a total of 15 samples per month and 180 samples per year. It is important to emphasize that the areas where the research was conducted were randomly selected. The samples used for microculture and taxonomic analysis were obtained from the colony-forming units (CFUs)[13,14].

### Statistical analysis

The results were analyzed using SPSS software, Chicago, USA, SPSS Inc® version 16.0 for Windows (2007). The study was cross-sectional and observational, and descriptive statistical techniques were used to assess all of the variables, with the aid of graphs and tables of frequencies. Two-tailed analysis of variance was performed to determine the relationships of the frequencies of the species (from among the five most frequent species) and the months of the year with the number of CFUs.

## RESULTS

Twenty genera of fungi were isolated in this study. Depending on the region, it was possible to isolate between 10 and 14 genera, as shown in **Table 1** ($\chi^2 = 535.95$, $p < 0.0001$).

The main genera found in all of the regions were *Aspergillus* (33.5%), *Penicillium* (18.8%), *Cladosporium (*14.2%), *Curvularia* (10.6%) and *Fusarium* (7.6%), and the detailed distributions are shown in **Table 1**.

**Figure 1** shows that the median number of CFUs/dish during the rainy season (January to June) was 20 (maximum = 279 and minimum = 0), and the corresponding number during the dry season was 14 (maximum = 227 and minimum = 0), using the Mann-Whitney test ($p = 0.96$).

A comparison of the average number of CFUs for the five most frequent fungi **(Table 2)**, using Tukey's test, showed that the number for *Aspergillus* differed significantly ($p < 0.05$) from the numbers for the genera *Cladosporium*, *Curvularia* and *Fusarium.*

TABLE 1 - Frequency distribution of colony-forming units of fungi by area in São Luis, State of Maranhão, between January and December 2007.

| | South | | East | | Center | | North | | West | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (CFUs/dish) | | (CFUs/dish) | | (CFUs/dish) | | (CFUs/dish) | | (CFUs/dish) | | (CFUs) | |
| Fungus | n | % | n | % | n | % | n | % | n | % | n | % |
| *Aspergillus* spp. | 105 | 29.4 | 118 | 36.2 | 78 | 31.6 | 96 | 39.7 | 109 | 32.2 | 506 | 33.5 |
| *Penicillium* spp. | 45 | 12.6 | 86 | 26.4 | 29 | 11.7 | 73 | 30.2 | 51 | 15.1 | 284 | 18.8 |
| *Cladosporium* spp. | 64 | 18.0 | 40 | 12.3 | 53 | 21.5 | 16 | 6.6 | 41 | 12.1 | 214 | 14.2 |
| *Curvularia* spp. | 29 | 8.2 | 19 | 5.8 | 11 | 4.5 | 28 | 11.6 | 73 | 21.6 | 160 | 10.6 |
| *Fusarium* spp. | 50 | 14.1 | 6 | 5.0 | 8 | 3.2 | 11 | 4.5 | 30 | 8.9 | 115 | 7.6 |
| *Drechslera* spp. | 38 | 10.7 | 10 | 3.1 | 15 | 6.0 | 1 | 0.4 | 0 | 0.0 | 64 | 4.2 |
| *Rhizopus* spp. | 5 | 1.4 | 5 | 4.6 | 14 | 5.7 | 0 | 0.0 | 3 | 0.9 | 37 | 2.4 |
| *Mucor* spp. | 0 | 0.0 | 5 | 1.5 | 27 | 11.0 | 0 | 0.0 | 0 | 0.0 | 32 | 2.1 |
| *Neurospora* spp. | 5 | 1.4 | 8 | 2.4 | 0 | 0.0 | 1 | 0.4 | 7 | 2.1 | 21 | 1.4 |
| *Alternaria* spp. | 2 | 0.5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 19 | 5.6 | 21 | 1.4 |
| *Geotrichum* spp. | 2 | 0.5 | 0 | 0.0 | 7 | 2.8 | 7 | 2.9 | 2 | 0.6 | 18 | 1.2 |
| *Nigrospora* spp. | 5 | 1.4 | 1 | 0.3 | 0 | 0.0 | 8 | 3.3 | 1 | 0.3 | 15 | 1.0 |
| *Cunninghamella* spp. | 0 | 0.0 | 6 | 1.8 | 1 | 0.4 | 0 | 0.0 | 0 | 0.0 | 7 | 0.5 |
| *Scedosporium* spp. | 3 | 0.8 | 0 | 0.0 | 1 | 0.4 | 1 | 0.4 | 1 | 0.3 | 6 | 0.4 |
| *Chaetomium* spp. | 2 | 0.5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 0.1 |
| *Verticillium* spp. | 2 | 0.5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 0.1 |
| *Trichosporon* spp. | 0 | 0.0 | 1 | 0.3 | 0 | 0.0 | 0 | 0.0 | 1 | 0.3 | 2 | 0.1 |
| *Exserohilum* spp. | 0 | 0.0 | 0 | 0.0 | 2 | 0.8 | 0 | 0.0 | 0 | 0.0 | 2 | 0.1 |
| *Acremonium* spp. | 0 | 0.0 | 1 | 0.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 0.07 |
| *Absidia* spp. | 0 | 0.0 | 0 | 0.0 | 1 | 0.4 | 0 | 0.0 | 0 | 0.0 | 1 | 0.07 |
| Total | 357 | 100.0 | 326 | 100.0 | 247 | 100.0 | 242 | 100.0 | 338 | 100.0 | 1,510 | 100.0 |

CFUs: colony-forming units.

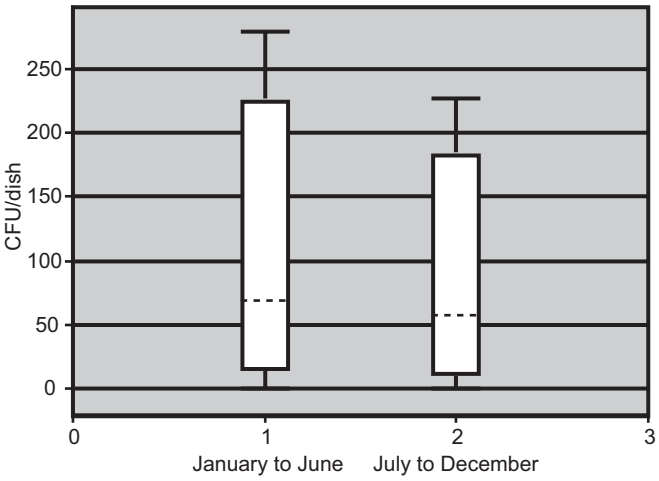


FIGURE 1 - Frequency distribution of the different genera of fungi isolated in the rainy season (January to June) and the dry season (July to December) in São Luis, State of Maranhão, between January and December 2007. Legend for Figure 1: Box plot. Medians and quartiles. CFUs per period/season. Mann-Whitney test, p = 0.96. CFUs: colony-forming units.

TABLE 2 - Comparison of the average number of CFUs for the five most frequent fungi and the frequency distribution of the recovered colony-forming units of fungi by area in São Luis, State of Maranhão, between January and December 2007.

| Fungus | Average ± SD | |
|---|---|---|
| *Aspergillus* | 42.17 ± 19.6 | |
| *Penicillium* | 23.67 ± 12.5 | |
| *Cladosporium* | 17.83 ± 22.1 | |
| *Curvularia* | 13.33 ± 10.0 | |
| *Fusarium* | 9.58 ± 10.8 | |
| Region | Total CFUs | |
| South | 357 | 23.6 |
| West | 338 | 22.4 |
| East | 326 | 21.6 |
| North | 256 | 17.0 |
| Center | 233 | 15.4 |
| Total | 1,510 | 100.0 |

CFUs: colony-forming units; SD: standart deviation.

Regarding seasonality, we observed the occurrence of fungi throughout the year, with a slight increase in the percentages of fungal genera in the months of May, August and September **(Figures 2** and **3)**.

When the percentages of CFUs collected in each of the five (northern, southern, central, eastern and western) areas of São Luis were analyzed, a statistically significant difference was observed (Table 2; $\chi^2 = 38.99$, $p < 0.0001$). The prevalence in the central and northern areas was lower than in the other areas.

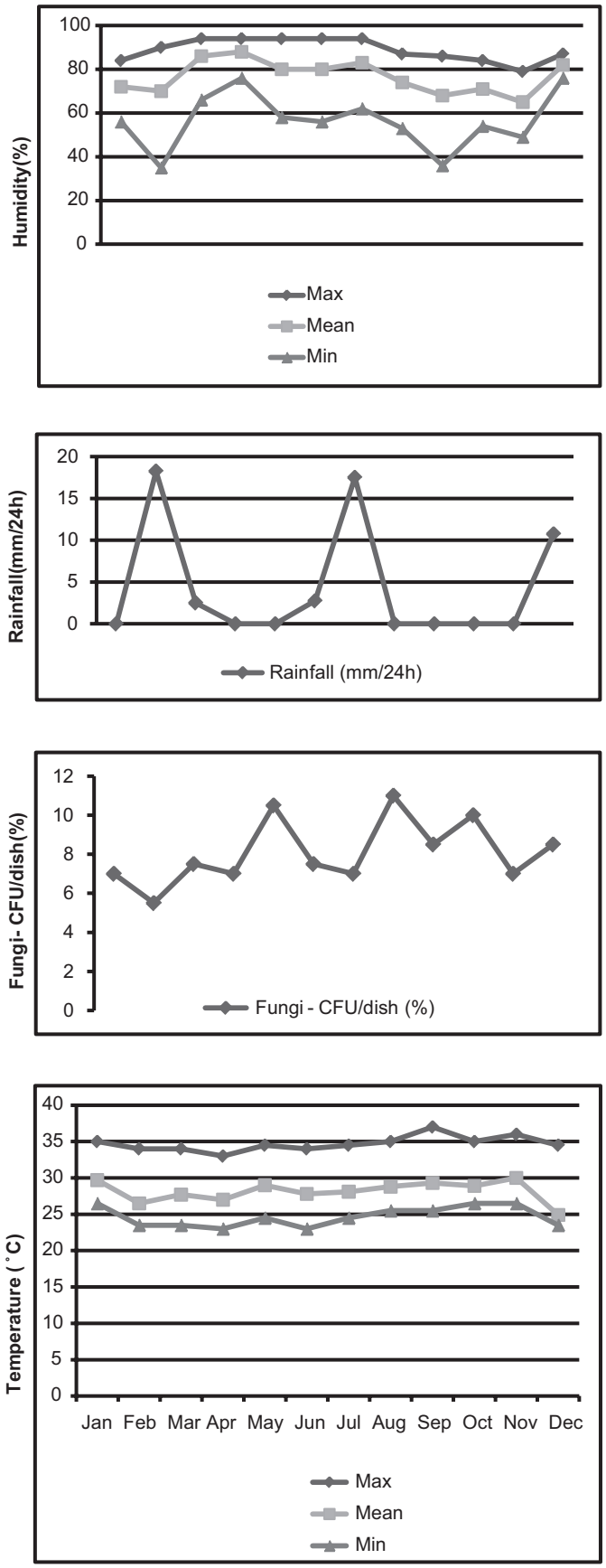


FIGURE 2 - Fungal growth and the climate variables *humidity*, *temperature* and *rainfall*, recorded monthly in São Luis, State of Maranhão, Brazil, in 2007. CFUs: colony-forming units.

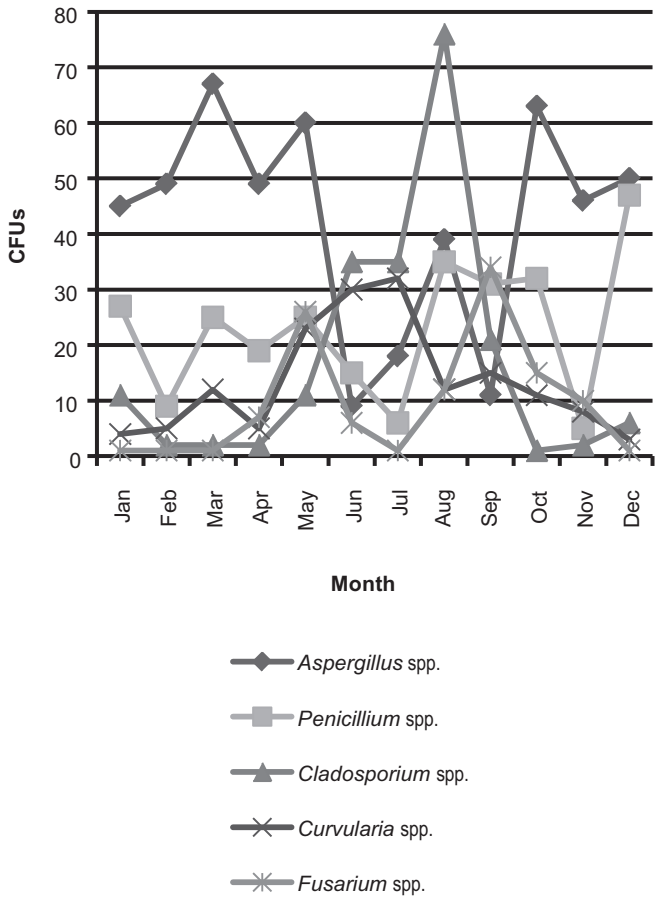


FIGURE 3 - Seasonal distribution of the five most prevalent genera isolated in São Luis, State of Maranhão, in 2007. CFUs: colony-forming units.

## DISCUSSION

*Aspergillus* was the most commonly isolated genus in the current study, and *Penicillium was* the second most commonly isolated genus. In Mexico City, *Penicillium* is also the second most common genus[15]. However, in studies performed in other countries, such as France, the USA, Chile and Cuba, *Cladosporium* has stood out as the most prevalent genus[16-18].

*Aspergillus, Penicillium*, *Cladosporium, Curvularia* and *Fusarium* are the most frequent outdoor species, according to previous research[19,20]. The genera of fungi identified in the present study were correlated with natural systems and could be useful in assessing the impact of environmental changes on the region studied.

In Brazil, the occurrence of airborne fungi in indoor and outdoor areas has been investigated in different regions[3,21,22]. In the Northeast, Fortaleza, Natal and Recife are climatically similar cities. In Fortaleza, Ceará reported that the genera *Aspergillus* and *Penicillium* prevailed[10]. Additionally, *Aspergillus* and *Penicillium* were more frequent genera in Recife and Natal, respectively[23,24]. *Curvularia* appeared with the highest frequency only in Fortaleza[8]. In Belém, Pará, *Aspergillus, Penicillium* and *Cladosporium* were reported to be the most prevalent genera isolated[25].

Despite differences in climate, in Porto Alegre, Rio Grande do Sul, *Aspergillus* was the second most frequent genus[26]. In Botucatu, São Paulo, *Cladosporium* was the most frequent genus[27]. In the metropolitan area of São Paulo, *Penicillium spp* and *Aspergillus* spp. were the dominant species both indoors and outdoors[28].

High relative humidity is essential for the development of fungi, and sunny weather favors the release of spores[29]. High temperature and humidity can result in increased concentrations of fungi[19]. A high concentration of spores in the air is important because this situation can result in increases in allergic diseases of the respiratory system[10].

In the South and the West, the greatest numbers of airborne fungal genera were isolated **(Table 1)**. These regions have greater areas of vegetation covering them. The North is near the sea and presents a low level of air pollution. In Centro (CE), a small number of airborne fungi were isolated, possibly due to higher levels of pollution.

The quantitative analysis of colony counts in the northern, southern, central, eastern and western areas was statistically significant ($p = 0.0002$) when assessing the five most frequent genera relative to the months of the year. Seasonal fluctuations were also reported in Santiago, Chile[30].

The literature reveals that *Cladosporium* has been repeatedly found indoors, particularly in house dust[13]. However, in the current paper, *Cladosporium* was found outdoors considerably more often, given that it was the second most isolated genus.

The occurrence of a great number of airborne fungi emphasizes the importance of studying airborne fungi in São Luis, Maranhão. The climate of tropical areas supports the growth of airborne fungi, resulting in high levels of fungal spores in the air, which can increase the incidence of allergic respiratory diseases related to these fungi.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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