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# AIRBORNE FUNGI IN THE CITY OF PORTO ALEGRE, RIO GRANDE DO SUL, BRAZIL

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## SUMMARY

Knowledge of anemophilous fungi in a given city or region is important for the ecological diagnosis and specific treatment of allergic manifestations induced by inhaled allergens. In order to diagnose the presence of anemophilous fungi, several qualitative and quantitative techniques are used depending on the study place. This study of fungal air spores was performed with a Rotorod Sampler®, an equipment which samples the air through a plastic rod attached to an electric engine that makes it spin fast enough to collect the particles in the air. The samples were collected once a week during 24 hours using the standard cycle of the manufacturers. A total of 52 samples were obtained from April 2000 through March 2001. The results revealed prevalence of ascosporos (50.49%), *Cladosporium* (17.86%), *Aspergillus/Penicillium* (15.03%), basidiosporos (3.84%), rusts (3.82%), and *Helminthosporium* (2.49%), and a lesser frequency of *Botrytis* (1.22%), *Alternaria* (1.19%), smuts (0.90%), *Curvularia* (0.87%), *Nigrospora* (0.61%), and *Fusarium* (0.08%). Also, 1.59% of the spores detected here could not be identified by the systematic key used. More fungal spores were observed during the summer than during the autumn.

**KEYWORDS**: Aeroallergens; Airborne; Anemophilous fungi; Spores.

## INTRODUCTION

Fungi disseminate their spores in the environment through the atmospheric air, water, insects, man, and animals. Anemophilous fungi are those whose spores are spread by the atmospheric air. Qualitative and quantitative knowledge of these fungi in a given region is of great importance and concern because they can cause several respiratory diseases in man such as asthma and rhinitis when inhaled[2].

Among the fungi groups that spread air spores and are important aeroallergens are *Zygomycota, Ascomycota, Basidiomycota* and *Deuteromycota*[18].

*Zygomycota* are represented by *Rhyzopus* and *Mucor*. *Ascomycota* deliver asci carrying ascospores and are represented by *Leptosphaeria*, *Chaetomium* and *Venturia*. *Basidiomycota* deliver basidiospores and are represented by fungi that are pathogenic for plants, i.e, rusts and smuts. *Deuteromycota* have the greatest number of fungi in their asexual reproduction phase, such as the *Aspergillus, Penicillium, Cladosporium, Alternaria* among others. *Ascomycota, Basidiomycota, Zigomycota* and *Deuteromycota* have the greatest number of well-known causative agents of allergic symptoms[5].

Spore formation by fungi go through a number of processes, and different types of spores are produced by the same fungus at the same time in response to varying environmental conditions. Spores are developed by sexual reproduction (teleomorphic or perfect phase) either through nuclear fission or without nuclear fissure, resulting in the anamorphic or imperfect phase. Thus, some spores of different fungi are morphologically similar, making them impossible to identify by gender (e.g., *Aspergillus* and *Penicillium*). Other spores are so small, transparent, or devoid of distinctive characteristics that they cannot be identified (*Phoma, Neurospora* and *Candida*). Many other spores, especially ascospores and basidiospores, are not identified as specific particles[1].

Qualitative and quantitative techniques are used to diagnose the presence of anemophilous fungi. Qualitative techniques include Petri dishes exposures or cover glass research. These are exposed for some time in the environment. This method is based on the sporulation characteristics of fungi, and thus cannot be used to identify them when fungi fail to sporulate on the medium, neither to quantitate them[8,11]. Quantitative techniques include the use of equipment such as Burkard®, Rotorod Sampler®, and others that can collect fungi spores on a continuous basis. Subsequently these can be identified through light microscopy according to their morphological characteristics and quantitated by cubic meter of air measured for 24 hours.

Research on fungi conducted in Brazil so far is mostly qualitative, and there are no data from surveys using intermittent sampling equipment like Rotorod Sampler®, Burkard® and others. Few are the publications about fungi prevalence in the atmospheric air of Brazilian cities. The published studies show a higher incidence of the following genera:

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*Cladosporium, Penicillium, Aspergillus, Rhodotorula, Aureobasidium, Candida, Fusarium, Curvularia, Rhizopus, Helminthosporium, Trichoderma*, and others at a lower incidence[9,10,12,13,14,15,16,18].

In Porto Alegre, HOMRICH (1961) investigated indoor environments and surroundings for the presence of fungi and found *Aspergillus* to be the most prevalent genus, followed by *Penicillium, Rhizopus* and *Mucor*[10]. It is important to emphasize that there is a difference between fungi present in indoor environments and in the atmospheric air. Of course, dwelling conditions determine a greater or lesser probability of fungal growth. About 300 species of fungi have already been described as allergenic. In the world, species belonging to *Alternaria, Cladosporium, Aspergillus*, and *Penicillium* are the most frequent ones[10].

Knowledge of anemophilous fungi of a given city or region is important for the ecological diagnosis and specific treatment of allergic manifestations induced by inhaled allergens[12].

The composition of the atmospheric fungal population in Porto Alegre was unknown. Since the spores of these fungi have clear antigenic properties, it is possible that such elements have played a sensitization role among atopic individuals in Porto Alegre. The identification of the fungal microbiota of our city was essential to obtain this knowledge.

The main purpose of this study was to investigate the prevalence, quantity, and seasonal variation of anemophilous fungi in the atmospheric air of Porto Alegre.

## MATERIALS AND METHODS

The sampling of fungal air spores was performed with a Rotorod Sampler® placed at 23 meters of height on the top of the building of the Federal Foundation of Medical Sciences of Porto Alegre, located downtown.

The samples were collected once a week using the standard cycle of the manufacturer (i.e., one-minute collection + 9-minute pause, for 24 hours). The equipment's collector, made of a plastic rod measuring 1.52 X 1.52 X 32 mm, was previously greased with silicone before starting the cycle. Only the external surface of the collector rods were used for the procedure, according to manufacturer's advice.

After the end of cycle, the collector rod was dyed with Calberla's dye, put under a cover glass (22 X 22 mm), and examined under a light microscope at a magnification of 40X or 100X. Counts were performed throughout the cover glass extension. The particle counts on this surface are related to the samples quantity of air per cubic meter in 24 hours sampling. The final concentration is expressed as number of particles per cubic meter of air.

In this study 52 samples were collected and 3,773 fungal air spores were detected and identified in the period of April 2000 through March 2001.

The identification of fungal air spores was performed using the systematic key recommended by the American Academy of Allergy, Asthma & Immunology (AAAAI), 1997[3].

## RESULTS

Our data on fungal air spores refer to the period of April 2000 through March 2001.

Wherever possible, fungi were identified as far as their genera, in genera groups such as *Aspergillus/Penicillium*, or only the spore form, such as ascospores and basidiospores, and their frequency was calculated in relation to the total count of spores.

Table 1 shows the results about the identification and number of anemophilous fungal spores found in this study. Note that the number of spores varied widely across the months.

**Table 1**
Total counts and percentage incidence of fungal genera recorded from April 2000 to March 2001 in Porto Alegre, Rio Grande do Sul, Brazil

| Fungi | 2000 | | | | | | | | | 2001 | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Total | Total(%) |
| *Alternaria* | 3 | 1 | 1 | 0 | 6 | 0 | 4 | 11 | 2 | 0 | 1 | 16 | 45 | 1.19 |
| Ascosporos | 10 | 7 | 30 | 40 | 319 | 51 | 44 | 177 | 19 | 74 | 61 | 1,073 | 1,905 | 50.49 |
| *Aspergillus/Penicillium* | 33 | 40 | 41 | 44 | 28 | 33 | 27 | 70 | 44 | 68 | 35 | 104 | 567 | 15.03 |
| Basidiosporos | 2 | 4 | 5 | 4 | 15 | 1 | 6 | 2 | 1 | 1 | 6 | 98 | 145 | 3.84 |
| *Botrytis* | 27 | 5 | 11 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 1.22 |
| *Cladosporium* | 9 | 2 | 6 | 4 | 315 | 25 | 16 | 116 | 9 | 6 | 4 | 162 | 674 | 17.86 |
| *Curvularia* | 1 | 1 | 9 | 2 | 3 | 2 | 2 | 4 | 2 | 2 | 1 | 4 | 33 | 0.87 |
| *Fusarium* | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0.08 |
| *Helminthosporium* | 0 | 4 | 9 | 2 | 8 | 2 | 4 | 6 | 2 | 7 | 2 | 48 | 94 | 2.49 |
| *Nigrospora* | 0 | 0 | 2 | 7 | 2 | 0 | 0 | 1 | 0 | 0 | 1 | 10 | 23 | 0.61 |
| Rusts | 3 | 4 | 1 | 4 | 11 | 0 | 3 | 17 | 3 | 7 | 7 | 84 | 144 | 3.82 |
| Smuts | 0 | 0 | 0 | 0 | 3 | 1 | 3 | 4 | 2 | 0 | 2 | 19 | 34 | 0.90 |
| Not identified | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 5 | 0 | 0 | 0 | 22 | 60 | 1.59 |
| **Total** | **88** | **70** | **116** | **110** | **743** | **115** | **109** | **413** | **84** | **165** | **120** | **1,640** | **3,773** | **100.00** |

As can be seen in Fig. 1, three peaks were observed in the months of August, November, and March within the studied period.

Table 2 shows the anemophilous spore frequency in the four seasons of the year within the studied period.

## DISCUSSION

The standardization of aeroallergens, according to CHAPMANN[6], generates comparative data from year to year and season to season. These data can allow to establish the beginning and the end of a period, to identify sporulation peaks, and to quantitate the number of spores in a given season of the year.

Our study was performed in only one site of Porto Alegre because of the impossibility of using the equipment in other sites in the same period.

**Table 2**
Total counts of fungal genera recorded in the four seasons of the year from April 2000 to March 2001 in Porto Alegre, Rio Grande do Sul, Brazil

| **Fungi** | **Seasons** | | | |
|---|---|---|---|---|
| | Autumn | Winter | Spring | Summer |
| *Alternaria* | 5 | 6 | **17** | **17** |
| Ascosporos | 47 | 410 | 240 | **1,208** |
| *Aspergillus/Penicillium* | 114 | 105 | 141 | **207** |
| Basidiosporos | 11 | 20 | 9 | **105** |
| *Botrytis* | 43 | 3 | 0 | 0 |
| *Cladosporium* | 17 | **344** | 141 | 172 |
| *Curvularia* | **11** | 8 | 8 | 7 |
| *Fusarium* | **3** | 0 | 0 | 0 |
| *Helminthosporium* | **113** | 12 | 12 | 57 |
| *Nigrospora* | 2 | 9 | 1 | **11** |
| Rusts | 8 | 15 | 23 | **98** |
| Smuts | 0 | 4 | 9 | **21** |
| Not identified | 0 | 33 | 5 | 22 |
| Total | 274 | 968 | 606 | 1,925 |

The results shown in Table 1 encompass all seasons of the year. The detected prevalence of spores was as follows (in descending order): ascosporos (50.48%), *Cladosporium* (17.86%), *Aspergillus/Penicillium* (15.03%), basidiosporos (3.84%), rusts (3.82%), and *Helminthosporium* (2.49%). Less prevalent were *Botrytis* (1.22%), *Alternaria* (1.19%), smuts (0.90%), *Curvularia* (0.87%), *Nigrospora* (0.61%), and *Fusarium* (0.08%). Also, 1.59% of the spores detected here could not be identified by the systematic key used. This difficulty is due to spores of different genera presenting the same morphologic aspect and no distinctive characteristics to allow their classification, a problem faced by everyone doing research on these fungi.

The frequency of anemophilous fungi in Porto Alegre showed an irregular pattern during the studied months (Table 1). Three peaks were identified in the months of August, November, and March (Fig. 1).

Our findings failed to correlate with the degree of environmental pollution or with winds speed.

In Rio of Janeiro, PASSARELLI *et al.*[16] detected *Rhodotorula* and *Cladosporium* with marked seasonal periodicity from May to October, *Penicillium* with the same incidence throughout the year except for December and January, when incidence rates were decreased. The distribution of *Aspergillus* was uniform with no seasonal variation, and *Fusarium* had no defined seasonal periodicity; the other fungi showed no seasonal variation.

In this study, 3,773 fungal spores were detected and identified. All genera were found in every season of the year (Table 2), except for *Fusarium, Botrytis, Nigrospora* and smuts. *Aspergillus/Penicillium*, ascosporos, basidiosporos, *Nigrospora,* rusts and smuts showed a higher incidence in summer, while *Cladosporium* had a higher incidence in winter and *Botrytis, Curvularia and Helminthosporium* in autumn; *Alternaria* was found at the same number in spring and summer. *Fusarium* was not found in winter, spring, and summer, *Botrytis* was not found in spring and summer, and smuts were not detected in autumn.

Results in Table 2 show a higher incidence of fungal spores in the summer, and a lower incidence during the fall months. According to BURGE *et al.*[5] the higher prevalence of fungal spores was observed

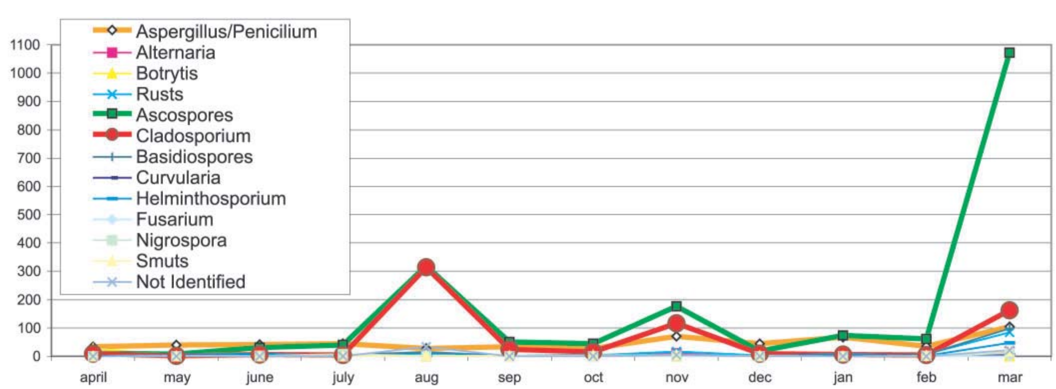


**Fig. 1** - Variation in total fungal genera recorded from April 2000 to March 2001 in Porto Alegre, Rio Grande do Sul, Brazil.

during the dry and hot seasons. In our study, however, we did not measure the air humidity to verify this observation.

In a study performed in a few Brazilian cities, OLIVEIRA LIMA *et al.*[14] concluded that the most frequent fungi in the atmosphere of these cities were *Aspergillus, Penicillium, Cladosporium, Fusarium, Rhodotorula, Rhizopus, Aureobasidium Curvularia, Helminthosporium, Candida, Trichoderma*, and *Phoma*, as well as others with a lesser incidence. The genera *Alternaria* showed an irregular pattern of incidence. According to the authors, these findings match those of other American and European countries.

CHAPMANN[6] pointed out that the prevalence of air spores does not always identify clinically important inhalant allergens. Indeed, in spite of the high number of *Cladosporium* spores found here, it presented low sensibility.

Our overall data indicate the presence of a large number of fungal air-spores in Porto Alegre, with a higher incidence of ascospores, *Cladosporium, Aspergillus*, and *Penicillium.* In general, those fungi with a higher incidence in our study are the same as those most frequently found in other Brazilian cities through qualitative studies[10,12,13,15,18].

The occurrence of a great number of fungal spores emphasizes the importance of studying anemophilous fungi in Porto Alegre. In addition, the anemophilous fungi found here reinforce Bernd's finding[4] that 13.8% of patients with respiratory allergies showed sensibility to air fungi.

The continuous presence of spores of genera *Cladosporium, Aspergillus*, and *Penicillium* alerts health professionals to the importance of continuously monitoring patients with allergies for these microorganisms.


## RESUMO

### Fungos anemófilos na cidade de Porto Alegre, Rio Grande do Sul, Brasil

O conhecimento dos fungos anemófilos em determinada cidade ou região é importante para o diagnóstico etiológico e o tratamento específico de manifestações alérgicas provocadas por estes alérgenos inalantes. Várias técnicas são preconizadas para coleta e identificação de fungos anemófilos na dependência do local estudado. Nesta pesquisa foi utilizado o equipamento Rotorod Sampler® que retira a amostra do ar através de um bastão preso a um motor elétrico que o faz girar rapidamente e as partículas suspensas no ar são recolhidas pelo bastão. A coleta foi realizada uma vez por semana, durante 24 horas, correspondendo a um ciclo de coleta. Totalizando 52 coletas entre abril 2000 a março de 2001. Os resultados apresentaram-se com prevalência de ascosporos (50,49%), *Cladosporium* (17,86%), *Aspergillus/Penicillium* (15,03%), basidiosporos (3,84%), rusts (3,82%) e *Helminthosporum* (2,49%), com menor freqüência *Botrytis* (1,22%), *Alternaria* (1,19%), smuts (0,90%), *Curvularia* (0,87%), *Nigrospora* (0,61%) e *Fusarium* (0,08%). Não foram possíveis de serem identificados 1,59% dos esporos de fungos anemófilos observados neste estudo. O maior número de esporos foi observado no verão e o menor no outono.


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