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# Fungi associated with three houses in Havana (Cuba)

MARIA JESÚS AIRA, TERESA IRENE ROJAS and VICTORIA JATO

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Aerobiological sampling was carried out during two consecutive years, 2000 and 2001, in order to ascertain the level of fungal spores present in indoor and outdoor environments in the city of Havana. The samples were collected using Burkard (Burkard Personal Culture and Burkard Personal Slide) and Andersen traps. Sampling was completed by using adhesive tape. We identified 19 spore types, of which the most frequent were *Aspergillus* (*A. flavus*, *A. niger* and *A. clavatus*), *Penicillium* (*P. citrinum*) and *Cladosporium* (*Cl. cladosporioides*). The other fungal types identified include important potential allergens and/or phytopathogens.

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The great abundance of fungi in different environments combined with the small size and ease of dispersion of their propagules favours the presence of high concentrations of fungal spores in the air, in both indoor and outdoor environments. The climatologic conditions in tropical countries, especially the high humidity and temperatures recorded during the rainy season, favour the development of fungi and increases the level of spores in the atmosphere.

Respiratory allergy to fungi was only described and verified 50 years ago due, among other reasons, to the degree of difficulty involved in its study and the limitations of diagnostic tests. However, this type of illness is becoming increasingly common, often accompanied by persistent symptoms that are frequently related to asthma (Palmas et al. 1997). Fungal spores, as well as mites, pollen and other biological particles in indoor environments play an important role in the development of this pathology, probably as a result of the prolonged duration of their presence in homes and the high concentrations that they may attain. The incidence of sensitisation to fungi is variable in different parts of the world and is in part due to the variability of extracts used as well as exposure considerations; however, a progressive increase of fungal sensitive persons has been detected (Cosentino & Palmas 1996).

To date there have been few studies of the atmospheric fungal content in Cuba and most of them have been carried out in libraries, archives and warehouses (Gómez et al. 1989, Fernández et al. 1990, Rojas et al. 1993 *a, b*, 1996, 2000). However, growing interest is now being shown in the levels of biological particles in the air of homes (Aira et al. 2001, Castañeda et al. 1996).

This interest is focussed, on evaluating possible impacts on respiratory health and, on determining which biological particles have the greatest effect on the deterioration of

cultural heritage. Consequently, collaboration was initiated between the University of Havana (Cuba) and the Universities of Santiago de Compostela and Vigo (Spain) to study these problems.

This paper presents the first results of this collaboration and focuses on the fungal content of houses located in the city of Havana and in the outdoor atmosphere.

## MATERIAL AND METHODS

The city of Havana is situated in the western part of the island (23°8'N, 82°22'W) and has more than 2 million inhabitants (Fig. 1). The climate is tropical and it is characterised by an annual mean temperature of 25.5°C and annual mean precipitation of 1,511 mm, and 78% relative humidity. Biogeographically it belongs to the Neotropical floral kingdom, West Cuba floral region.

Sampling was carried out in three houses located in the urban area of Havana. House A and B were sampled sequentially in the morning (11 am to 14 pm) during the second fortnight of February 2000, using a Personal Culture (B.P.C.) sampler and a Personal Slide (B.P.S.) sampler (Burkard Manufacturing, England). The area of these houses is around eighty square meters and in each of them live four people. The room with minor ventilation was chosen in both houses to indoor sampling. The outdoor sampling was carried out in the street.

In the year 2001 a new study was made in the same hour and date of February using an Andersen culture plate impactor (Andersen 1958), (Lanzoni manufacturing). Monitoring of house B was impossible to do in this year and it was replaced by another house (C), which was chosen because of its similar characteristics. It is near the other houses and their area and construction materials are similar, but only three people live there. All the rooms in this house are communicated with a terrace where the outdoor sampler was made.

Every year samples were collected inside and outside the houses, and the values of relative humidity and temperature have been recorded (Table I). Sunny and rain-free days were selected to sampling in every case. Sabouraud dextrose agar medium was used in both BPC and Andersen samplers. The sampling by B.P.C.

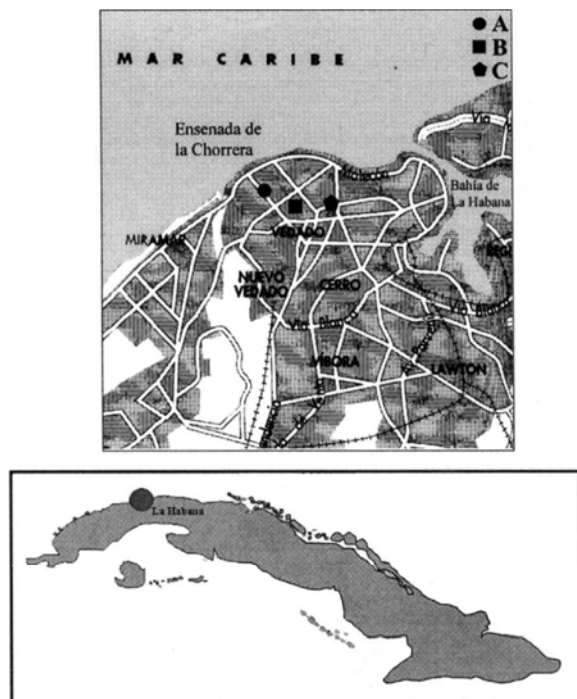


Fig. 1. Location of collection site.

sampler was carried out for five minutes, at a previously calculated sampling rate of 10 litres/minute. The Andersen trap also sampled for five minutes, at a rate of 24 litres/minute.

Samples were also collected from walls using adhesive tape. The tape was lightly pressed against the area to be sampled, usually damp and with discoloured patches.

To identify the colonies, isolated cultures were prepared and the plates were incubated in specific medium for 5–7 days at a temperature of 27° C, using specialised bibliography (Ellis 1971, 1976, Gravesen et al. 1994, Klich & Pitt 1994, Hoog et al. 2000, among others).

The data of the B.P.C. and Andersen sampler are expressed as colony forming units CFU. All the surface exposed in the B.P.S. sampler was read and the fungal spores identified and the result is presented as spores  $m^{-3}$ . The spores collected with adhesive tape have only of qualitative value.

## RESULTS

After applying the correction factor, the results obtained in February 2000 (B.P.C.) and 2001 (Andersen) in the three houses are showed in Fig. 2. The values obtained in February 2000 ranged between 150 and 660 CFU  $m^{-3}$  in indoor environments and between 260 and 820 CFU  $m^{-3}$  in outdoor

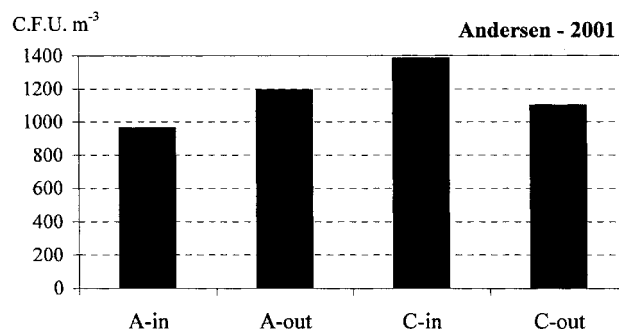
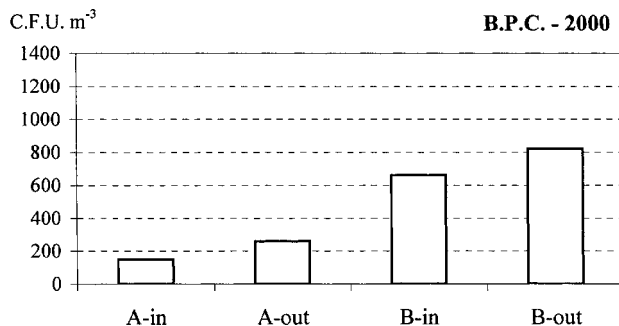


Fig. 2. Total number of CFU  $m^{-3}$  collected with B.P.C. in 2000 and Andersen traps in 2001 year (In – indoors; Out – outdoors).

environments (Houses A and B respectively). These results indicate a much higher total content of colony-forming units in the sampling carried out with the Andersen trap in 2001. In this case, the levels ranged between 960 and 1381 CFU  $m^{-3}$  in indoor environments and between 1190 and 1098 CFU  $m^{-3}$  in outdoor environments (Houses A and C respectively). The different sampler used and the higher relative humidity registered in 2001 could be the reason of the notable increase in the number of CFU  $m^{-3}$ .

Indoor/outdoor comparisons show that the number of CFU  $m^{-3}$  is higher outside the houses in both years. These differences are smaller in the sampling carried out in 2001 with the Andersen sampler. The indoor level of spores was only higher than the outdoor level in House C, probably due to the fact that the windows of this house, which overlook a spacious terrace roof, are left open all day.

The most frequent fungal types in both indoor and outdoor environments belonged to the genera *Aspergillus*, *Cladosporium* and *Penicillium* (Fig. 3), specifically the 444 CFU  $m^{-3}$  of *Penicillium* in House B (outdoors) and the high

Table I. Reference of samples and meteorological parameters.

T = temperature; H = Relative humidity.

| Year 2000 |            |           | Year 2001 |          |           |
|-----------|------------|-----------|-----------|----------|-----------|
|           | T/H        | Reference |           | T/H      | Reference |
| Indoor    | 25°C/58%   | A-in      | Indoor    | 27°C/73% | A-in      |
| Outdoor   | 26°C/54%   | A-out     | Outdoor   | 26°C/72% | A-out     |
| Indoor    | 26.7°C/64% | B-in      | Indoor    | 28°C/64% | C-in      |
| Outdoor   | 26.9°C/61% | B-out     | Outdoor   | 26°C/70% | C-out     |

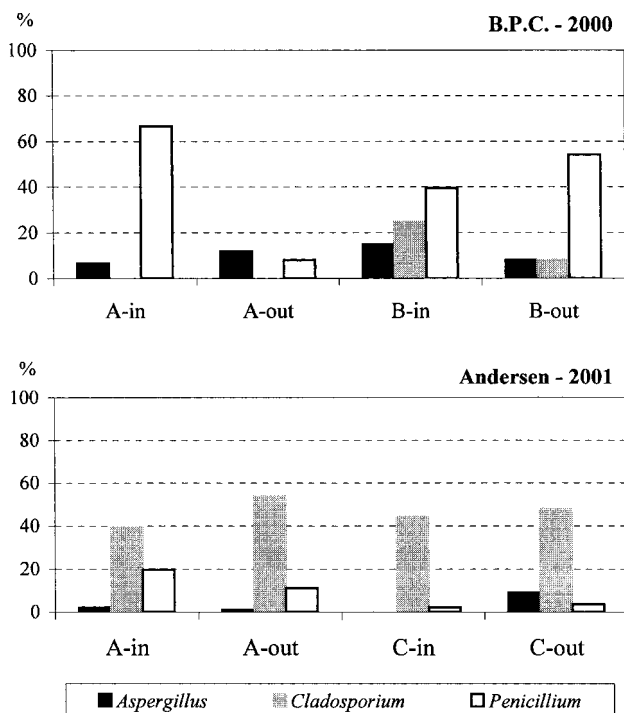


Fig. 3. Percentage of CFU m<sup>-3</sup> of the principal fungal types (In – indoors; Out – outdoors).

quantities of *Cladosporium* in A-out (2001) and C-in (outdoors House A and indoors House C respectively). The CFU m<sup>-3</sup> levels obtained in the different stages of the Andersen sampler (Table II) showed a greater concentration on plate 2 (the size of these particles would enable their deposition in the trachea) and on plates 4 and 5 (whose spore sizes would enable them to reach the bronchi), which are precisely those levels where a greater quantity of *Aspergillus*, *Cladosporium* and *Penicillium* colonies accumulated. The percentages of these three types with regard to total identified colonies on the different culture plates was 73% (A-in, 2000), 20% (A-out, 2000), 79% (B-in), 70% (B-out), 61% (A-in, 2001), 66% (A-out, 2001), 47% (C-in) and 57% (C-out).

Three species of *Aspergillus* were abundant. *Aspergillus clavatus* forms greyish or bluish green colonies and has ellipsoidal conidia. It is cosmopolitan and abundant in soil and may cause aspergillosis and lung infections. *Aspergillus flavus* is characterized by its yellowish green colonies and globular conidia. It is an opportunistic pathogen, which contains allergens and produces carcinogenic aflatoxin (Deshpande & Gangawane 1997). *Aspergillus niger* forms colonies that stand out because of their black conidiophores; its conidia are globular, rough or have spines. It is cosmopolitan and very frequent in house dust, on the ground in fruits and seeds and in textile material. It may cause damage in the storage of plant products, especially in warm climates, as well as causing respiratory allergies.

Among the identified *Penicillium* species, it is worth highlighting the presence of *P. citrinum*, which has cotton-like colonies of bluish grey colour and spherical conidia with smooth or slightly rough walls. It is known to have caused

Table II. Number of CFU m<sup>-3</sup> of each level of the Andersen trap in 2001.

A, C – houses.

As – *Aspergillus*; C – *Cladosporium*; P *Penicillium*.

| Level | A-in | A-out | C-in | C-out |
|-------|------|-------|------|-------|
| 1     | 60   | 91    | 36   | 45    |
| 2     | 40   | 36    | 436  | 54    |
| 3     | 100  | 82    | 73   | 100   |
| 4     | 380  | 482   | 645  | 454   |
| 5     | 300  | 445   | 173  | 436   |
| 6     | 80   | 54    | 18   | 9     |

| Level | A-in  | A-out | C-in  | C-out |
|-------|-------|-------|-------|-------|
| 1     | –     | –     | –     | –     |
|       | 20 P  | 20 C  | –     | –     |
| 2     | 10 As | –     | –     | 20 As |
|       | –     | 20 C  | –     | –     |
|       | –     | –     | –     | 40 P  |
| 3     | 10 As | –     | –     | –     |
|       | 10 C  | 40 C  | –     | 100 C |
|       | 60 P  | –     | 10 P  | –     |
| 4     | –     | 10 As | –     | 40 As |
|       | 280 C | 320 C | 610 C | 430 C |
|       | 30 P  | –     | –     | –     |
| 5     | –     | –     | –     | 40 As |
|       | 90 C  | 240 C | –     | 410 C |
|       | 50 P  | 130 P | 30 P  | –     |
| 6     | –     | –     | –     | –     |
|       | 30 P  | –     | –     | –     |

Table III. Others fungal spores identified in 2000 and 2001 apart from *Aspergillus*, *Cladosporium*, and *Penicillium*.

|                          | House A | House B | House C |
|--------------------------|---------|---------|---------|
| Agaricales               | *       | –       | –       |
| <i>Alternaria</i>        | *       | *       | *       |
| <i>Circinella</i>        | –       | –       | *       |
| <i>Curvularia</i>        | *       | *       | *       |
| <i>Drechslera</i>        | –       | *       | –       |
| <i>Exserohilum</i>       | *       | *       | –       |
| <i>Fusarium</i>          | *       | *       | *       |
| <i>Gliomastix</i>        | *       | *       | *       |
| <i>Leptosphaeria</i>     | –       | *       | *       |
| <i>Monodictys</i>        | *       | *       | –       |
| <i>Myxomycetes</i>       | *       | *       | *       |
| <i>Paraphaeosphaeria</i> | –       | *       | *       |
| <i>Pleospora</i>         | –       | *       | *       |
| <i>Spegazzinia</i>       | *       | *       | –       |
| <i>Stemphylium</i>       | –       | *       | *       |
| <i>Torula</i>            | *       | –       | *       |

urinary tract and lung infections, as well as keratitis (Perry 1964, Mori et al. 1987, Gughani et al. 1976).

Finally, *Cladosporium cladosporioides* was the commonest fungal types within the genus. Characterized by its smooth conidia, it is cosmopolitan, a saprophyte and a plant parasite, and has been cited as the cause of lung, skin and nasal infections.

Other fungal types that were identified (Table III) in smaller quantities on the culture plates or adhesive tape, by means of the morphology of their conidia, in the three houses were: *Alternaria*, *Curvularia*, *Fusarium*, *Gliomastix* and *Myxomycetes*. The following were identified in two of the houses: *Exserohilum*, *Monodictys*, *Spegazzinia* (Houses A and B), *Leptosphaeria*, *Paraphaeosphaeria*, *Pleospora*, *Stemphylium* (Houses B and C) and *Torula* (Houses A and C). Agaricales spores were identified in House A, *Dreschlera* spores in House B and *Circinella* spores in House C. In addition to these, different kinds of yeast colonies, especially *Rhodotorula*, were also observed.

## DISCUSSION

We identified 19 fungal types in the indoor and outdoor samples taken in Havana, the common genera were: *Aspergillus*, *Cladosporium* and *Penicillium*. Other fungal types identified were *Alternaria*, *Curvularia*, *Fusarium*, *Gliomastix*, *Myxomycetes*, *Exserohilum*, *Monodictys*, *Spegazzinia*, *Leptosphaeria*, *Paraphaeosphaeria*, *Pleospora*, *Stemphylium*, *Torula*, *Dreschlera*, *Circinella* and numerous yeasts, mainly *Rhodotorula*. *Nigrospora*, *Bipolaris*, *Periconia*, *Staphylo-trichum* and *Tritirachium* are fungal types identified in Cuba's atmosphere by different authors that did not appear in this sampling (Arnold et al. 1987, Rojas 1998).

The majority of these fungi are cosmopolitan; many are saprophytes that live on plants, wood or on the ground. Some are pathogens that may produce allergies or plant pathogens.

The levels of CFU m<sup>-3</sup>, especially those obtained in the 2001 samples, can be considered high, more so taking into account that these results were obtained in the dry season (February), during which the levels of spores in the air are expected to decrease due to the lower atmospheric humidity. Although the sampling carried out in House A with the B.P.C. and the Andersen trap correspond to different years, the difference in the levels obtained with the two systems reinforce what different authors have cited as regards the greater efficiency of the Andersen method (Buttner & Stetzenbach 1993).

A high percentage of the identified propagules were found in stages 4 and 5, corresponding to the deposition of particles measuring from 1.1 to 3.3 microns, which would be able to reach the trachea and bronchi.

Some of the species identified in this study (*Gliomastix*, *Exserohilum*, *Monodictys*, *Spegazzinia*, *Leptosphaeria*, *Paraphaeosphaeria*, *Pleospora*, *Stemphylium*, *Torula*, *Dreschlera*, *Circinella*) were collected by lightly pressing a piece of adhesive tape against patches or altered areas of walls. Numerous authors have highlighted the difficulty of trapping fungal propagules present in indoor and/or outdoor environments with the customary suction methods; certain propagules can even inhibit the growth of others and thereby prevent the development of their colonies in viable traps (Bush 1989). Likewise, the limited dispersion capacity of the spores of some fungi makes them difficult to trap. The use of complementary methods, such as adhesive tape, is therefore very important in order to complete the information obtained.

## CONCLUSIONS

In relation to the efficiency of the different sampling methods employed, the high number of colonies recorded by the Andersen trap stands out in comparison with those of the B.P.C. The samples obtained using the B.P.S. trap, with the same duration as the previous ones (5 minutes), were sterile, probably due to the fact that this trap has a longer exposure time. It is also worth highlighting the usefulness of collecting samples in a direct way by using adhesive tape in addition to suction methods, although the information obtained is only of a qualitative nature.

This paper is a first step towards the identification of fungi whose spores are present, to a greater or lesser degree, in the atmosphere. The obtained results, evaluated from a quantitative point of view, indicate in general values similar to those obtained by other authors in different sampling areas, and they are considerably higher in outdoor environments. However, the presence of important plant pathogens and the abundance of fungi of recognized clinical significance, call for further research in the Cuban capital.

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