

Fungal biodiversity in indoor environments in Havana, Cuba

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Abstract A study was made of the indoor mycobiota in a range of public and private buildings (libraries, museums, laboratories and offices, warehouses, homes and a school) in the city of Havana (Cuba). Culturable airborne fungi were sampled using a slit-to-agar impactor (Chirana aeroscope). High levels of contamination were recorded, with more than 700 colony-forming units per cubic metre of air in 85% of the buildings sampled, and more than 1,000 in 55% of buildings. Of the 28 genera and 31 species identified, the most common were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum*, *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*. Genus and species diversity was analysed, and new findings were obtained regarding Cuba's atmospheric ecosystem.

Keywords Airborne viable fungi · Indoor environment · Havana

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1 Introduction

The biodiversity of fungi is immense; they are found over the whole range of climates and in every geographical area. In tropical climates, where prevailing heat and humidity conditions favour growth and sporulation, airborne fungal propagule concentrations tend to be high; in indoor environments, this can pose a risk to human health, prompting biodeterioration of materials (De Lucca 2007).

Between 10 and 15% of allergic respiratory diseases are linked to fungal contamination, and especially to opportunistic pathogens including *Aspergillus fumigatus*, *A. niger*, *A. terreus* and *A. flavus*. Spores from these fungi are easily released into the air, and their small size allows them to penetrate the lower airways when inhaled, giving rise to serious diseases including pulmonary aspergillosis (Hedayati et al. 2005). Monitoring of air quality in the workplace is becoming an increasingly essential measure for the prevention of occupational diseases (Guglielminetti et al. 2001).

A number of authors have noted that the most common fungi in indoor environments are *Aspergillus*, *Penicillium* and *Cladosporium*; the dominance of one or another of these genera depends largely on local climate and on the specific conditions of the sampling site (Shelton et al. 2002).

Few aeromycological studies in tropical climates have used volumetric sampling methods; this hinders the interpretation of findings and the comparison of

results with those obtained using standard methods. In Cuba, the earliest research addressed the link between fungi and allergies, but failed to provide identification at species level (Cadrecha and Fernández 1955; Seidel and Rauber 1970). Since then, a number of studies have focussed on airborne filamentous fungi, both outdoors (Arnold et al. 1987; Castañeda et al. 1996; Herrera et al. 2003; Rojas et al. 2007) and indoors (Aira et al. 2002; Borrego et al. 2008, 2010; Labarrere et al. 2003; Rojas et al. 2002, 2008; Sánchez et al. 1988; Vaillant et al. 1989). While these papers give an idea of the most abundant genera, and in some cases species, they deal with only one type of environment.

This study sought to characterise the indoor mycobiota in a range of public and private buildings, to identify the most abundant species and to assess fungal biodiversity.

2 Materials and methods

Air sampling was carried out at 20 points in six different types of building (libraries, museums, laboratories and offices, warehouses, homes and schools), in the city of Havana (Cuba). The number of sampling points selected in each building was governed by its surface area. Three replicates were performed for each sampling point. Results were expressed as the average number of colony-forming units per cubic metre (cfu/m^3) of air.

Culturable airborne fungi were sampled using a slit-to-agar impactor aeroscope (Chirana, Czechoslovakia) with a suction capacity of 29 l of air/min; this method is comparable to conventional techniques for viable spore sampling (Rojas et al. 2008).

Taxonomic identification was based on standard monographs and taxonomic keys for the genera and species isolated (e.g. Barnett and Hunter 1998; Domsch et al. 1993; Samson et al. 2002) and on specialist literature focussing on *Aspergillus*, *Penicillium* and *Cladosporium* (Ellis 1971; Ho et al. 1999; Klich and Pitt, 1994; Pitt 2000). The taxa identified were classified by abundance and relative frequency using the ecological categories formulated by Esquivel et al. (2003).

Temperature and relative humidity at each sampling point were measured in situ during sampling.

3 Results

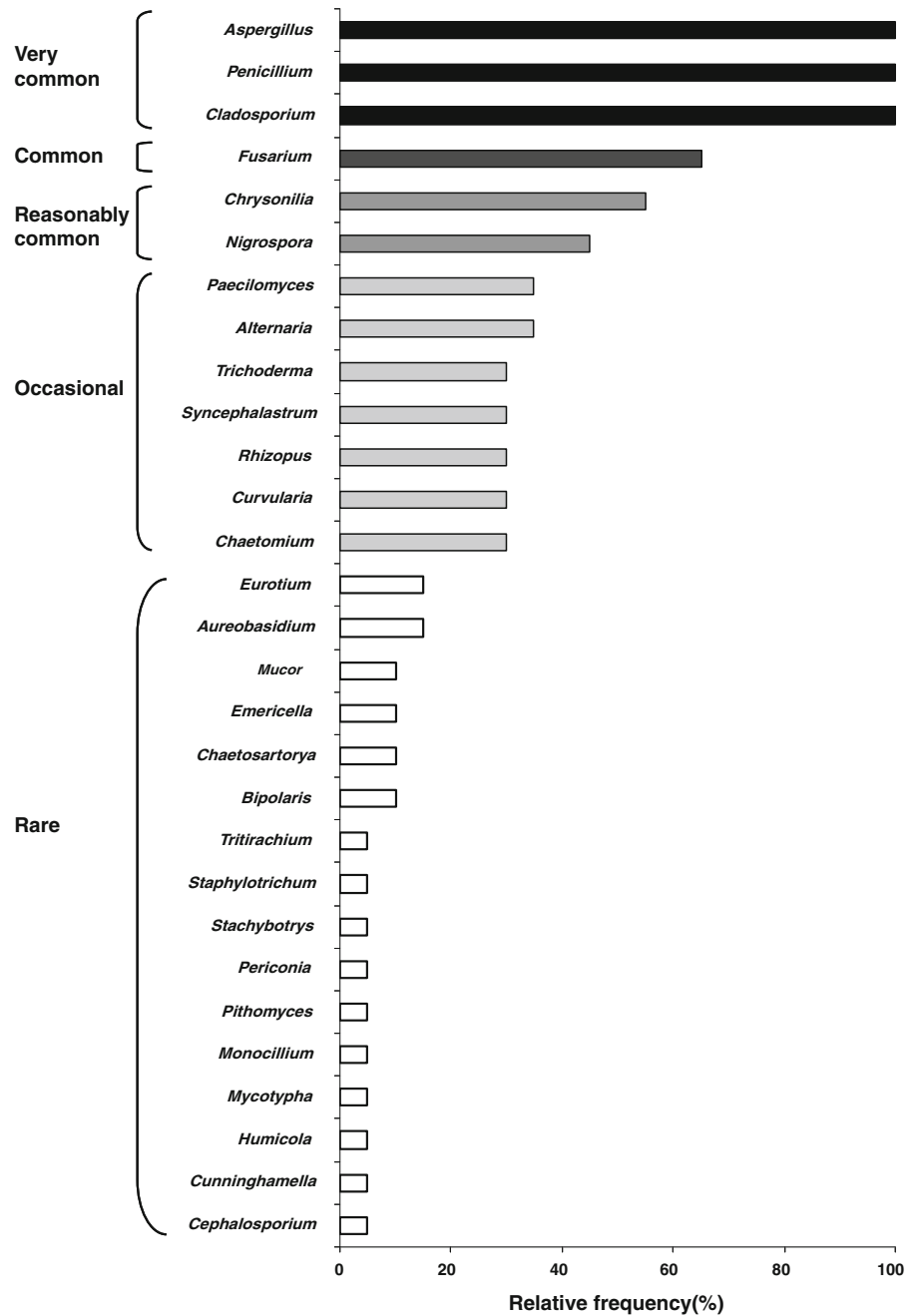
Fungal concentrations at the 20 sampling points tested ranged from $423 \text{ cfu}/\text{m}^3$ in one of the museums to $3,738 \text{ cfu}/\text{m}^3$ in one of the laboratories (Table 1). Concentrations exceeded $700 \text{ cfu}/\text{m}^3$ at 85% of sampling points, and $1,000 \text{ cfu}/\text{m}^3$ at 55%. Fungal contamination was greatest in laboratories and offices ($2,585 \text{ cfu}/\text{m}^3$), followed by homes ($1,879 \text{ cfu}/\text{m}^3$) and schools ($1,796 \text{ cfu}/\text{m}^3$).

The 1,231 fungal isolates obtained belonged to 28 genera, with a clear predomination of *Aspergillus*, *Penicillium* and *Cladosporium* (457, 263 and 249 isolates, respectively). The number of isolates identified as *Chrysonilia*, *Fusarium*, *Chaetomium*, *Emericella*, *Paecilomyces*, *Nigrospora*, *Curvularia*, *Syncephalastrum*, *Alternaria*, *Trichoderma* and *Rhizopus* ranged between 10 and 39, while the remaining genera (*Aureobasidium*, *Periconia*, *Eurotium*, *Bipolaris*, *Mucor*, *Cephalosporium*, *Tritirachium*, *Monocillium*, *Cunninghamella*, *Staphylotrichum*, *Stachybotrys*, *Pithomyces*,

Table 1 Colony-forming units per cubic metre (cfu/m^3), number of isolated and meteorological parameters in 20 places of study

	Places	cfu/m^3	No. isolated	Tmean	RH mean
Libraries	Li1	1,050	43	28.0	68.0
	Li2	834	36	28.4	67.8
	Li3	714	224	26.0	71.0
	Li4	693	54	29.5	74.0
	Li5	552	34	30.0	74.0
Museums	Mu1	966	23	27.4	70.0
	Mu2	928	47	27.6	73.4
	Mu3	920	32	29.3	73.0
	Mu4	423	62	28.9	81.0
Laboratories and offices	La1	3,738	45	27.0	78.0
	La2	3,118	37	27.0	78.0
	La3	2,226	29	27.0	85.0
	La4	1,260	15	24.0	78.0
Stores	St1	1,580	124	25.3	78.0
	St2	1,000	75	23.8	98.0
	St3	912	87	22.6	94.4
Home	Ho1	2,070	38	31.0	61.6
	Ho2	1,980	82	27.0	72.0
	Ho3	1,589	88	29.5	72.4
School	Sc1	1,796	56	28.0	71.9

Fig. 1 Relative frequency of fungal genus identified in 20 places sampled



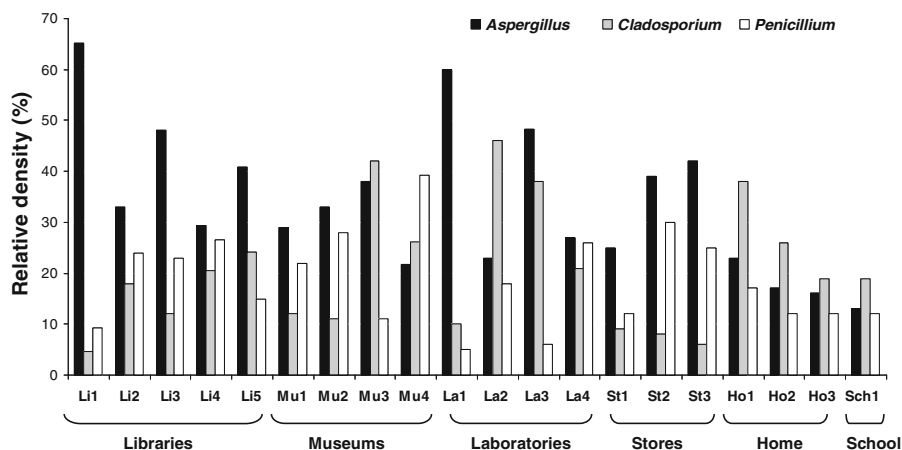
Mycotypha and *Humicola*) were represented by fewer than 10 isolates each.

In terms of relative frequency, *Aspergillus*, *Penicillium* and *Cladosporium* were classed as “very common” (relative frequency = 100%); *Fusarium* was classed as “common”, *Chrysonilia* and *Nigrospora* as “reasonably common”, *Paecilomyces*,

Alternaria, *Trichoderma*, *Syncephalastrum*, *Rhizopus*, *Curvularia* and *Chaetomium* as “occasional”, and the remaining genera as “rare” (Fig. 1).

The abundance of the various fungi identified at each sampling point was analysed in terms of relative density. *Aspergillus* predominated in libraries, museums, laboratories and offices, and warehouses, while

Fig. 2 Relative density of *Aspergillus*, *Cladosporium* and *Penicillium* in 20 places sampled



Penicillium was the most abundant genus in homes and schools. The greatest relative density for *Aspergillus* (65%) was recorded in one of the libraries; maximum density for *Penicillium* (39%) was noted in one of the museums, while *Cladosporium* exhibited peak density (46%) in a laboratory (Fig. 2). Of the remaining genera, high values for relative density were found only for *Fusarium* and *Syncephalastrum* in libraries, for *Chrysionilia* in museums and schools, for *Rhizopus* and *Curvularia* in laboratories and offices, for *Emericella* in warehouses, and for *Fusarium* and *Chrysionilia* in homes.

Of the 31 species identified, 18 belonged to the genus *Aspergillus* and two of its teleomorphs, 4 to *Cladosporium* and 7 to *Penicillium* (Table 2). The species most commonly isolated at all sampling points were *A. flavus* and *A. niger* (relative frequency, 100%), followed by *Penicillium citrinum*, *P. chrysogenum*, *Cladosporium cladosporioides* and *Cladosporium sphaerospermum* (RF, 77.7–88.8%). The most abundant species in libraries were *A. flavus*, *A. niger*, *P. chrysogenum* and *C. cladosporioides*, with relative densities of 18–21%; in museums, *A. niger*, *P. chrysogenum* and *C. cladosporioides* (5–15%), and in warehouses *Aspergillus versicolor*, *P. chrysogenum* and *C. cladosporioides* (10–16%).

The greatest genus and species diversity (Fig. 3) was recorded in warehouses (13–19 genera; 14–19 species), in one of the museums (14 genera) and in one of the libraries (21 species). A number of genera and species were recorded here for the first time in indoor ecosystems in Cuba: *Cunninghamella*, *Humicola*, *Monocillium* and *Staphylotrichum*, and the species *Aspergillus clavatus* Dezm, *Aspergillus parasiticus*

Speare, *Aspergillus puniceus* Kwon & Fennell, *Aspergillus sclerotiorum* Huber, *Aspergillus tamarii* Kita, *Aspergillus ustus* (Bainier) Thom & Church, *Emericella nidulans* (Eidam) Vuill. (anamorph *A. nidulellus* Samson & W. Gams), *Eurotium chevalieri* Margin (anamorph *A. equitis* Samson & W. Gams) and *Penicillium simplicissimum* (Oudem.) Thom.

4 Discussion

4.1 Quantitative analysis

Elevated fungal propagule concentrations were recorded at most of the sampling points. Similar findings are reported for other countries, with average concentrations of around 1,000 cfu/m³ both in Mexico (Ponce-Caballero et al. 2010) and in Colombia (Mateus et al. 2004).

Although there are no generally accepted standards regarding the health implications of different fungal concentrations, threshold values have been put forward by a number of authors. Burge (2002) suggests that levels <200 cfu/m³ can be considered harmless, while levels >1,000 cfu/m³ are unhealthy, and levels >2,000 cfu/m³ pose a serious health risk. Using those thresholds, the greatest health risk would be posed by one of the laboratories in the present study, where counts exceeded 2,000 cfu/m³, but levels of contamination were also high enough to be rated “unhealthy” in homes, in the other three laboratories, the school and one of the libraries (>1,000 cfu/m³).

Reponen et al. (1992) report that fungal spore levels >1,000 cfu/m³ in indoor environments are indicative

Table 2 Relative density of *Aspergillus*, *Penicillium* and *Cladosporium* species identified in 20 places sampled

	Relative density (%)									
	Li1	Li2	Li3	Mu2	Mu3	Mu4	St1	St2	St3	RF (%)
<i>Aspergillus flavus</i> Link	29.4	5.1	19.8	15.6	3.1	12.5	3.1	3.7	10.6	100
<i>Aspergillus niger</i> van Tieghem var <i>niger</i>	44	6.7	14.6	12.5	15.6	9.4	3.1	7.4	13.8	100
<i>Aspergillus terreus</i> Thom.		3.4	6.2	6.2		3.1	12.8		4.5	66.6
<i>Aspergillus fumigatus</i> Fresenius		1.7	3.2	6.2		12.5			3	55.5
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi				6.2	28.2		12.6	9.4	9	55.5
<i>Aspergillus oryzae</i> (Ahlb) Cohn			3.2	3.2	18.7			1.8		44.4
<i>Aspergillus candidus</i> Link	9			6.2		3.1				33.3
<i>Aspergillus clavatus</i> Dezm.				3.2		6.2			3	33.3
<i>Eurotium chevalieri</i> Margin (**)			1		4.7				6	33.3
<i>Aspergillus ustus</i> (Bain.) Thom & Church			1			3.1	1.2			33.3
<i>Emericella nidulans</i> (Eidam) Vuill. (*)							3.1		7.6	22.2
<i>Aspergillus ochraceus</i> Wilhelm		3.4							3	22.2
<i>Aspergillus sclerotiorum</i> Huber			2					3.7		22.2
<i>Aspergillus sydowii</i> (Bain & Sart.) Thom & Church			3.1				3.1			22.2
<i>Aspergillus wentii</i> Wehmer			1						3	22.2
<i>Aspergillus</i> spp.			2.2					12.9		22.2
<i>Aspergillus caespitosus</i> Raper & Thom						9.6				11.1
<i>Aspergillus parasiticus</i> Speare			1							11.1
<i>Aspergillus puniceus</i> Kwon & Fennell			1							11.1
<i>Aspergillus tamarii</i> Kita					7.8					11.1
<i>Penicillium citrinum</i> Thom	11.8	3.4		3.2	4.7	3.1	1.2	1.8	3	88.8
<i>Penicillium chrysogenum</i> Thom		16.9	26	6.2		25	24	22.3	3	77.7
<i>Penicillium glabrum</i> (Wehmer) Westling		10		9.3			18.9	7.4	3	55.5
<i>Penicillium purpurogenum</i> Stoll		6.7		3.2	3.1			3.7	1.5	55.5
<i>Penicillium simplicissimum</i> (Oudem.) Thom		3.4				3.1	1.2	1.8	3	55.5
<i>Penicillium corylophilum</i> Dierckx		3.4						12.9		22.2
<i>Penicillium janthinellum</i> Biourge		3.4								11.1
<i>Cladosporium cladosporioides</i> (Fres.) de Vries		32.5	8.3	9.4	3.1	3.1	12.6	9.4	18.5	88.8
<i>Cladosporium sphaerospermum</i> Penz	5.8		3.2	3.2	1.6	3.1	3.1	1.8	3	88.8
<i>Cladosporium oxysporum</i> Berk. & Curt.			3.2	6.2	4.7	3.1			1.5	55.5
<i>Cladosporium herbarum</i> (Pers.) Link ex Gray					4.7					11.1

* Anamorph *A. nidulellus*, ** Anamorph *A. equitis*

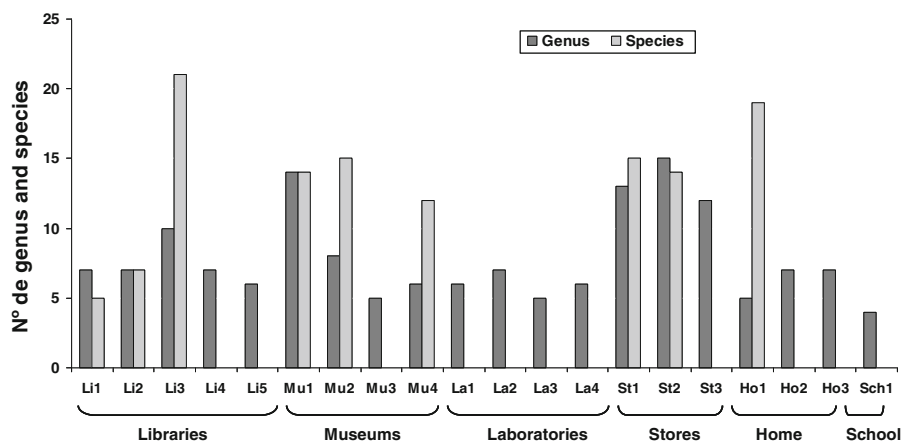
of fungal colonisation of materials, which would be of particular concern in libraries and museums, where humidity levels of over 70% would further encourage fungal development.

The high levels of contamination detected in the present study are attributable to a number of factors, including poor hygiene, the accumulation of dust, insufficient or intermittent air conditioning, ventilation using outside air, and the presence of substrates providing suitable nutrients for fungal development (e.g. wood, cellulose and textiles). All these factors,

which are known to contribute to an increase in microbial concentrations (Codina et al. 2008; Pasanen et al. 2000; Shelton et al. 2002), are likely to have influenced the levels recorded at most sampling points.

The high relative humidity levels (>90%) recorded at two of the warehouses were not associated with higher fungal propagule concentrations, perhaps because the increased aerodynamic particle size also prompts an increase in particle settling velocity and thus a drop in airborne spore concentrations (Reponen 1995).

Fig. 3 Biodiversity of fungal genus and species identified in 20 places sampled



The only previous study of indoor fungal levels in Cuba using a volumetric sampling system was carried out in the National Archive by Vaillant et al. (1989), who reported that fungal propagule concentrations never exceeded 100 cfu/m³, a value far lower than that recorded in the present study. By contrast, later studies using other techniques report concentrations of around 1,000 cfu/m³, that is, similar to those observed here, in archives and libraries in Havana (Rojas et al. 2002), and in homes in that city (Aira et al. 2002).

4.2 Qualitative analysis

The present results pointed to a considerable degree of genus diversity, the predominant genera at all sampling points being *Aspergillus*, *Penicillium* and *Cladosporium*. Similar findings are reported by other authors, both in Cuba (Aira et al. 2002; Borrego et al. 2010; Labarrere et al. 2003; Rojas et al. 2002) and elsewhere (Codina et al. 2008; Hedayati et al. 2005; Medrela-Kuder 2003). This study confirmed that these cosmopolitan genera are characteristic of Havana and that variations in abundance are attributable to the materials present at the sampling points and to the prevailing conditions at the time of sampling.

The predominance of *Aspergillus* at 80% of the sampling points indicates that relative humidity levels of over 68% favour development of this fungus, which is reported to be a primary coloniser readily releasing spores at an air velocity as low as 0.5 m s⁻¹ (Pasanen et al. 1991).

A correlation was found between genus diversity and substrate composition in the different types of building sampled. The presence of cellulose and

lignocellulose materials, particularly in warehouses, contributed to a greater abundance of *Aspergillus*, *Penicillium*, *Cladosporium*, *Chaetomium*, *Monocillium*, *Mucor*, *Nigrospora*, *Rhizopus*, *Stachybotrys*, *Trichoderma*, *Curvularia*, *Chaetomium*, *Paecilomyces*, *Fusarium*, *Chrysionilia* and *Syncephalastrum*. All these fungi have been isolated in libraries and museums (Aira et al. 2007; Borrego et al. 2010; Maggi et al. 2000) and identified as the cause of biodeterioration in documents and other cellulose-containing items (Lugauskas et al. 2003; Portugal et al. 2009). The presence of *Cunninghamella*, *Pithomyces*, *Periconia* and *Bipolaris*, genera more commonly detected outdoors, may be linked to the ventilation systems in use (Rojas et al. 2008).

Of the genera classed as “rare”, *Stachybotrys* was isolated in the building exhibiting the highest relative humidity, suggesting that—as indicated by Horner et al. (2004)—elevated moisture levels favour its development. *Alternaria*, generally common in environments rich in cellulose-containing materials, was also rare, perhaps due to the abundance of *A. niger* and *A. flavus*, which have been shown—at least in vitro—to inhibit *Alternaria* growth (Singh et al. 1995).

At species level, *A. niger* and *A. flavus* predominated, as also reported for other environments in Havana; by contrast, *C. cladosporioides*, *C. sphaerospermum*, *P. chrysogenum* and *P. citrinum*, which were particularly abundant in museums and warehouses, have not been widely reported (Aira et al. 2002; Borrego et al. 2010).

In terms of the biosafety levels suggested by de Hoog (1996), *A. flavus* and *A. fumigatus* would be considered the species most harmful to human health;

however, *A. niger*, *A. terreus*, *A. tamarai* and *C. cladosporioides* have also been implicated in a range of pathologies (De Lucca 2007). Appropriate measures should therefore be implemented to reduce fungal concentrations in the environments tested, with a view to improving air quality and avoiding potential adverse effects.

The present findings add to our knowledge of fungal diversity in Cuba's atmospheric ecosystems, identifying four new genera and nine species.

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