

Indoor and outdoor airborne fungal propagule concentrations in Mexico City

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Abstract

Thirty homes of asthmatic adults located in Mexico City were examined to determine the predominant culturable fungi and the changes in their airborne concentrations. Fungi were cultured and identified microscopically from air samples collected in naturally ventilated homes, during both wet (July–August) and cool dry (November–December) seasons, and from settled dust from the same homes. Airborne dust from indoor yielded 99–4950 cfu m⁻³, and settled dust 10²–10⁶ cfu g⁻¹ on DG18 agar. The indoor geometric mean concentration of airborne fungi during the cool dry season was 460 cfu m⁻³ while in the wet season it was 141 cfu m⁻³. Similarly, numbers of airborne fungal propagules out of doors decreased 60% between the dry and wet season. In general, the total fungal concentrations in indoor air were less than 10³ cfu m⁻³ and a large proportion of them was collected in Stage-2 of the Andersen sampler. Moreover, the ratio between indoor and outdoor concentrations was < 3:1. Five of the 30 sampled homes yielded > 500 cfu m⁻³ of one genus, with up to 1493 *Cladosporium* cfu m⁻³ or 2549 *Penicillium* cfu m⁻³. Also, these two genera were predominant in both airborne and settled dust, and their concentrations were greater indoors than out, indicating a possible indoor source of fungal propagules. The predominant species were *Cladosporium herbarum*, *Penicillium aurantiogriseum* and *P. chrysogenum*. These results suggest that exposure to large concentrations of fungi occurs indoors and is associated with both seasons of the year and with particular home characteristics. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

The incidence of respiratory disease and exposure to allergens in indoor environments has been widely recognized (Solomon, 1975; Burge, 1990; Lacey, 1991; Gravesen, 1994), and is particularly important for children and householders who spend more than 70% of the day in these environments. The fungal propagules in tropical cities include major allergens that can cause asthma and allergenic rhinitis (Ogunlana, 1975; Bunnag et al., 1982). It has been reported that about 18% of asthmatic patients, both adults and children, living in

Mexico City, have positive skin reactivity to some fungi (Pedroza et al., 1984; Enríquez, 1995), but this needs to be studied using standardized fungal extracts.

Mexico City has a tropical climate and is situated 2240 m above sea level. It has a dry season from November to April and a wet season from May to October (Jáuregui, 1987). Mexico City is also one of the most populous cities in the world, having 18 million inhabitants, a large percentage of whom live in small and badly ventilated dwellings (Ponciano, 1994; Ezcurra and Mazari-Hiriart, 1996). Qualitative studies (Cueva and Téllez, 1958; Espinoza et al., 1993) and a recent volumetric aeromycological study (Escamilla et al., 1995), have shown that large concentrations of

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Table 1

Concentrations of fungi in airborne and settled dust in 30 homes, determined on DG18 culture medium

Fungi	Airborne dust fungi (cfu m ⁻³)				Settled dust fungi (cfu g ⁻¹ × 10 ⁻³) indoor	
	Outdoor		Indoor			
	Geom. mean	Max.	Geom. mean	Max.	Geom. mean	Max.
Total	143	720	264	4950	155	4100
<i>Alternaria</i>	2	24	2	31	0.05	133
<i>Aspergillus</i>	2	14	7	173	0.5	366
<i>Cladosporium</i>	63	416	102	1493	26	2000
<i>Penicillium</i>	17	242	38	2549	1.6	500

fungi can be associated with respiratory diseases. However, little is known of the number and types of airborne allergens nor of factors affecting their occurrence. The present study sought to quantify and identify fungal propagules in air and dust from the homes of asthmatic patients at different seasons of the year.

2. Materials and methods

2.1. Homes

To select asthmatic patients to participate in this study, a time-activity questionnaire, including location of their homes, was completed by 150 asthmatic adults that attended the Instituto Nacional de Enfermedades Respiratorias (INER).

Each patient selected was requested to answer two further questionnaires, about their home characteristics (wall to wall carpet, furred pets, visible mould growth, etc.) and to determine their socio-economic background, during the sampling period.

2.2. Sampling

Thirty asthmatic females, aged 30–45, who spent more than 65% of the day inside their homes and lived in the southern part of Mexico City were selected. Airborne and settled dust were sampled at 10:00 h in undisturbed conditions in the living rooms of 30 homes on successive days, once during the cool dry season (November–December) and, in the same order, once in the wet season (July–August) during 1994. Simultaneously, an air sample was collected outside the house.

Airborne dust was collected at a flow rate of 28 l min⁻¹, using a two-stage Andersen sampler (Graseby Andersen, Atlanta, GA) for 15 min. Samplers were mounted on towers, 2 m above ground level. The samplers were loaded with Petri dishes containing Czapek dichloran-18% glycerol agar (DG18, Hocking and Pitt, 1980). Exposed plates were incubated for 1 week at

25°C. Fungal colonies were counted daily during incubation, before slower growing colonies could become overgrown, and their morphological characteristics were observed microscopically. Using tables provided by the sampler manufacturer, counts were transformed to allow for multiple deposition of particles at individual impaction sites. Results for each stage were then expressed as colony forming units (cfu) per cubic meter (m³) of air. Total concentrations were obtained by totalling the cfu m⁻³ from both stages.

Settled dust samples were obtained using a vacuum cleaner (Vorwerk VK 121), modified to collect dust onto a filter paper (Verhoeff et al., 1994). A floor area of 2 m² from each livingroom was sampled for 4 min. Stock suspensions were prepared with 100 mg of sieved dust in 10 ml sterile distilled-water (American Public Health Association, 1985) adding polysorbтан 80 (Tween 80) as a detergent (0.05%); these were then used to prepare 10-fold dilution series down to 10⁻⁴. After

Table 2

Percentage of air samples indoor and outdoor (*N* = 60) and settled dust samples yielding viable fungi on DG18

Fungi	Air		Dust indoor
	Outdoor	Indoor	
<i>Cladosporium</i>	100.0	100.0	100.0
<i>Penicillium</i>	95.0	100.0	79.0
<i>Alternaria</i>	76.0	67.0	59.0
<i>Aureobasidium</i>	48.0	47.0	41.0
Non-sporulating mycelia	38.0	69.0	53.0
<i>Aspergillus</i>	33.0	48.0	48.0
<i>Fusarium</i>	30.0	18.5	5.0
<i>Monilia</i>	28.0	19.0	5.0
<i>Epicoccum</i>	21.0	19.0	5.0
<i>Eurotium</i>	17.0	52.0	38.0
<i>Helminthosporium</i>	17.0	33.0	5.0
<i>Phoma</i>	13.0	10.0	3.0
<i>Paecilomyces</i>	10.0	0.0	0.0
<i>Rhizopus</i>	9.0	25.0	25.0
<i>Mucor</i>	7.0	30.0	3.0
<i>Botrytis</i>	7.0	11.0	3.0
<i>Trichoderma</i>	7.0	9.0	3.0

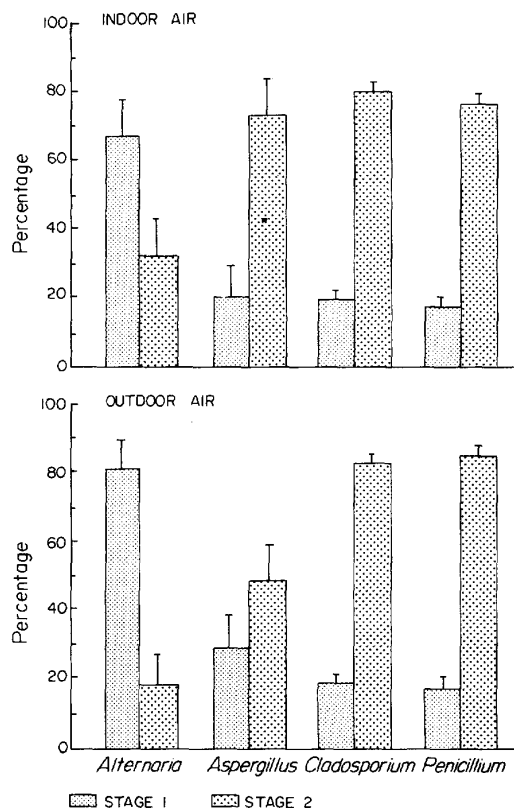


Fig. 1. Distribution of airborne fungal propagules between the two stages of an Andersen sampler in indoor and outdoor environments.

shaking each suspension for 2 min, 300 μ l were spread on duplicate plates of DG18. Plates were incubated in darkness for 1 week at 25°C and the colonies growing were counted and classified. The results were expressed as colony forming units (cfu) per g of dust. Representative colonies from both airborne and settled dust were then transferred to appropriate media for identification to species level, using standard mycological procedures.

Wet and dry bulb temperatures were measured inside and outside each house at the time of sampling, and used to calculate relative humidity and vapour pressure.

2.3. Statistical methods

Because the frequency distribution of the raw data failed to correspond to a normal distribution, the non-parametric Wilcoxon's signed-ranks test was applied to test the differences between fungal concentrations from different seasons. Spearman's rank correlation coefficients were also calculated between airborne fungal concentrations indoors and outdoors, and between indoor airborne and settled dust. Associations between home characteristics and airborne fungal concentrations were investigated using the Mann-Whitney test.

3. Results

The sampled homes were naturally ventilated and windows and doors were opened at their discretion. On the other hand, 90% of these patients belonged to middle-class and only 10% were lower class (Bronfman et al., 1988).

In general, the total fungal counts obtained from indoor environments were similar to those from outdoors, with geometric means, respectively, of 264 cfu m^{-3} and 143 cfu m^{-3} . However in 18% of the samples, airborne fungal concentrations showed an indoor/outdoor ratio > 3:1; the largest count was 4950 cfu m^{-3} indoors, and 720 cfu m^{-3} outdoors, during the dry season (Table 1).

The fungi most frequently isolated from indoor air and present in all samples were *Cladosporium* spp. Non-sporulating mycelia were isolated from 69% of the samples, *Alternaria* spp. from 67%, and *Eurotium herbariorum* from 52%. *Aspergillus* spp., *Aureobasidium pullulans* and *Helminthosporium* spp. were present in about 30% of the samples (Table 2).

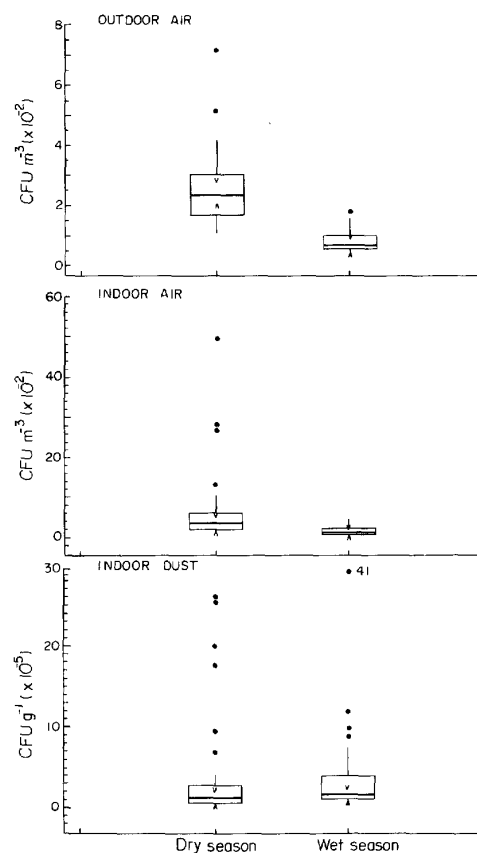


Fig. 2. Seasonal variation of fungal propagules from dust samples in indoor and outdoor environments.

Table 3

Geometric mean (GM), maximum (Max) airborne fungal concentrations, in/out (I/O) ratio range, and Spearman correlation (r) between airborne fungal concentrations from indoors and outdoors during the dry and wet seasons

Genera	Indoor		Outdoor		<i>r</i> [*]	I/O ratio range
	GM (cfu m ⁻³)	Max	GM (cfu m ⁻³)	Max		
Dry season						
<i>Cladosporium</i>	181	1493	121	416	0.63*	0.5–7
<i>Penicillium</i>	79	2549	41	242	0.10	0.0–18
<i>Aspergillus</i>	6	54	2	14	0.08	0.0–10
<i>Alternaria</i>	3	31	3	22	0.05	0.0–3
Wet season						
<i>Cladosporium</i>	55	147	33	163	0.40*	0.5–10
<i>Penicillium</i>	23	104	7	54	0.41*	0.0–18
<i>Aspergillus</i>	4	173	1	12	0.08	0.0–13
<i>Alternaria</i>	4	17	3	24	0.34	0.0–2

* $P < 0.05$.

The distribution of airborne fungal propagules between the two stages of the Andersen sampler is shown in Fig. 1. Except for *Alternaria*, most colonies grew on plates from Stage 2 of the sampler ($\leq 4.5 \mu\text{m}$ aerodynamic size). However, 12% more *Alternaria* were recovered on Stage 2 from indoor air than from outdoor air.

Concentration of total fungal propagules in settled dust samples ranged from 10^2 to 10^6 cfu g⁻¹, with a geometric mean concentration of 10^3 (Table 1). The mean coefficient of variation between duplicate samples was $20 \pm 17\%$.

Species of *Cladosporium* and *Penicillium*, and in smaller numbers, of *Aspergillus*, *Fusarium* and *Aureoba-*

sidium, were among the predominant fungi isolated. *Eurotium herbariorum* was also present in more than half of the indoor samples (Table 2).

The effect of season on total fungal concentrations in indoor and outdoor environments is shown in Fig. 2. Differences between total airborne propagules in wet and dry seasons were statistically significant ($z = 4.2$ and 4.7 respectively, $P < 0.01$) in both in- and outdoor environments, with largest concentrations occurring during the dry season. By contrast, the fungal content of settled dust did not differ significantly between seasons ($z = 1.4$ in indoor and $z = 0.4$ in outdoor, $P > 0.05$).

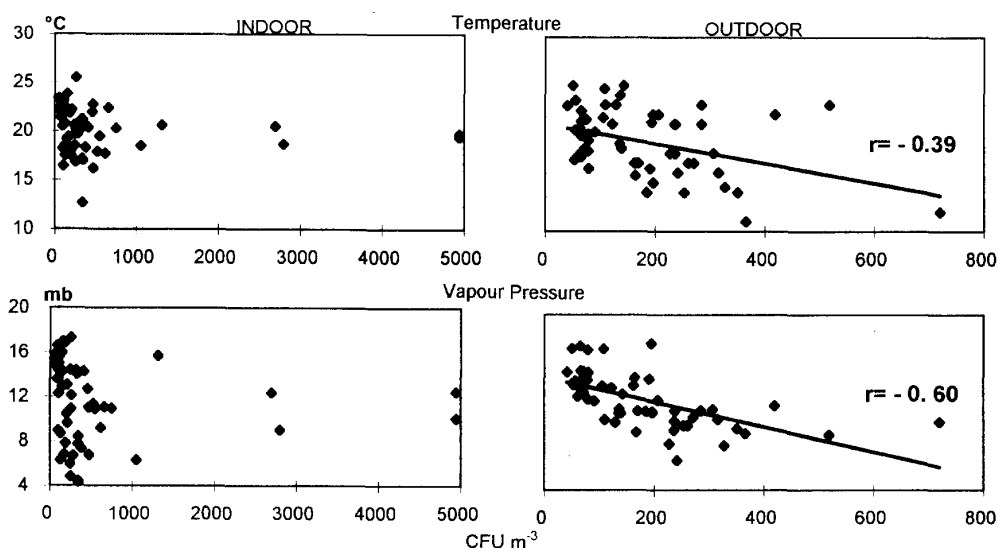


Fig. 3. Correlations between airborne fungal concentrations and some weather variables in indoor and outdoor environments.

The occurrence of the most common fungal genera in outdoor and indoor air in each season is shown in Table 3. *Cladosporium* represented 50% of the total fungi in both environments, with concentrations indoors ranging from 17 to 1493 cfu m⁻³ in the dry season. Concentrations in and out of doors were significantly correlated in both seasons ($r = 0.63$ dry, 0.40 wet season). *Penicillium* constituted about 20% of the total indoor, with concentrations in the dry season ranging from 7 to 2549 cfu m⁻³. However, concentrations in indoor and outdoor are only correlated significantly in the wet season. *Alternaria* and *Aspergillus* were only isolated in small concentrations and showed no differences between seasons. The greatest differences in the dry season between concentrations indoors and those out of doors were with *Penicillium* (in/out ratio 18:1) (Table 3).

Temperature ranged from 12.7–25.5°C indoors and 11–25°C outdoors. Vapour pressures ranged from 4.3–17.3 mb indoors and 6.7–17.4 mb outdoors. Correla-

tions between airborne fungal concentrations and both air temperature and vapour pressure were statistically significant only in outdoor environments (Fig. 3).

Associations between some home characteristics and total fungal concentrations are shown in Table 4. During the dry season, concentrations of airborne fungal propagules correlated significantly ($P < 0.05$) with the presence of pets ($z = 1.97$) and concentrations in settled dust with wall to wall carpeting ($z = 1.98$). In 5 of the 30 homes, visible mould growth was observed on the walls and airborne *Cladosporium* or *Penicillium* exceeded 500 cfu m⁻³.

Altogether 6 *Penicillium*, 8 *Aspergillus*, 3 *Alternaria*, and 2 *Cladosporium* species were isolated (Table 5).

4. Discussion

Although conidiospores can be present in the air all the year round in tropical regions, a seasonal variability is usually evident (Abdalla, 1988; Halgway, 1989; Tan et al., 1992). In Mexico City larger conidiospore concentrations have been found at the end of the wet season (October) and during the cool dry season (November and December) (Calderón, personal communication). There is only one previous report of fungal concentrations in homes located in Mexico City which showed similar concentrations to those found in this study at the start of the cool dry season (Escamilla et al., 1995). An important factor in the present study, was that the maximum concentration thresholds of the two-stage Andersen sampler were exceeded in 6% of the samples in the dry season during the 15 min sampling period. This suggests that some concentrations were consequently underestimated. However, to avoid sample variability, the sampling period should not be decreased but the two-stage sampler should be substituted by a six-stage Andersen sampler.

During the wet season, concentrations of airborne *Cladosporium* and *Penicillium* in outdoor air decreased to about half that found in the dry season. These smaller fungal concentrations could have resulted from unfavorable environmental conditions for their release and dispersal or from the washout of their propagules from the atmosphere (Rosas et al., 1986). Concentrations of fungal propagules decreased similarly indoors, perhaps because few spores came from out of doors or because the increased air humidity hindered their liberation. However, moist conditions might also be expected to encourage fungal growth and, perhaps to change the balance of species.

The prevalent fungal genera isolated on DG18, from the homes sampled, were *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*, all of which have been reported as the most common indoor fungi in other studies (Solomon, 1976; Wickman et al., 1992; Ebner

Table 4
Comparison of total fungal concentrations according to some home characteristics

Home characteristics	Seasons			
	Dry		Wet	
	Airborne dust			
	N	Median (cfu m ⁻³)	N	Median (cfu m ⁻³)
Wall to wall carpet				
Yes	15	343	15	145
No	15	381	15	111
Z ^a	0.76		0.76	
P ^b	> 0.05		> 0.05	
Furred pets				
Yes	22	425	22	132
No	8	247	8	170
Z ^a	1.97		0.60	
P ^b	< 0.05		> 0.05	
Settled dust				
	N	(cfu g ⁻¹ × 10 ⁻³)	N	(cfu g ⁻¹ × 10 ⁻³)
Wall to wall carpet				
Yes	15	166	15	149
No	15	70	15	156
Z ^a	1.98		0.99	
P ^b	< 0.05		> 0.05	
Furred pets				
Yes	15	145	15	176
No	15	97	15	143
Z ^a	0.84		0.50	
P ^b	> 0.05		> 0.05	

^aMann-Whitney, Z test.

^bSignificance level, two-tailed test.

Table 5
Frequency of isolation and abundance of fungal species in both in- and outdoors

Species	Frequency (% of samples)			Abundance (% of total genus)		
	Air		Dust	Air		Dust
	Out	In	In	Out	In	In
<i>Alternaria</i>	75.8	67.4	59.3	3.8	1.4	0.02
<i>A. alternata</i>						
<i>A. tenuissima</i>						
<i>A. raphani</i>						
<i>Aspergillus</i> *						
Subgenus <i>Aspergillus</i>						
Section <i>Aspergillus</i>	23.9	62.8	37.5	29.5	32.6	31.3
<i>A. glaucus</i> (<i>Eurotium herbariorum</i>)						
Subgenus <i>Nidulantes</i>						
Section <i>Versicolores</i>	41.6	55.7	48.2	25.1	44.8	54.1
<i>A. versicolor</i>						
<i>A. sydowi</i>						
Subgenus <i>Circumdati</i>						
Section <i>Flavi</i>	22.8	10.4	8.9	20.7	7.6	1.3
<i>A. flavus</i>						
<i>A. flavo-furcatis</i>						
Section <i>Circumdati</i>	11.6	27.3	14.3	12.8	10.9	13.8
<i>A. melleus</i>						
<i>A. petrakii</i>						
Section <i>Nigri</i>	10.8	15.8	10.7	7.2	4.3	2.8
<i>A. niger</i>						
<i>Cladosporium</i>						
<i>C. herbarum</i>	100.0	100.0	100.0	96.0	94.4	99.8
<i>C. cladosporioides</i>	30.0	20.0	1.0	4.0	5.6	0.2
<i>Penicillium</i>						
<i>P. aurantiogriseum</i>	57.1	88.4	41.2	26.0	46.4	13.3
<i>P. chrysogenum</i>	53.6	78.6	48.2	35.5	44.2	76.1
<i>P. spinulosum</i>	39.4	41.1	3.5	22.8	7.9	8.0
<i>P. purpurogenum</i>	3.6	7.1	1.8	1.3	0.2	0.1
<i>P. minoluteum</i>	1.8	1.8	5.3	2.1	0.2	0.02
<i>P. crustosum</i>	8.9	1.0	0.0	1.6	0.1	0.0
<i>Penicillium</i> spp.	62.5	4.2	30.3	7.2	1.8	8.0

*Taxonomy according to Kuraishi et al., 1990.

and Haselwandter, 1992; Beguin, 1995). However, the culture medium permitted the isolation of some xerophilic species of *Aspergillus* and *Penicillium* which have been reported as potentially allergenic moulds (Morring et al., 1983; Verhoeff et al., 1988; Hamada and Morita, 1990).

During the dry season, the large concentrations of airborne fungi inside homes were associated with the presence of furred pets, which give off skin scales, fecal particles and hair that could form good substrates for fungal growth (Hoffman, 1980; Su et al., 1992). Also, homes with wall to wall carpet had large concentrations of fungi in settled dust, which shows that dust can accumulate in carpets despite cleaning.

It was not possible to observe the effect of temperature or vapour pressure on indoor airborne fungal concentrations, even in mouldy homes. It could be associated with the short time of the sampling that does not represent the indoor climatic conditions and proba-

bly a weather parameter that represents the additive effect of temperature and vapour pressure must be included. Also, it has been reported that the water contents of indoor air may be a poor indicator of the moisture penetration and condensation on cold walls and ceilings that favour the growth of fungi (Strachan et al., 1990; Becker, 1994; Nevalainen et al., 1994).

The size distribution of fungal propagules collected outdoors was similar to that found indoors, with a large proportion collected in the stage-2 ($\leq 4.5 \mu\text{m}$ in aerodynamic size) of the sampler, indicating that they could be deposited in the lower respiratory tract (Licorish et al., 1985; Rantio-Lehtimäki, 1989; Reponen et al., 1993).

There are inadequate dose/response data to permit accurate risk analyses. Therefore, an alternative approach is to characterize the kind of environment and to define the normal concentrations, based on the mean concentrations of fungal propagules. In north temper-

ate areas, Reponen et al. (1993) suggested 500 cfu m⁻³ of airborne fungi as a 'normal level' for indoor environments in winter, and Passanen et al. (1992) suggested 10 000 cfu m⁻³ as an indicator of likely mould problems.

In the present study, concentrations of airborne propagules of one genus (*Cladosporium* or *Penicillium*) were > 500 cfu m⁻³, and large indoor/outdoor ratios could be good indicators of some indoor fungal source. The measurement of airborne fungal propagules during undisturbed periods does not provide an accurate estimation of the exposure of the residents to fungi in indoor environments because large concentrations may be created by disturbing previously settled dust (Holmberg, 1984; Passanen et al., 1992). In this study, no correlation could be observed between concentrations of fungal propagules in airborne and settled dust, probably because the homes were sampled during periods of inactivity or because such propagules derived from other sources.

Recognition of the factors that lead to elevated indoor mould counts is important in the control and treatment of respiratory disease and could lead to the development of corrective environmental measures and educational programmes.

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