

Prevalence of fungi in carpeted floor environment: analysis of dust samples from living-rooms, bedrooms, offices and school classrooms

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Abstract

The results of 100 carpet dust analyses from atopic individuals' environment were compared according to the sampling period or the location. Dust samples were collected with a standard domestic vacuum cleaner, in locations with carpeted floor: in residences (living-room and/or bedroom), in school classrooms and in offices. The quantities of fungi vary from 5000 CFU/g to 66 000 000 CFU/g of dust. More than 100 species were isolated by dilution plating. The main species found in carpet dust were *Eurotium repens*, *Penicillium chrysogenum*, *Alternaria alternata*, *Aureobasidium pullulans* and *Phoma herbarum*. Strict xerophilic species were rather rare and detected in small quantities. Differences in the distribution of the CFU concentrations were examined for the four different sampling locations and were statistically significant ($P = 0.0174$). In this study, schools were open spaces, and offices, mostly with air conditioning systems, were locations in which air is not confined. This, added to frequent professional carpet cleaning, probably explains the lowest levels of fungal concentration found in these locations. The majority of the homes had the largest fungal concentration in the living-room (median: 2×10^5 CFU/g) while some bedrooms (median: 7×10^4 CFU/g) had the highest concentrations. It is suggested that, when fungi are suspected to be the origin of respiratory allergy or irritating symptoms, the mycoflora of the bedroom, principally, should be investigated first.

Keywords: Fungi; Carpet; Dust; Home; Allergy; Aerobiology

1. Introduction

Moulds from indoors have been implicated in respiratory allergy. Different surveys showed a relationship between home dampness and mould growth on surfaces and perennial rhinitis, atopic dermatitis, asthma, or hypersensitivity pneumonitis (Strachan, 1988; Platt et al., 1989; Brunekreef et al., 1989; Dales et al., 1991; Brunekreef, 1992; Nolard et al., 1994).

Other non-allergenic mechanisms due to the inhalation of low molecular weight fungal compounds may also be the origin of various health effects. Indeed some moulds produce volatile compounds which can affect the eyes or nose and provoke throat irritation, headache or fatigue (Samson, 1985; Miller et al., 1988). Moulds have on their surface a polyglucose (1–3) β -D-

glucan which could explain respiratory as well as non-respiratory symptoms (Rylander et al., 1992; Rylander et al., 1994; Auger et al., 1994). Moreover, some moulds produce spores containing toxins as do various species of *Penicillium*, *Trichoderma viride*, *Aspergillus versicolor* or *Stachybotrys atra* (Tobin et al., 1987; Larsen and Frisvad, 1994). This last species has been reported to be responsible for conjunctivitis, irritation of the respiratory system and of the skin (Croft et al., 1986; Johanning et al., 1993).

On the basis of medical files of a number of atopic individuals, we selected a number of patient-occupied sites and sampled, all year round, the mycoflora as described previously (Beguin and Nolard, 1994). Knowing that (i) the indoor mould burden may be roughly estimated by establishing the number of viable mould

spores that grow in a dust specimen (Hamilton et al., 1992); (ii) many reports have demonstrated the greater occurrence of moulds (or allergens) in dust from floors with wall-to-wall carpets compared with smooth floor covering (Gravesen et al., 1986; Dybendal et al., 1989; Verhoeff et al., 1994); and that, (iii) there is a larger amount of airborne moulds measured 1 m above carpets than above bare floors (Gravesen et al., 1983), analysis of carpet dust, of course, completes the mycological information.

With a view to determining which species are able to grow in carpet dust (or capable of accumulating in it), 100 analyses were compared according to the sampling period or the location.

2. Methods

2.1. Sample collection

Dust samples were collected in a wide range of homes or institutions from various localities. The samples were collected using a standard domestic vacuum cleaner (Miele type S247 i), in locations with carpeted floor; in dwellings: living-room ($n = 19$) and/or bedroom ($n = 33$), in school classroom ($n = 18$) and in office ($n = 30$). The end of the vacuum cleaner hose was modified to collect dust on a 5-cm diameter circular cloth filter (3 M/Filtrete). Approximately, two square meters of floor area were vacuumed for 1 min with a power rating of 1200 W. Filters with floor dust were sealed in plastic bags and kept at 5°C until analysis. Samples were taken during the years 1993–1994. The distribution for the seasons is specified in Table 1.

2.2. Culture methods

Dilution plate technique with an aqueous solution of Tween 80 (0.02%) was used for isolating moulds from a weighed sample of sieved dust as described previously (Beguin, 1995). Three culture media with added chlo-

Table 2

Fungal contamination level of the carpets

Concentration of fungi in carpets (CFU/g)	Number of dust samples
< 10 000	5
10 000–< 100 000	50
100 000–< 1 000 000	39
≥ 1 000 000	6

ramphenicol (0.05%) were used in parallel: MEA, M40Y and M40Y + 5% NaCl. Inoculated Petri dishes were incubated at 25°C for a period ranging from 7 to 14 days. The colonies counted at each dilution were only taken into account if the previous dilution, or the corresponding dilution made on another medium, confirmed the result. Fungal counts are given in terms of CFU (colony forming units).

2.3. Statistical analysis

Since the fungal concentrations from bedroom were not normally distributed even after transformation into base 10 logarithms (Kolmogorov–Smirnov test: $P = 0.007$), comparisons of mould counts between seasons or locations were performed with the Kruskal–Wallis test. The Mann–Whitney U -test was used to compare the data between two locations.

3. Results

3.1. Concentration level

Every wall-to-wall carpet contained fungi, some more than others: concentrations varied from 5×10^3 CFU/g to 6.6×10^7 CFU/g of dust. Carpet dust could be categorized in accordance with the level of fungal concentration as in Table 2: more than 50% of the analyzed dust samples contained less than 10^5 CFU/g. The median level of concentration was 6×10^4 CFU/g.

3.2. Prevalence of fungi

The species isolated are listed in Table 3. Most are ubiquitous saprophytes commonly found on numerous kinds of organic matter or facultative parasites of plants: they have, of course, no particular preference for any specific substrate. Some require high moisture for growth, whereas others grow on substrata of high osmotic pressure. Fungi which did not reveal high concentrations were not always identified up to species level. Among more than 100 species isolated, *Eurotium repens*, detected in 87 dust samples. *Penicillium* spp. in 79 (the main species being *Penicillium chrysogenum*),

Table 1
Distribution of sampling according location and season

Sampling period	Number of carpets sampled				
	Living-room	Bedroom	Office	Classroom	Total
Spring	1	6	20	3	30
Summer	7	7	4	5	23
Autumn	5	13	4	6	28
Winter	6	7	2	4	19
Total	19	33	30	18	100

Table 3
Fungi isolated from 100 samples of carpet dust

Species	% of carpets with $\geq 10^5$ CFU/g	% of dust samples	Maximum concentration CFU/g
<i>Absidia corymbifera</i>	1	6	10^5
<i>Acremonium strictum</i>	0	1	10^4
<i>Acremonium</i> spp.	0	12	10^4
<i>Alternaria alternata</i> gr.	0	53	10^4
<i>Aposphaeria</i> sp.	0	1	10^4
<i>Arthrinium phaeospermum</i>	0	2	10^3
<i>Aspergillus candidus</i>	0	4	10^3
<i>Aspergillus flavus</i>	0	5	10^2
<i>Aspergillus fumigatus</i>	0	23	10^3
<i>Aspergillus niger</i>	1	34	2.4×10^6
<i>Aspergillus ochraceus</i>	0	9	10^4
<i>Aspergillus penicilloides</i>	1	3	3×10^5
<i>Aspergillus restrictus</i>	0	4	10^4
<i>Aspergillus tamarii</i>	0	5	10^3
<i>Aspergillus unguis</i>	0	2	10^2
<i>Aspergillus ustus</i>	0	4	10^3
<i>Aspergillus versicolor</i>	0	36	10^5
<i>Aureobasidium pullulans</i> var. pullulans	6	50	6.1×10^6
<i>Botryotrichum piluliferum</i>	0	1	10^3
<i>Botrytis cinerea</i>	0	7	10^3
<i>Candida guilliermondii</i>	1	1	10^5
<i>Candida parapsilosis</i>	3	3	2×10^5
<i>Candida zeylanoides</i>	0	1	10^4
<i>Chaetomium</i> spp.	0	10	10^4
<i>Cladosporium cladosporioides</i>	0	2	10^3
<i>Cladosporium herbarum</i>	2	13	10^5
<i>Cladosporium sphaerospermum</i>	0	34	10^4
<i>Cladosporium</i> spp.	0	25	10^4
<i>Cryptococcus laurentii</i>	1	2	10^5
<i>Cryptococcus</i> sp.	0	1	10^3
<i>Debaryomyces hansenii</i>	5	6	4×10^5
<i>Dinemasporium</i> sp.	0	1	10^4
<i>Drechslera</i> sp.	0	1	10^3
<i>Emericella nidulans</i>	0	3	10^3
<i>Epicoccum purpurascens</i>	0	19	10^4
<i>Eurotium chevalieri</i>	0	9	10^3
<i>Eurotium amstelodami</i>	0	6	10^5
<i>Eurotium repens</i>	0	87	10^4
<i>Exophiala jeanselmei</i>	1	1	3×10^5
<i>Fusarium culmorum</i>	0	1	10^3
<i>Fusarium oxysporum</i>	0	1	10^2
<i>Fusarium proliferatum</i> var. proliferatum	0	1	10^3
<i>Fusarium solani</i>	0	5	10^3
<i>Fusarium</i> spp.	0	11	10^4
<i>Geotrichum candidum</i>	2	3	6.6×10^7
<i>Gliocladium</i> sp.	0	1	10^4
<i>Gliomastix luzulae</i>	1	1	10^5
<i>Gliomastix murorum</i>	0	1	10^4
<i>Humicola grisea</i> var. grisea	0	1	10^3
<i>Metschnikowia pulcherrima</i>	1	1	10^5
<i>Monilia sitophila</i>	0	2	10^3
<i>Mucor circinelloides</i>	0	4	10^4
<i>Mucor hiemalis</i> f. hiemalis	3	3	10^5
<i>Mucor mucedo</i>	0	1	10^2
<i>Mucor</i> spp.	0	44	10^4
<i>Myrothecium roridum</i>	0	1	10^4
<i>Nectria</i> sp.	0	3	10^4
<i>Neosartorya fischeri</i>	0	1	10^4
<i>Paecilomyces carneus</i>	0	1	10^3
<i>Paecilomyces variotii</i>	0	1	10^4

Table 3 (continued)

Species	% of carpets with $\geq 10^5$ CFU/g	% of dust samples	Maximum concentration CFU/g
<i>Penicillium aurantiogriseum</i>	1	4	9×10^5
<i>Penicillium brevicompactum</i>	0	1	10^3
<i>Penicillium chrysogenum</i>	8	24	2×10^5
<i>Penicillium digitatum</i>	0	1	10^4
<i>Penicillium olsonii</i>	0	2	10^4
<i>Penicillium rugulosum</i>	0	1	10^4
<i>Penicillium spinulosum</i>	2	4	10^5
<i>Penicillium thomii</i>	1	1	1.2×10^6
<i>Penicillium</i> spp.	1	45	10^5
<i>Pestalotia</i> sp.	0	1	10^4
<i>Phoma eupyrena</i>	0	1	10^4
<i>Phoma herbarum</i>	4	5	1×10^6
<i>Phoma pomorum</i>	0	1	10^3
<i>Phoma</i> spp.	3	39	10^5
<i>Pithomyces chartarum</i>	0	15	10^4
<i>Pleospora herbarum</i>	0	3	10^3
<i>Pyrenochaeta</i> sp.	0	1	10^4
<i>Rhizopus stolonifer</i>	0	24	10^4
<i>Rhodotorula mucilaginosa</i>	1	6	4×10^5
<i>Saccharomyces cerevisiae</i>	1	1	10^5
<i>Scopulariopsis acremonium</i>	0	1	10^3
<i>Scopulariopsis brumptii</i>	0	1	10^3
<i>Scopulariopsis candida</i>	0	1	10^3
<i>Scopulariopsis</i> spp.	0	7	10^4
<i>Sesquicillium candelabrum</i>	1	1	10^5
<i>Sporothrix</i> sp.	0	2	10^3
<i>Stachybotrys atra</i>	0	3	10^3
<i>Trichoderma hamatum</i>	0	1	10^3
<i>Trichoderma harzianum</i>	0	2	10^4
<i>Trichoderma koningii</i>	0	1	10^4
<i>Trichoderma viride</i>	1	1	8×10^5
<i>Trichoderma</i> spp.	0	41	10^5
<i>Trichophyton terrestre</i>	1	1	10^5
<i>Trichosporon cutaneum</i>	0	1	10^4
<i>Trichosporon pullulans</i>	1	2	5×10^5
<i>Ulocladium botrytis</i>	0	14	10^4
<i>Ustilaginales</i> anamorph of	1	1	10^5
<i>Wallemia sebi</i>	0	3	10^4
<i>Yarrowia lipolytica</i>	1	1	2×10^5
<i>Mycelia sterilia</i>	1	—	2×10^5
Unidentified species of yeasts	0	15	10^4

Species with $\geq 1\%$ of carpets $\geq 10^5$ CFU/g in bold face.

Alternaria alternata in 53 and *Aureobasidium pullulans* in 50 were the most frequently recovered moulds. The genus *Mucor* was recorded with a frequency of 52%.

3.3. Environmental conditions

Some dominant species in aeromycological surveys were absent or not very numerous in carpet dust. For example, the phylloplane fungi, *Cladosporium herbarum* was only found twice in high concentrations (in summer). Inversely, some taxa were very frequent in carpet dust such as *Phoma* (mainly *P. herbarum*) which occurred in 46% of the dust, several times in high levels of contamination. The common soil fungus *Aspergillus fumigatus* occurred in 23 carpets and reached only 10^3

CFU/g. Others species reached high concentrations, like *Penicillium thomii* which is fairly common in soil and was isolated only once, but at 12×10^5 CFU/g.

Fig. 1 shows the fungal concentrations in carpet dust for the four locations throughout the year. Within each location, total fungal levels did not fluctuate significantly with the seasons of the year ($P > 0.05$). Nevertheless, for all carpets, there was an uneven seasonal distribution of the higher levels of contamination: among 44 carpets contaminated with 10^5 CFU/g or more, eight (26.6%) occurred in spring, 14 (60.8%) in summer, 13 (46.4%) in autumn, and nine (47.3%) in winter (Table 4). Among the approximately 30 species responsible — separately or not — for these contaminations, *A. pullulans*, *Penicillium chrysogenum* and

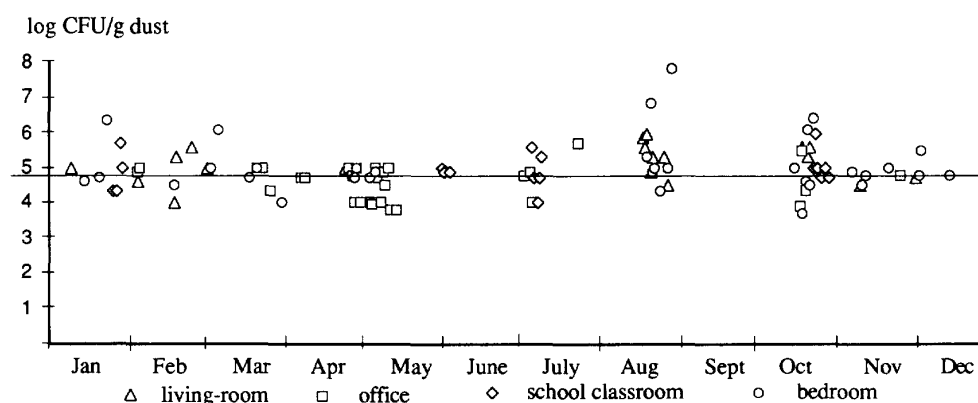


Fig. 1. Fungal contamination levels in carpeting throughout the year. The horizontal line indicates the median.

Phoma herbarum were the main moulds with upper values of 6.1×10^6 , 2×10^5 and 10^6 CFU/g dust, respectively. The two last species were present almost constantly during the year while *A. pullulans* was mainly found in summer. Yeasts were frequently recovered and some of them were present in dust in high quantities ($\geq 10^5$ CFU/g) such as *Debaryomyces hansenei* and *Candida parapsilosis*.

Despite the use of different media and proven techniques of dilution plating to attempt to quantify a maximum of viable fungi (Beguin, 1995), in this study, strict xerophilic species, requiring lower moisture conditions for their optimal growth, only once outnumbered less xerophilic species in carpets: *Aspergillus penicilloides* reached 3×10^5 CFU/g in winter. *Aspergillus restrictus* and *Wallemia sebi* were absent in the samples at concentrations $\geq 10^5$ CFU/g dust.

Distributions of the CFU concentrations for the four different sampling locations, displayed in Fig. 2, were significantly different ($P = 0.0174$). The fungal concentrations in the living-rooms were higher than other locations. The geometric mean (GM) of living-rooms was 135 586 CFU/g dust versus 40 703 CFU/g dust for offices ($P = 0.0021$). Although the median values of the bedrooms and offices, represented in Fig. 2, are close, the mould content of dust was also significantly higher in bedrooms than in offices ($P = 0.0469$). The

concentration level in carpet dust samples from homes (living-rooms or bedrooms) was generally higher than from classrooms (GM = 84 080 CFU/g), but this difference was not statistically significant ($P > 0.05$). Despite a lower geometric mean in bedrooms (119 462 CFU/g) compared to living-rooms, the six highest concentrations $\geq 10^6$ CFU/g occurred in bedrooms. These six highest levels were equally distributed between three seasons: the main species, for each of them, were *Aspergillus niger* (1 $\times$, in autumn), *A. pullulans* (2 $\times$, in summer and in winter), *Geotrichum candidum* (1 $\times$, in summer), *P. thomii* (1 $\times$, in autumn) and *P. herbarum* (1 $\times$, in winter).

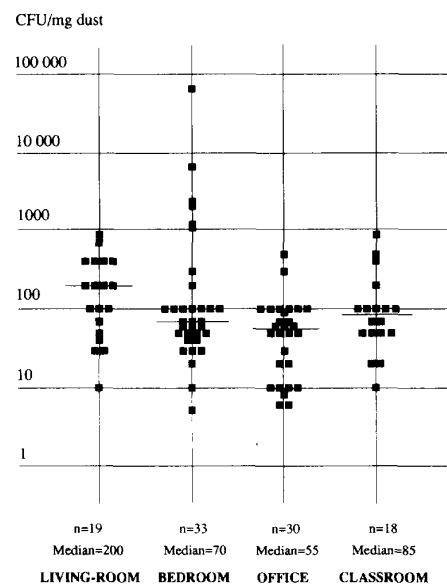


Fig. 2. Distribution of fungal concentrations in carpet dust samples from the four locations. The horizontal lines indicate the median values. Minimum and maximum fungal counts: 5000 CFU/g, 66 000 000 CFU/g.

Table 4
Comparison of fungal concentrations in carpet with the sampling season

	n	Median CFU/mg	Range CFU/mg	Number of car- pets with $\geq 10^5$ CFU/g
Spring	30	55	6–100	8
Summer	23	100	10–66 000	14
Autumn	28	65	5–2400	13
Winter	19	100	10–2100	9

4. Discussion

Even when, for some taxa, unfavourable conditions in carpeting did not induce growth, in Petri dishes some fungi could become antagonists for others. It must be emphasized that competition and inhibition phenomena in plates at low dilutions resulted in some underestimated frequencies.

Carpeted floors accumulate fungal species which are brought indoors with shoes, pets, food, etc., and obviously those which originate from the air. But the mycoflora in this ecological niche will be composed especially of species which find suitable conditions for their growth. It was not always possible to determine if a significant recovery was the result of fungal growth in carpet or was due to contamination from a different origin. It is assumed that contamination in carpeting did not result from sedimentation of spores from the air because the predominant species isolated from dust are, for the most part, not generally considered as dominant types in the air indoors or outdoors. For instance, *Cladosporium herbarum* which is the most common species in air indoors as well outdoors in Belgium (Beguin and Nolard, 1994) was not very represented in dust samples. The inability to grow can be due to adverse conditions like the moisture level in the carpet and microbial antagonism. On the other hand, the common Coelomycetes, *Phoma herbarum*, rare in the air, was frequently isolated in this study. Similarly, in carpet dust samples across the USA, Burge et al. (1992) reported that Coelomycetes were recovered, overall, in the highest levels with a median value greater than 120 000/g, whereas these moulds are rarely dominant in air in the US. It is on record that there is an association between indoor fungus prevalence and indoor humidity (Wood et al., 1988). In this study, concentrations of the most xerophilic species are usually lower than less xerophilic species probably because the relative humidity of carpets is too high for their optimal growth. Indeed, Schober (1991) reported that the different distributions of hydrophilic and xerophilic fungi in dust from carpeting and furniture can be considered to be the result of a difference in moisture availability of the sites. Considering the diversity of the investigated carpets, *E. repens* was the most frequent species (87%) because this halotolerant fungus (Kelavkar et al., 1993) is able to adapt its metabolism to grow in a wide range of osmotic pressure. In mattress dust, with the same methodology, strict xerophilic species as *A. penicilloides* and *A. restrictus* were more frequently isolated and often revealed higher concentrations than non-xerophilic species (Beguin, 1995).

Of the four locations, wall-to-wall carpets from living-room and bedroom had the highest geometric mean concentrations. Indoor environmental conditions such as temperature and humidity have an influence on the

number of fungal species (and mites) which can differ from carpet to carpet. (In fact, the relative humidity corresponding to the optimal growth of mites, xerophilic or hydrophilic fungal species differs for each group of these microorganisms.) As with moulds in this study, *Der p 1* (major allergen of the house dust mite) content of dust collected from classroom floors was much lower than of dust collected from homes (Voûte et al., 1994).

Indoor humidity depends very much on the building characteristics such as single-storey individual houses where absolute indoor humidity (AIH) is higher than in multi-storey individual houses or apartments in flats (Wickman et al., 1991); concrete slab construction also contributes to high AIH (Wickman et al., 1994). Since water vapour condensation occurs on cooler surfaces such as the window panes during the cold season where it becomes a useful indicator of vapour production, condensation can occur on the carpets covering cold floors. In recommendations about humidity control, ventilation of the house is one of the main factors, and the production of water inside a confined space requires effective ventilation in order to avoid significant condensation. In addition to the ventilation rate, the lack (usually) of cooking, clothes washing, and showering in offices and schools results in much less water vapor generated than in homes. This, added to frequent professional carpet cleaning in offices, probably, explains the low level of fungal concentration.

In home environments, the level of the fungal concentration from living-room dust was higher than from bedroom dust. This difference is also true for the case of mite allergen levels which were higher in dust samples from living-room floor than in dust from bedroom floor (Smith et al., 1985; van Strien et al., 1994). Even in the air, Strachan et al. (1990) reported a higher level of fungal propagule counts m^{-3} in living-room than in bedroom. Although the median of the living-rooms (median: 2×10^5 CFU/g) was higher than that of the bedrooms (median: 7×10^4 CFU/g), overall, six bedrooms revealed the highest concentrations. In an air-monitoring study, Su et al. (1992) reported that common soil fungal concentrations (*Aspergillus* and *Penicillium* spp.) were higher for homes with crawl spaces (usually with a dirt floor, often damp, which can be a contamination source) than for homes with occupied basements. The content of soil particles in carpet dust can depend on the proximity to outdoors, and also as a result of walking on the carpet with shoes on rather than with shoes off. These situations are more frequent in living-rooms than in bedrooms, yet the highest contamination ($\geq 10^6$ CFU/g) occurred in the bedroom and consequently, could be attributed to fungal growth in carpet dust rather than an influx from other sources such as air or soil. Growth in carpeting was a likely explanation for three of five species respon-

sible for these contaminations i.e. *A. niger*, *A. pullulans* and *P. herbarum* whose high frequencies (34%, 50% and 46% for *Phoma* spp.) bore witness to their predilection for this biotope. Contaminations by *G. candidum* and *P. thomii* were sporadic and causes remained unclear. The magnitude of the fungal concentrations by *A. niger*, *A. pullulans* and *P. herbarum* could be attributed to the fact that some people were reluctant to air their bedrooms during the night, because of the noise of the street, burglaries, etc., or cold weather in winter. Indeed, in bedrooms, condensation on window panes is frequent, although opening windows is the easiest way to reduce humidity (because condensation can occur outdoors in cold weather).

Domestic activities can generate aerosols of dust allergens: Hunter et al. (1988) showed that high counts of propagules in the air can be the result of dust-raising activities such as vacuum cleaning of carpets, which could release large quantities of spores of allergenic or toxigenic fungi into the air. Cole et al. (1993, 1994) reported that a mean carpet concentration of 8×10^5 CFU/g dust does not appear to contribute to problem levels of airborne biocontamination in the course of routine cleaning. However, they noted that neglected and uncleaned carpets can affect the quality of the indoor air. Nevertheless, it must be emphasized that vacuum cleaners can differ significantly in their capacity for disseminating particles into the air, as reported by Sly et al. (1985). Buttner and Stetzenbach (1993) determined the effect of human activity on air sampling in an experimental room: with a carpet as source of *P. chrysogenum* spores, walking or vacuuming resulted in retrieval of significantly higher concentrations of airborne spores and when there was an increased spore load in the carpet, disturbance by walking resulted in the retrieval of higher concentrations of airborne spores. It is suggested that it is in the bedroom, where the most heavily contaminated dust samples occur (and which could be at the origin of adverse health effects), that the mycoflora must be principally investigated.

In conclusion, this study of fungi in carpet from different locations in Belgium revealed that: (i) Xerotolerant species such as *Eurotium repens* and hydrophilic species were commonly found in carpet dust, probably owing to the high humidity in the carpets; (ii) Wall-to-wall carpets from homes had higher contamination levels than less confined spaces such as offices or school classrooms which had low contamination levels; (iii) The highest contamination occurred in some bedrooms, and, when fungi are suspected to be the origin of respiratory or other illness symptoms, the mycoflora of this location, in particular, should be investigated.

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