

Characteristics of dwellings contaminated by moulds†

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Dwellings showing a presence of moulds are considered to be unhealthy both by the inhabitants and by sanitary authorities. Although the thresholds of pathogenicity have not yet been established, the toxic, allergic and infectious risk of indoor moulds is better understood today. A study on indoor fungi contamination for 128 dwellings was done between October and May in France. It concerned 69 dwellings, the occupants of which either complained to the sanitary authorities about problems of moulds and humidity or consulted a doctor who related their symptoms to housing conditions. Fifty-nine other dwellings, the occupants of which were healthy, constituted the control group. We present the statistical analysis of questionnaires, which aimed to clarify characteristics of dwellings associated with high concentrations of airborne moulds. Air samples were taken with an impactor in 500 rooms. On visiting dwellings, investigators obtained answers to 25 questions concerning characteristics of inhabitants and living space, as well as the presence of mould indicators. Indoor and outdoor temperature and indoor relative humidity of air measurements were taken. The total concentration of fungi in the air was significantly higher in ground floor apartments *versus* those on other floors ($p = 0.047$), in small and highly occupied dwellings ($p = 0.03$ and 0.003), in dwellings with electric heating ($p = 0.04$), without a ventilation system ($p = 0.003$), with water damage ($p = 0.003$), and finally, in those where the investigator noted an odour of moisture or visible moulds ($p < 0.001$). The efficacy of the latter criteria in the evaluation of insalubrity is discussed.

Introduction

The presence of moulds in the domestic environment is a source of concern for health authorities and the scientific community because they are at the origin of disorders ranging from acute discomfort such as Sick Building Syndrome¹ to potentially fatal problems, such as pulmonary hemosiderosis.² From a social perspective, the standard dwelling is incompatible with the presence of moulds, and their presence is likened to unworthy housing or insalubrity. This has led various committees of experts worldwide to meet in order to define methods for measuring indoor fungal contamination and to estimate health risk for the occupants.^{3,4} Medically, fungal exposure inside the home has been associated with allergic reactions, infectious diseases that occur in immuno-suppressed subjects and symptoms attributed to the toxic effects of fungi.^{5–8} The diseases about which most information is available are respiratory allergy symptoms in sensitized individuals: asthma, rhinitis, allergic bronchopulmonary aspergillosis (ABPA) and hypersensitivity pneumonitis. Personal factors (age, individual susceptibility)

combined with exposure parameters (nature and concentration of contaminants, duration and type of exposure) constituted data that can have an influence on health problems after exposure. The main factors likely to increase fungal concentration in indoor air are water infiltration,^{9,10} problems of poor insulation, thermal bridges and condensation,¹¹ lack of ventilation,^{12,13} carpet for floor covering^{14,15} and presence of pets.^{14,16}

From a legal standpoint, the presence of fungi in dwellings as a factor of insalubrity is a notion that remains to be defined. In industrialized countries, citizens with indoor problems are directed to local public bodies that deal with domestic hygiene, who may order an inspection of the dwelling. The type of samples taken on this occasion varies from a simple measure of relative humidity of air to thorough investigations of the environment, as done by the “Service d’Analyse des Milieux Intérieurs” (SAMI) of Liège in Belgium.¹⁷ In France, the SRU (Solidarité Renouvellement Urbain) law proposes to evaluate the presence of moulds by subjective criteria or indirect measurement (humidity, dampness odour and visible moulds); the presence of other compounds, such as carbon monoxide and lead, would indicate the need for direct measurement.

In view of the lack of studies on the fungal contamination of dwellings in France,¹⁸ we undertook a study on airborne contamination in 500 rooms from 128 dwellings. We present the statistical analysis of a housing questionnaire, the objective of which was to clarify the physical or incidental characteristics of dwellings associated with high concentrations of moulds. This study also had to judge the efficiency of a questionnaire in assessment of indoor environment and utility of microbiological measures.

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† Electronic supplementary information (ESI) available: Table A: Mean ratio and 95% confidence intervals as determined by weighted regression according to the month of sampling. Table B: Geometric means, geometric standard deviation for total concentration of fungi in the air, means ratio and 95% confidence intervals according to particular room characteristics. See DOI: 10.1039/b718909e

Materials and methods

Dwelling selection

The 128 dwellings investigated were recruited from three sources between October 2004 and May 2006. For 29 dwellings, indoor air investigations for fungi were requested by a physician for allergologic or pneumologic problems. These cases were indicated by the “Réseau Franc-Comtois d’Allergologie (RAFT)” or by the university hospital Department of Respiratory Disease. For 40 dwellings, investigation was requested following a complaint registered either at the city health department (“Service Hygiène et Santé”), for urban areas, or at the county health department (“Direction Départementale des Affaires Sanitaires et Sociales”), for rural areas. Controls were selected for these 69 dwellings with the following criteria: same geographic area, size (number of rooms $\pm$ 1) and occupation (number of inhabitants $\pm$ 1). Fifty-nine of the 69 controls selected agreed to participate in the study.

Indoor investigation

Indoor investigations were done by trained investigators who visited the dwellings. They used a questionnaire that covered characteristics of inhabitants and living space: geographic zone (urban or rural), living level (apartment on the ground floor or another floor, or independent home with several floors), number of rooms, occupant-surface ratio (number of inhabitant/surface area), age of the home (less or more than 10 years in 2006), smoking habits inside the home, heating source (gas, electric or central heating), ventilation type (functional air extractor, simple air vent or no ventilation), presence of pets, and water damage.

For each room involved in the sampling, the following characteristics were collected: volume (in cubic metres), presence of a washing machine in room, room used to dry laundry, windows that open, air vents on windows, time of airing per day, floor covering (tiles, linoleum, carpet, wooden floor, other), and the presence of plants (at least five). Furthermore, a visual inspection for moisture and moulds was done by the investigator for each sampling room and included evaluation of surfaces for visible moulds (level 1: 0 to 100 cm², level 2: 100 to 500 cm², level 3: 500 cm² to 1 m², level 4: 1 to 5 m², level 5: more than 5 m²) as well as the absence or presence of the following variables: visible moulds, dampness odour, perceived humidity, the remains of steamed-off wallpaper and blistered paint.

Temperature and relative humidity of air measurement

Outdoor temperature was the temperature recorded the day of investigation by the nearest local meteorological station and published on the Meteorological Office web site. Indoor temperature and relative humidity of air were recorded with a portable thermohygrometer in two rooms of the dwelling—the bathroom and the living room. A mean of the two values was used for the analysis.

Microbiological samples and analyses

Dwellings were visited between October and May to exclude the summer season, when the concentration of microorganisms in

outdoor air increases and windows are often opened. Four rooms were investigated per dwelling, when available: the bathroom and the kitchen (rooms with water) and the bedroom and living room (living areas). Air sampling was done in rooms that had not been vacuumed for at least two days and in which windows had been closed for at least one hour. From each room, 100 litres of air were taken with a biocollector MAS100™ (Merck®, Darmstadt, Germany), placed in a central position, 0.75 m from the floor and using Malt agar (Oxoid, Unipath, Basingstoke, England) and Dichloran-Glycerol (Oxoid, Unipath, Basingstoke, England) culture media added with 0.5% chloramphenicol (Merck, Darmstadt, Germany). The malt agar medium was incubated at 25 °C and Dichloran-Glycerol at 30 °C. Microorganisms were counted and identified at 7 days. For each species, the culture media used was that in which colonies were most numerous. Results were expressed in colony-forming units per cubic metre of air (CFU m⁻³) after correction with the “positive hole conversion table MAS100”.

Statistical analyses

STATA Statistical Software release 9 (StatCorp LP 2005) was used for statistical analysis. The total concentration of fungi and concentration for genus *Aspergillus*, *Penicillium* and *Cladosporium* were first tested for normality. Values were then transformed by natural logarithm to normalize the distribution so that parametric tests could be performed. After logarithm transformation, all skewness were smaller than 1.3. We expressed mean concentrations as geometric means (GM) with a geometric standard deviation (GSD). Weighted regression adjusted for a month of sampling was used to identify statistical predictors of fungi concentration in the air. The association between fungi levels and explanatory variables is presented as the means ratios (MR). For continuous independent variables, MR represents the factor by which the geometric mean is increased (or decreased) per interquartile range. For categorical independent variables, MR is the ratio of the geometric mean in the category concerned versus the reference category. Statistical significance was set at the conventional 5% level.

Results

Microbiological analyses

The 7 species present in more than 10% of samples (50/500) are described in Table 1. Other species in decreasing percentage of frequency were: *Ulocladium* sp. (7.8%), *Fusarium* spp. (7.2%), *Botrytis nivea* (6.8%), *Mucor* spp. (5.4%), *Paecilomyces* spp. (4.6%), *Trichoderma viride* (3%), *Rhizopus* sp. (3%), *Beauveria* sp. (2.6%), Basidiomycetes (2.4%), *Acremonium* spp. (2.4%), *Scopulariopsis* spp. (2.2%), *Aureobasidium* sp. (2.2%), *Absidia* spp. (2.2%), *Epicoecum* sp. (2%), *Papularia* sp. (1.8%), *Aphanocladium* sp. (1.6%), *Phoma* sp. (1.2%), *Pithomyces* sp. (0.8%), *Verticillium* sp. (0.4%), *Scedosporium* sp. (0.4%), *Syncephalastrum* sp. (0.4%), *Trichosporon* sp. (0.4%), *Humicola* sp. (0.2%), *Rhizomucor* sp. (0.2%), *Geomyces pannorum* (0.2%), *Ramichloridium* sp. (0.2%).

The 3 genus *Cladosporium*, *Penicillium* and *Aspergillus* represented 86.16% of the total fungi isolated (in CFU m⁻³) in the air

Table 1 Details from the 7 species most often present in samples ($n = 500$) in CFU m⁻³

	Complaint and health problem dwellings		Control dwellings	
	PS (%)	Mean (SD)	PS (%)	mean (SD)
<i>Penicillium</i> spp. ^a	90.8	215 (579)	87.9	229 (778)
<i>Aspergillus</i> spp. ^b	98.9	232 (697)	82.7	54 (140)
<i>Cladosporium</i> spp. ^c	87.5	196 (685)	77.5	72 (119)
White yeasts	27.2	14 (49)	30.7	11 (29)
<i>Wallemia sebi</i>	15.8	24 (149)	11.3	2 (6)
<i>Alternaria</i> spp.	13.2	2 (9)	8.2	1 (4)
<i>Rhodotorula</i> spp.	9.9	1 (4)	12.1	3 (9)

^a PS: positive samples; mainly *P. chrysogenum* and *P. olsonii*. ^b In decreasing percentage of frequency: *A. versicolor*, *A. glaucus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. nidulans*, *A. candidus*, *A. flavus*, *A. terreus*, *A. wentii*. ^c Mainly *C. cladosporioides* and *C. herbarum*.

of dwellings with subjects presenting with complaints and health problems and 85.94% for controls dwellings.

Influence to the month of sampling

A significant increase in total concentration of fungi was observed in October ($p = 0.006$) (see table A in electronic supplementary information†). When taken individually, genus *Aspergillus* decreased significantly in May ($p = 0.03$), whereas *Penicillium* increased in October ($p = 0.03$) and *Cladosporium* in May ($p = 0.01$) and October ($p > 0.001$).

General housing characteristics

Four general housing variables associated with dampness presence significantly affected the total concentration of fungi (Table 2): living level, heating mode, ventilation type and water damage. Thus, the total concentration of fungi was significantly higher in apartments located on the ground floor compared to those located above the ground floor or in individual homes. Genus *Cladosporium* was significant ($p = 0.02$) and *Aspergillus* and *Penicillium* were not. The total concentration of fungi was higher in houses with electric heating versus central heating. Individually, genus *Aspergillus*, *Penicillium* and *Cladosporium* were not significant. The total concentration of fungi was higher in dwellings without ventilation than in those with a functional air extractor. Genus *Penicillium* ($p = 0.008$) and *Cladosporium* ($p = 0.001$) were significantly higher, contrary to genus *Aspergillus*. Conversely, the total concentration of fungi did not differ between dwellings with simple air vents and functional air extractors.

The total concentration of fungi did increase significantly when water damage was present in a dwelling, with a significant increase in genus *Penicillium* ($p = 0.03$), but not *Aspergillus* or *Cladosporium*.

Other general housing variables associated with an increase in total concentration of fungi in airborne samples were number of rooms and occupant-surface ratio. The number of rooms was significantly and negatively associated with the total concentration of fungi. Considered separately, genus *Aspergillus*, *Penicillium* and *Cladosporium* were not significantly associated with this

Table 2 Geometric means (GM), geometric standard deviation (GSD) for total concentration of fungi in the air, and means ratio (MR) and 95% confidence intervals (CI) according to general housing characteristics^a

Variables	N/128	GM (GSD)	MR (95%CI)	P value
Geographic zone				
Urban	65	321.07 (1.26)	Ref.	
Rural	63	296.84 (1.09)	0.95 (0.62–1.44)	0.80
Living level				
Ground floor	22	573.93 (1.51)	Ref.	
Next floor	56	281.31 (1.24)	0.55 (0.31–0.99)	0.047
Individual house	49	264.06 (0.83)	0.50 (0.27–0.92)	0.03
Number of rooms	126	—	0.84 (0.72–0.98)	0.03
House age				
<1996	103	299.12 (1.08)	Ref.	
>1996	14	329.13 (1.73)	1.25 (0.63–2.50)	0.52
Occupant-surface ratio	126	—	2.66 (1.42–4.98)	0.003
Smoking in dwelling				
Yes	43	265.48 (1.10)	Ref.	
No	85	333.52 (1.21)	1.28 (0.83–1.97)	0.28
Heating mode				
Electric	22	494.84 (1.43)	Ref.	
Gas	45	304.75 (1.28)	0.65 (0.36–1.17)	0.15
Central	55	254.90 (0.94)	0.53 (0.29–0.95)	0.04
Ventilation type				
Air extractor	74	249.82 (1.21)	Ref.	
Air vent	40	329.96 (0.94)	1.10 (0.69–1.75)	0.69
No ventilation	14	785.90 (1.22)	2.78 (1.45–5.36)	0.003
Presence of pets				
Yes	64	294.93 (1.15)	Ref.	
No	64	323.55 (1.21)	1.08 (0.71–1.66)	0.71
Water damage				
Yes	34	523.25 (1.52)	Ref.	
No	94	255.30 (0.96)	0.50 (0.32–0.78)	0.003

^a N: number of answers out of 128 dwellings; Ref: reference category.

variable. The total concentration of fungi increased with the number of inhabitants per cubic metre. *Penicillium* ($p = 0.048$) and *Cladosporium* ($p = 0.01$) were significant, *Aspergillus* ($p = 0.06$) was not.

The following characteristics were not associated with a significant change in the total concentration of fungi: outdoor and indoor temperature ($p = 0.10$ and 0.11), indoor relative humidity of air ($p = 0.21$), geographic zone (urban/rural), age of the house, smoking habits in the dwelling, heating mode and presence of pets.

Particular room characteristics

The following room characteristics were not associated with a significant increase in total concentration of fungi in airborne samples (see table B in electronic supplementary information†): the nature of the sampled room (bathroom, kitchen, bedroom, living room), volume, if it was used to dry laundry, the presence of a washing machine, of windows or air vents in windows, airing time per day, floor covering and the presence of plants.

Visual inspections for moisture and moulds

All variables in connection with visual inspections for dampness and moulds were associated with a significant change in the total concentration of airborne fungi (Table 3), but because these variables are strongly linked, only dampness odour and visible moulds were significant in the adjusted model. 169 rooms had dampness odour and/or visible moulds and 331 rooms did not. For adjusted variables, dampness odour was associated with an increase in *Aspergillus* ($p = 0.03$), *Penicillium* ($p = 0.04$) and *Cladosporium* ($p = 0.04$) and the presence of visible moulds was associated with a significant increase in *Aspergillus* ($p = 0.02$), *Cladosporium* ($p = 0.002$) but not *Penicillium* ($p = 0.09$).

The distribution for total concentration of fungi in airborne samples was 50 CFU m⁻³, 130 CFU m⁻³, 230 CFU m⁻³, 470 CFU m⁻³, and 2440 CFU m⁻³ for the 5th, 25th, 50th, 75th and 95th percentiles, respectively. If we view the percentage of rooms with and without dampness odour or visible moulds, as defined by six ranks corresponding to these distributions (Fig. 1), we see

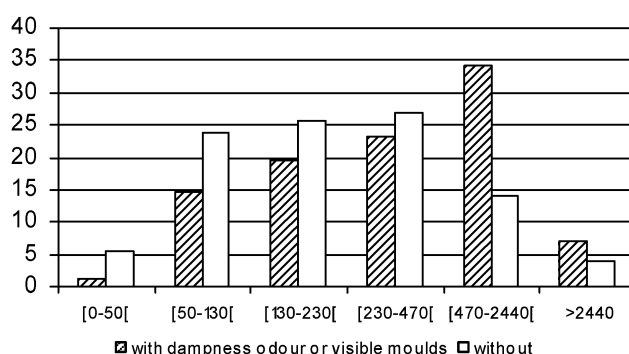


Fig. 1 Distribution (in %) for rooms with and without dampness odour or visible moulds according to 6 ranks for total concentration of fungi in airborne samples (in CFU m⁻³).

that 16% of the rooms (27/169) with dampness odour or visible moulds have less than 130 CFU m⁻³ of total fungi in airborne samples and 17.8% (59/331) of the rooms without dampness odour or visible moulds have more than 470 CFU m⁻³. No significant differences were found between these rooms and the other rooms with regard to the explanatory variables presented in this study (characteristics of dwellings). Eight of the 59 rooms with high fungal spore concentrations but no visible moulds were sampled in October. Eleven of the 272 rooms with high fungal spore concentrations and visible moulds were sampled in October. The χ^2 test results were significant ($p < 0.01$): homes that had high fungal spore concentrations but no visible moulds were significantly more frequently sampled in October.

Assessment of surface with visible moulds was done for 5 levels of contamination. 356 rooms had less than 100 cm² of visible moulds (level 1), 86 between 100 and 500 cm² (level 2), 31 between 500 cm² and 1 m² (level 3), 8 between 1 m² and 5 m² (level 4) and 9 rooms had more than 5 m² of visible moulds (level 5). An increase in level for the surface of visible moulds was associated with an increased microbial concentration in the air (Fig. 2) but the correlation was low ($R^2 = 0.06$). Because the box plots presented in Fig. 2 showed a plateau after level 3, we can assume that a 3-level evaluation (<100 cm²; 100–500 cm²; >500 cm²) would have sufficed to predict high concentrations of fungi in the air.

Table 3 Means ratio (MR) and adjusted mean ratio according to visual inspections for moisture and molds^a

Variables	N/500	MR (95%CI)	P value	Adjusted MR (95%CI)	P value (adjusted)
Dampness odour					
Yes	53				
No	447	0.36 (0.26–0.50)	<0.001	0.45 (0.29–0.68)	<0.001
Perceived humidity					
Yes	66				
No	434	0.55 (0.40–0.75)	<0.001	1.19 (0.79–1.80)	0.40
Steamed-off wallpaper					
Yes	62				
No	438	0.63 (0.47–0.87)	0.005	0.91 (0.64–1.31)	0.63
Blistered paint					
Yes	38				
No	462	0.44 (0.29–0.62)	<0.001	0.70 (0.46–1.08)	0.11
Visible moulds					
Yes	156				
No	344	0.54 (0.43–0.68)	<0.001	0.67 (0.52–0.86)	0.002

^a Ref.: reference category; N: number of answers on 500 rooms; 95%CI: 95% confidence interval.

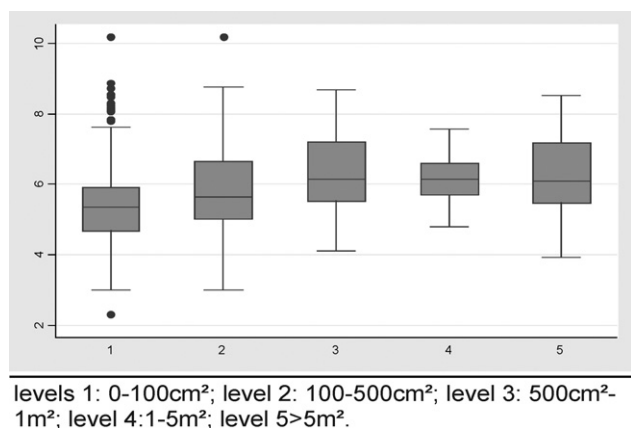


Fig. 2 Box plots for total concentration in moulds (in $\ln \text{CFU m}^{-3}$) in rooms according to the surface of visible moulds observed.

The dispersal of values, particularly in rooms presenting few or no traces of visible moulds (level 1, Fig. 2), shows that high concentrations of moulds in the air were also obtained in rooms with no visible traces of moulds.

Discussion

As shown in other studies carried out in various regions,^{19,20} the three genus most frequently isolated in the air were *Penicillium*, *Aspergillus* and *Cladosporium*. The latter fungi is typically found in outdoor samples. *Penicillium* and *Aspergillus* grow more commonly in an indoor environment (houseplants, food, carpet etc.).^{20,21} To minimize the influence of the season, samples were taken between October and May, when indoor mould concentrations are lower and occupants readily close windows.^{16,22} Furthermore, the statistical model was adjusted for month of sampling.

Six variables were significantly associated with an increased concentration of moulds in the air: living on the ground floor, without a ventilation system, with electric heating, in a small, highly occupied apartment. Before we began our study, public health inspectors had reported that the presence of moulds was often due to an unsuitable use of the housing and to non compliance with basic rules of hygiene. Hence, we expected the significant variables to be: little aeration per day, laundry put to dry in a room or the presence of plants. This was not the case. The significant variables were related to excess humidity in the dwelling due to causes that were either structural (lack of ventilation, apartment in the ground floor) or accidental (water damage). It is essential to ascertain the risk factors for the development of moulds in order to provide effective means of prevention and remedies.

In 16% of the rooms sampled, mould concentrations in the air were low ($<130 \text{ CFU m}^{-3}$) despite the presence of a mouldy odour or visible moulds on the walls. Indeed, the dispersal of spores from a substrate depends on numerous factors such as their size, roughness, density, electric load, movements of air or nature of support.²³ Therefore bathrooms, where the presence of moulds is often visible on shower joints, are no more contaminated than bedrooms, kitchens and living rooms. On the other hand, 18% of the rooms sampled, in which the investigator

detected neither a dampness odour nor visible moulds on walls, were highly contaminated ($>470 \text{ CFU m}^{-3}$). Here, we can presume that the mould source was hidden (behind walls, under the carpet) or that moulds present on the walls were neither colourful, nor fragrant. Moulds that produce melanin in layers are more visible, and those that produce volatile organic compounds (VOCs) are more fragrant.^{24,25}

Microbiological results of air samples are known to vary widely, although microbiologists have not reached a consensus as to an acceptable variability.^{10,26} We think it can be assumed that any episode of mould in a dwelling is not temporary and persists through the season.

Airborne fungi from outdoor air have an influence on airborne fungi from indoor air. Peak of proliferation of airborne fungi depends on geographical zones.^{27,28} In France, one peak arises from June until September for the main microorganisms.²⁹ Twice in our study, October appears as a factor of increased airborne fungi from indoor air. We can assume that this increase is due to above normal seasonal temperatures in October, 2006 (temperature averages 3.3°C above normal according to the French meteorological office). Meteorological factors such as temperature are significantly correlated with the atmospheric concentration of spores, and the quantity of spores (*Cladosporium* for example) is positively correlated to temperature and hours of sunlight.³⁰

Because they can cause health problems, moulds in dwellings are now taken into account in insalubrity criteria established by legislation in larger industrialized nations. The need for environmental samples and the utility of a simple questionnaire is still a matter of debate. French legislation considers the presence of dampness odour or visible moulds as to be a criteria of insalubrity. These criteria can be obtained by a simple questionnaire. Auto-questionnaires performed by occupants are not reliable.^{9,31} Investigations by a trained investigator are of better quality, even if only for dampness odours or visible moulds. In our study, dampness odour and visible moulds criteria were a predicting factor of high mould concentration in the air ($>470 \text{ CFU m}^{-3}$) in 80% of cases. High concentrations are not revealed in 20% of the cases (one investigation on five). On the other hand, subjective consideration by questionnaire does not allow the determination of fungal concentrations in the air or the determination of fungal species. This is also the case for samples and analyses concerning global compound quantifications such as ergosterol or VOCs dosage. Fungal species provide important information because they do not have the same pathogenicity. For example, *Alternaria alternata* is one of the main allergens in allergic rhinitis and asthma. *Stachybotrys chartarum* is probably one of the specific agents of pulmonary hemosiderosis.² Concentrations also are important, so an increase in allergen concentration can be associated with an increase in allergic sensitization.³² An association between the quantity of fungal spores in the atmosphere and the number of cases of hospitalization for asthma has been strongly suspected.³³ To implement epidemiological studies that will allow the determination of thresholds of pathogenicity, fungal species must be taken into account. Moreover, health professionals may need a list of fungal species present in the dwellings of the patients they see. Indeed, the allergologic prick tests, which are specific to given species, could be adapted to the patient's environment. In hypersensitivity pneumonitis, serology

is one of the diagnosis criteria, and determining fungal species is very useful in adapting the panel of antigens to the real exposure and in setting up means of prevention and remedies.^{34,35}

Conclusions

In conclusion, if the occupant is sick, if the source of fungal contamination is not clear, or if the presence of moulds is strongly suspected without odour or visible spots, air samples must be taken.

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