



The importance of combining air sampling and surface analysis when studying problematic houses for mold biodiversity determination

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Abstract

Houses that underwent water damages are often responsible for health problems of the occupants. Since there is no universally used protocol for the analysis, we wanted to verify the usefulness of surface sampling versus air sampling for the evaluation of mold diversity in problematic houses and the value of the number of visible mold growth zones to predict air quality. Seventeen houses were sampled for culturable molds in the air and on the surfaces showing contamination. We compared the mold taxa found in the air and on the surfaces and verified the correlation between the number of moldy surfaces and airborne mold concentration. This study demonstrated that, surprisingly, some of the so called wet spore molds (e.g. *Stachybotrys*) were found more often from air than surface samples whereas, some dry spore molds (e.g. *Asp. fumigatus*) was more easily isolated from surface samples. There was a good correlation between the number of visible mold growth zones and the concentration of airborne molds. We conclude that air and surface sampling are necessary to evaluate mold diversity in problematic houses and that the number of mold growth zones is a good predictor of airborne mold concentration.

Introduction

Outdoor air normally contains various types of molds and other organic particles. Many sources such as decaying organic material, water sources, and animals are responsible for these so called bioaerosols. In the Canadian cold climate, outdoor bioaerosols concentrations under snow cover are almost zero. The indoor environment contamination originates from sources different from the outdoor: human, animals, house dust, different substrates supporting the growth of microorganisms. The abundance of the sources, the dispersion and decay factors and the release of indoor spores will determine the concentration of aerosols (Burge, 1995). When houses undergo water damages, mold proliferation may become very important and decontamination of these houses is inevitable. Inhalation of mold spores may induce diverse health effects such as allergic problems (rhinitis, allergic alveolitis, asthma) and systemic symptoms related to inhalation

of mycotoxins produced by certain types of molds (Burge, 1990). Air and building analyses are often performed in order to determine the salubrity of homes and to find potential responsible agents for health problems and determine the exposure levels (Macher and Huang, 1991; Beguin and Nolard, 1991; Icenhour and Levetin, 1997; DeKoster and Thorne, 1995). Air sampling should be the most representative of human exposure (Burge, 1995). It is difficult to achieve a representative sampling because the sampling period is limited, it does not represent a real dose evaluation and the methods used do not allow the recovery of all mold species. Surface sampling can also be performed in order to determine the levels of biological contaminants in houses and help to establish the building's history. Miller and co-workers (Miller et al., 1988) studied Canadian homes using bulk and air samples and concluded that the different methods used to analyse the contamination in houses provide different information.

One can divide the spore types into two different groups: wet spores (e.g. *Stachybotrys* sp., *Acremonium* sp., *Trichoderma* sp.) and dry spores (e.g. *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp.). The dry spore molds can produce large quantities of airborne spores and are therefore easily inhaled whereas wet spores, which are produced in a mucilaginous mass, are not always easily propelled into the air. The “dry” or “wet” state of spores are not related with water activity requirements for growth but are a spore production characteristic. The wet spore molds are sometimes associated with visible growth patches on walls and other surfaces (Beguín and Noland, 1994). Some consultation companies, when having to study complaints houses, will use just surface sampling because, in their opinion, some of the important toxigenic mold (e.g. *Stachybotrys chartarum*) may be found more easily on walls and surfaces than in the air.

The major aims of this study were (1) to compare, qualitatively, the population of molds recovered with air sampling and sampling of visible surface mold growth (2) determine the predictive value of the abundance of moldy zones for airborne mold concentration and (3) determine the importance of air sampling for culturable molds versus the surface sampling in the evaluation of the house mycoflora.

Materials and methods

Houses selection and sampling sites

Seventeen houses from eastern Canada (province of Quebec) were selected because of self-reported health problems of occupants following water damages. All the sampled houses underwent cleaning and renovation after the water damage without solving the occupants health problems. They were sampled during the 1998–1999 winter (under snow cover). Samplings were performed during the winter to minimize the influence of outdoor molds on the results obtained. Samples were taken in the main room of each floor or in the bedroom of the occupants with health problems. The mold growth sources were analysed in every room of the sampled floor.

Sampling

Six-stages Andersen microbial samplers (AMS) (Graseby Andersen, Atlanta, GA) were used for air sampling. The samplers were put on a table, about

one meter above the floor. Doors and windows were closed prior to sampling but other room doors were kept open during sampling. The ventilation was kept as in the normal situation. AMS were loaded with rose bengal agar (RBA) and malt extract agar (MEA). The flow rate was set at 28.3 l/min with a Drycal DC-1 flow meter (Bios International Corporation, Pompton Plains, NJ). Samples with each media were performed for 5 and 20 minutes. The plates were incubated at 30 °C for 7 to 10 days. Colonies were counted on RBA for quantitative analysis and molds were isolated and identified or identified directly on the original plate, if possible, from each media. Molds were identified at the genus or group level.

Temperature and humidity were measured with a thermometer/hygrometer (Fisher Scientific, Montreal, Canada). Surfaces were sampled on every visible mold growth source found on the sampled floor. Samples were taken by one or all the following methods according to the availability of the zone: scotch tape direct sampling, cotton swabs and RBA Rodac contact plates (Replicating Organism Direct Agar Contact). If available, bulk samples were taken (piece of carpet, wall, wall paper). The swab and bulk samples were plated on the same culture media as AMS. The scotch tape was analysed directly by microscopy after staining with lactophenol blue. The Rodac plates were incubated in the same conditions as AMS plates. Molds were identified using different reference books (St-Germain and Summerbell, 1996; Wang and Zabel, 1990; Samson et al., 1996; Watabene, 1994).

Statistical analyses

Correlations between air levels of molds and number of visible mold growth zones were performed with Spearman test since the data were not normally distributed.

Results

We studied the relationship between the number of mold growth zones and the concentration of molds in the air. Table 1 shows the values obtained for each floor (second, main and basement) for those values. Also shown are the values for the house, all floors pooled together: average for airborne molds and sum of the number of sources. The correlation found for the floors taken separately is significant ($p = 0.002$, $r_s = 0.53$) but the correlation for the global house analysis is not ($p = 0.06$).

Table 1. Relationship between mold levels in the air and the number of visual sources. The data are given separately per floor and the total number of sources per house and the house average old levels.

House	Airborne molds (number of visual sources) CFU/m ³ (number)			Average Airborne molds CFU/m ³	Total sources/ house
	2nd Floor	Main Floor	Basement		
1		470 (2)	1579 (3)	1025	5
2		44 (1)	47 (0)	46	1
3		1586 (2)	2961 (4)	2274	6
4		47 (2)		47	2
5	10 (0)	0 (0)	² 323 (0)	108	0
6		49 (0)	25 (0)	37	0
7		32 (3)	² 16 (2)	24	5
8		32 (4)	58 (3)	45	7
9		67 (2)		67	2
10	9 (0)	7 (0)		8	0
11	60 (1)	46 (0)		53	1
12	30 (1)	25 (0)	1123 (3)	393	4
13	2196 (0)	3916 (3)		2696	3
14		32 (1)		32	1
15	19 (0)	567 (0)	1839 (5)	808	5
16		1165 (1)	603 (4)	884	5
17		21 (0)	22 (0)	22	0
Floor CFU/m ³ and sources p = 0.002 (r _s = 0.53)				House CFU/m ³ and sources p = 0.06 (r _s = 0.48)	

¹0 represents value lower than the detection limit (2 CFU/m³)

²Denotes unfinished basement consisting of dirt and/or gravel floor, exposed structural wood-work/masonry, unfinished walls, and little or no insulation

We identified several mold taxa from the surface and air samples. Figure 1 shows the number of houses where each taxa was recovered from at least one surface or air sample. The wet spores molds are identified on the plot.

Discussion

In order to find the source of health problems in houses that underwent water damages, air and surface sampling are often performed. Air sampling is thought to have its weaknesses because some mold species produce spores that are not easily aerosolized because of their sticky nature. In several studies, *Stachybotrys* spores were not recovered from air samples but from bulk or surface samples (Cooley et al., 1998). In other studies, this same mold was recovered from air samples but showed low concentrations even in the presence of moldy patches and visible growth

(Beguin and Nolard, 1994). In our study, it was very interesting to observe that this same taxa was found in twice as many houses in the air than on the visible growth zones. *Exophiala* was found in the same number of houses from air or surface samples. For the other wet spore molds, the surface allowed their recovery in more houses. From air samples, the very high frequency of isolation of *Penicillium* strains confirms several studies that showed the importance of this taxa in complaints houses or buildings (McGrath et al., 1999; Cooley et al., 1998; De Koster and Thorne, 1995). Taxa such as *Asp. versicolor* and *Eurotium* were found in many houses. These molds were recovered more often from air samples whereas *Asp. fumigatus* was more often found in surface samples. Since *Asp. fumigatus* has water activity (a_w) needs that are higher, it is possible that its growth occurred on more humid surfaces and that the spores are not aerosolized as easily as *Asp. versicolor* or *Eurotium* that can grow on drier substrates (Raper et al.,

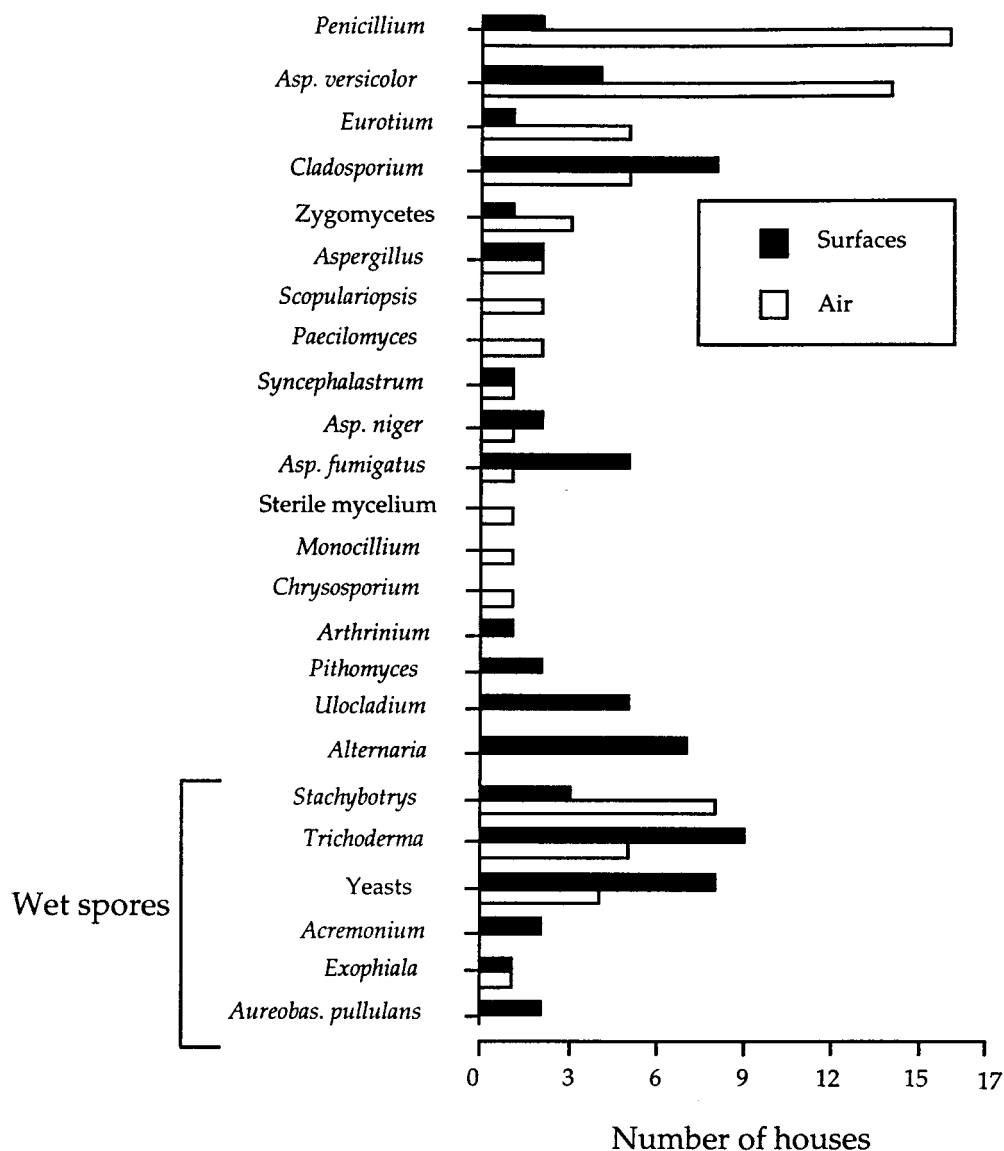


Figure 1. Frequency of recovery and identification of different mold taxa from air and surface samples from 18 houses. The frequency indicates the number of houses where the taxa was recovered from at least one sample, on at least one floor. The wet spore molds were separated from the dry spore molds.

1965). *Alternaria* produces spores in small amounts when compared to *Aspergillus* or *Penicillium*: it was not surprising to observe that no air samples showed their presence in several hours, except from surface samplings.

The correlation observed between the number of moldy zones and the air concentration of molds is very interesting. The great majority of the zones were small (a few centimeters). This is why the size of the zones were not taken into account. The

number of those zones interested us more since those houses recently underwent renovation following water damage and the mold proliferation was not abundant yet. Quantitatively, this correlation suggests that the number of observable moldy patches can predict (semi-quantitatively) the concentration of airborne molds. However, to determine the type of molds, surface molds analyses is needed as well as air analysis since the species recovered from those two types of samples may be different and hardly predictable.

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