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Characterizing Mold Problem Buildings – Concentrations and Flora of Viable Fungi

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Abstract

The purpose of this study was to characterize mold problem buildings by determining concentrations and flora of viable fungi. The composition and concentrations of fungal flora in mold problem (n = 9) and reference buildings (n = 9) were determined by means of four different sampling methods: six-stage impactor (Andersen sampler), sedimentation plate, surface and house dust samples. Samples were taken in the fall and in the winter, and the concentrations and flora in mold problem buildings were compared with those of matched reference buildings. The differences between mold problem and reference buildings were most clearly seen with the impactor samples. The total concentrations of airborne fungi were higher in moldy buildings. In addition, the concentrations of the genera Aspergillus *and* Oidiodendron *in the fall and the concentrations of* Aspergillus *and* Penicillium *in the winter were higher in mold problem than in reference buildings. In the winter, certain fungal genera (*Stachybotrys, Acremonium, Oedocephalum *and* Botryosporium*) were detected only in the problem buildings in impactor samples. These results indicate that there may be an unusual composition of fungal flora in mold problem buildings. The results of the sedimentation plate samples showed a trend similar to that of impactor samples in the winter. In addition, the results of surface samples supported the data on the fungal flora in the winter-time air samples. The house dust samples did not reveal any differences between mold problem and matched reference buildings.*

KEY WORDS:
Viable fungi, Concentration, Genera, Methods, Mold problem

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Introduction

People occupying mold problem houses often suffer from respiratory symptoms and other health problems (Platt et al., 1989; Waegemaekers et al., 1989). It is usually assumed that the exposure to high concentrations of airborne microorganisms, especially fungi, is the principal causative factor. However, total concentrations of airborne viable fungi observed in mold problem houses are not necessarily higher than those in control houses (Strachan et al., 1990; Flannigan et al., 1991; Nevalainen et al., 1991). Generally the viable fungal counts have great temporal and spatial variations (Hunter et al., 1988; Pasanen, 1992). Fungi fail to release spores continuously, and the release depends on ambient conditions (Pasanen et al., 1991). Therefore, short-time samples of viable fungi do not always indicate exceptionally high exposure to fungi or the existence of mold problems in buildings. The concentrations and genera of fungi as components of exposure in moldy buildings need, therefore, more detailed characterization.

In this study, concentrations and composition of viable fungi were determined in mold problem buildings (n = 9) and in matched reference (n = 9) buildings. Four different sampling methods were used: six-stage impactor, sedimentation plates, house dust and surface samples. Impactor and sedimentation samples were used to determine total concentrations and the composition of viable fungi in the air. An impactor was chosen to sample the overall range of bioaerosol particles > 1 μm. An impactor sample represents quite a short sampling time (2–15 min) and therefore sedimentation plate samples were taken to increase the timely representativeness of the sampling. Although the sedimentation plate method overestimates larger particles, a significant correlation between the results with

settling plate and an Andersen N6 sampler has been reported (Verhoeff et al., 1992). In addition, our previous studies have shown that the standard deviation of sedimentation plate samples decreases when 6 parallel petri dishes and a longer sampling time (60 min) are used (Jantunen et al., 1989). House dust samples were taken to represent a time-integrated sample, as suggested by Miller et al. (1988). The fungal flora of moist or moldy surfaces was determined to obtain qualitative information about the fungal genera in damaged building materials. This exposure characterization was made in parallel with health data collection by means of questionnaires (Husman et al., 1993; Koskinen et al., 1993).

Materials and Methods

The Buildings Studied

The study was carried out in 18 buildings; six day-care centers and 12 dwellings. Eight of the dwellings were apartments in block of flats and the rest were apartments in semi-detached houses. Three of the day-care centers and six of the dwellings were mold problem buildings and the rest were reference buildings. For each mold problem building, a reference building was chosen matching the age, site, use, construction materials and architecture. All the mold problem buildings had a history of dampness and microbial growth on the surfaces of building materials confirmed during site visits. Dampness problems were caused by water leaks in the construction, inadequate ventilation, defective drainage or moisture condensation resulting from faulty thermal insulation. No major repairs were made in the buildings. However, in some cases, visible mold was removed by washing or replacing the indoor surface materials. No attempts to repair the actual cause of moisture penetration were made. During the sampling, microbial growth was still visible in one of the day-care centers and four dwellings. The reference buildings were also inspected for moisture and mold problems. No signs of such problems were found.

Sampling and Analysis

Samples were taken in the fall, October–December 1990 and in the winter, January 1991. Indoor air samples were taken in 2–4 different rooms in each house. Outdoor air samples were taken in the fall, one for each sampling day. In the winter, outdoor air samples were not taken, because in subarctic winter, the outdoor air levels of fungi are extremely low due to the snow cover, and the contribution of outdoor air to indoor air fungal levels is then negligible (Reponen et al., 1992).

Air samples were taken to determine the concentrations and composition of the fungal flora using a six-stage impactor (Andersen 10–800) and sedimentation plates. Both impactor and sedimentation plate samples were taken on malt-extract agar (MEA) (DIFCO) and incubated at 20–23 °C for 5–7 days. Impactor samples were taken in the middle of the room. Sampling time was 10 min. When taking sedimentation plate samples, 6 agar plates, located on tables, at a height of 1 m around a room, were simultaneously kept open for an hour. For impactor samples, concentrations were calculated using Andersen correction table for multiple impactions on individual deposition sites (Andersen, 1958).

House dust samples were obtained by using the vacuum cleaner of each building studied. The vacuum cleaner was fitted with an unused filter bag before collection. The collection time was 2–4 weeks. Dust samples of 1 g were suspended into 100 ml of dilution water (distilled water with 42.5 mg/l KH_2PO_4, 250 mg/l $MgSO_4$, 8 mg/l NaOH, and 0.02% Tween 80), and the sample was shaken for 60 s. The solution was filtered and then diluted to 10^{-5}. Aliquots (0.5 ml) were plated on MEA. Surface samples (100 cm^2) were taken from surfaces of building materials with a swab into sterile water and the suspension was plated on MEA. The incubation conditions of dust and surface samples were the same as for air samples.

In the analysis, the data were divided into two series: the fall and the winter. In the analysis of the impactor samples, the outdoor air data were analyzed separately. The normality of the distributions of fungal spore concentrations was checked with the Kolmogorov-Smirnov test. Distributions were lognormal, therefore, geometric means of concentrations were used. The logarithmic transformation to each value x was made as $\log(x+1)$. Wilcoxon test was used to test the significance of differences between mold problem and reference buildings for total concentrations of viable fungi and each of the fungal genera isolated. The frequencies of the most common fungal genera were compared with the McNemar test. In both tests each result in a mold problem building was compared with the corresponding value in a reference building.

Results and Discussion

Impactor Samples

In the fall, the indoor air concentrations of viable fungi varied between 19 and 7900 cfu/m^3 in mold problem buildings and between 40 and 580 cfu/m^3 in reference buildings (Table 1). The total concentrations of viable fungi were higher in mold problem buildings than in reference buildings ($p < 0.05$). In addition, a similar difference was found in the concentration of *Aspergillus* and *Oidiodendron* spores ($p < 0.05$). The higher concentrations may be caused by indoor sources of fungi. There was also a statistically significant difference in frequencies of *Aspergillus* between problem and reference buildings ($p < 0.05$) (Table 2). However, the outdoor air concentrations of viable fungi were 37–11 000 cfu/m^3 (Table 1). The total concentrations of viable fungi as well as the concentrations of non-sporing isolates, *Polyscytalum*, and *Cladosporium* were higher in outdoor than in indoor air. Thus, the outdoor air had a clear effect on indoor air fungi. It is possible that also *Oidiodendron* originates partly from outdoor air.

In the winter, the total concentrations of viable fungi were 26–530 cfu/m^3 in mold problem buildings and 15–220 cfu/m^3 in reference buildings (Table 1). The concentrations were higher in mold problem buildings than in the reference buildings ($p < 0.01$). This may indicate indoor sources. The absolute levels of the viable fungi were not especially high, so these results support those of the previous studies where levels of the viable fungi in mold problem buildings have also been low (Strachan et al., 1990; Flannigan et al., 1991; Nevalainen et al., 1991). In winter-time samples in our climate, the outdoor air concentrations of viable fungi are < 100 cfu/m^3 and therefore their biasing effect on indoor air concentrations of viable fungi is minimal (Pasanen et al., 1990).

In addition of the difference in total concentrations of viable fungi, there were also differences in fungal genera between mold problem and refer-

Table 1 Geometric means (GM) and ranges of total concentration of viable fungi and the most common genera or groups of fungi in mold problem and reference buildings determined with an impactor. Statistical significance (S) was tested with the Wilcoxon test.

	Concentrations of viable fungi (cfu/m^3)				S
	Problem		Reference		
	GM	Range	GM	Range	
Fall indoor (n = 25)					
Total	250	19–7900	160	40–580	*
Penicillium	31	0–7900	16	0–72	N.S
Cladosporium	18	0–160	20	0–140	N.S
Aspergillus	4	0–76	2	0–20	*
Yeasts	14	0–74	16	0–200	N.S
Non-sporing isolates	54	0–1700	51	5–280	N.S
Polyscytalum	3	0–40	3	0–38	N.S
Oidiodendron	3	0–49	1	0–9	*
Fall outdoor (n = 9)					
Total	410	37–11000	190	37–630	N.S
Penicillium	14	0–95	12	0–76	N.S
Cladosporium	74	11–430	43	15–160	N.S
Aspergillus	1	0–11	2	0–15	N.S
Yeasts	23	0–790	10	0–83	N.S
Non-sporing isolates	190	19–9300	93	19–300	N.S
Polyscytalum	16	5–93	3	0–54	N.S
Oidiodendron	1	0–12	2	0–42	N.S
Winter indoor (n = 21)					
Total	120	26–530	58	15–220	**
Penicillium	38	0–480	13	0–220	*
Cladosporium	4	0–28	5	0–26	N.S
Aspergillus	3	0–19	1	0–5	*
Yeasts	5	0–78	4	0–62	N.S
Non-sporing isolates	7	0–200	9	0–83	N.S

N.S. $p > 0.05$
* $p < 0.05$
** $p < 0.01$
n = number of matched sample pairs.

Table 2 Summary of frequencies of most common fungal genera and groups of fungi in different samples in mold problem (P) and reference buildings (R). Results indicate the percentage of samples in which each genus was present.

Fungal genera	Percentage frequency of samples (%)							
	Impactor		Sedimentation		Dust		Surface	
	P	R	P	R	P	R	P	R
Fall indoor	(n=25)		(n=9)		(n=9)		(n=19)	
Penicillium	96	96	89	67	78	89	47	24
Cladosporium	80	84	78	100	67	89	82	29
Aspergillus	67	20	22	0	22	22	29	12
Yeasts	84	20	89	89	89	89	53	53
Non-sporing isolates	92	100	56	67	67	67	18	24
Acremonium	32	12	0	11	0	0	0	0
Alternaria	12	12	11	0	0	0	0	6
Aureobasidium	24	32	33	22	56	67	12	24
Geotrichum	20	36	11	22	0	0	0	0
Oidiodendron	36	20	0	0	0	0	0	0
Polyscytalum	52	44	22	0	0	0	0	0
Stachybotrys	8	0	11	0	11	0	0	0
Fall outdoor	(n=9)							
Penicillium	78	78						
Cladosporium	100	100						
Aspergillus	11	33						
Yeasts	78	67						
Non-sporing isolates	100	100						
Acremonium	0	11						
Aureobasidium	22	33						
Geotrichum	44	33						
Oidiodendron	11	22						
Polyscytalum	100	44						
Winter indoor	(n=21)		(n=13)		(n=9)		(n=16)	
Penicillium	95	86	85	77	89	89	63	44
Cladosporium	53	71	69	62	56	31	63	38
Aspergillus	42	19	31	15	33	33	0	6
Yeasts	71	57	46	77	100	100	50	56
Non-sporing isolates	62	76	54	38	11	33	19	13
Acremonium	19	0	15	0	0	11	6	0
Alternaria	14	10	8	0	0	0	6	31
Aureobasidium	14	10	15	23	33	33	13	19
Stachybotrys	14	0	8	0	22	0	19	13
Hyalodendron	14	10	23	0	0	0	0	0

n = number of matched sample pairs.

ence buildings. Concentrations of *Aspergillus* and *Penicillium* were higher in mold problem buildings than in references ($p < 0.05$). The mean ratio between a mold problem and a matched reference building was 3 for the concentration of *Aspergillus* and 10 for *Penicillium*. For *Aspergillus*, the result agrees with our previous studies (Pasanen, 1992). However, both *Aspergillus* and *Penicillium* have been reported to be common fungi in indoor air (Hunter et al., 1988; Miller et al., 1988; Pasanen, 1992; Verhoeff et al., 1991). The reason for this may be that both these fungi release spores easily (Pasanen et al., 1991) and *Penicillium* can develop micro-colonies on occasionally wet indoor surfaces (Pasanen et al., 1992). Their significantly higher concentrations in the mold problem buildings may indicate unusual indoor sources.

There were also certain fungal genera that were only detected in the problem buildings: *Botryosporium, Acremonium, Oedocephalum* and *Stachybotrys*. The genus *Stachybotrys* has been regarded as an indicator of mold problems, but the indicator value of the other genera has still to be established. These results indicate that there might be an unusual composition of fungal genera in mold problem buildings.

Sedimentation Plate Samples

In the fall, the concentrations of sedimentation plate samples were 2–220 cfu/6 plates/h in mold problem

Table 3 Geometric means (GM) and ranges of total concentrations of viable fungi and the most common genera and groups of fungi in mold problem and reference buildings determined with sedimentation plate samples. Statistical significance (S) was tested with the Wilcoxon test.

	Concentrations of viable fungi (cfu 6 plates/h)				S
	Problem		Reference		
	GM	Range	GM	Range	
Fall indoor (n = 9)					
Total	25	2–220	26	4–71	N.S
Penicillium	4	0–210	3	0–29	N.S
Cladosporium	4	0–31	7	2–22	N.S
Aspergillus	1	0–3	–		N.S
Yeasts	4	0–20	7	0–27	N.S
Non-sporing isolates	4	0–39	5	0–22	N.S
Winter indoor (n = 13)					
Total	17	6–98	7	2–24	N.S
Penicillium	6	0–86	3	0–6	*
Cladosporium	2	0–6	2	0–15	N.S.
Aspergillus	1	0–3	1	0–2	N.S
Yeasts	2	0–8	3	0–6	N.S
Non-sporing isolates	2	0–4	2	0–5	N.S

N.S. $p > 0.05$
* $p < 0.05$
– not found
n = number of matched sample pairs.

Table 4 Geometric means (GM) and ranges of total concentrations of viable fungi and the most common genera and groups of fungi in mold problem and reference buildings determined with dust samples. The differences between mold problem and reference buildings were not statistically significant (Wilcoxon test).

	Concentrations of viable fungi (10^3 cfu/g)			
	Problem		Reference	
	GM	Range	GM	Range
Fall (n = 9)				
Total	98	7–1200	72	17–420
Penicillium	3	0–1100	2	0–22
Cladosporium	0.21	0–11	2	0–36
Aspergillus	0.01	0–180	0.01	0–230
Yeasts	12	0–160	7	0–95
Non-sporing isolates	0.54	0–100	0.27	0–36
Aureobasidium	0.09	0–18	0.20	0–27
Winter (n = 9)				
Total	140	38–680	98	30–400
Penicillium	15	0–230	19	3–300
Cladosporium	0.10	0–18	0.08	0–120
Aspergillus	0.02	0–63	0.02	0–28
Yeasts	58	8–450	39	13–130
Non-sporing isolates	0	0–6	0.02	0–10
Aureobasidium	0.01	0–10	0.02	0–10

n = number of matched sample pairs.

buildings and 4–71 cfu/6 plates/h in reference buildings (Table 3). There were no statistically significant differences in either total concentrations of viable fungi or in concentrations of any genus between mold problem and reference buildings.

In the winter, the concentrations of sedimentation samples were 6–98 cfu/6 plates/h in mold problem buildings and 2–24 cfu/6 plates/h in reference buildings (Table 3). The results of the sedimentation samples showed a trend similar to that of the impactor samples: total concentrations in the mold problem buildings were low, but higher than in the reference buildings. This difference was not statistically significant. The difference in concentration of *Penicillium* between mold problem and reference buildings was statistically significant ($p < 0.05$). This result supports the results of the impactor samples.

House Dust Samples

In the fall samples, the concentrations of viable fungi in house dust varied from $7*10^3$ to $1.2*10^6$ cfu/g in mold problem buildings and from $1.*10^4$ to $4.2*10^5$ cfu/g in reference buildings. In the winter, the concentrations were $3.3*10^4$–$6.8*10^5$ cfu/g in problem buildings and $3.0*10^4$–$4.0*10^5$ cfu/g in reference buildings (Table 4). Thus, the concentrations in mold problem buildings were only slightly higher than in reference buildings. The difference was not statistically significant. The concentrations were almost at the same level as that reported by Miller et al. (1988), but slightly higher than reported by Burge et al. (1992) and Verhoeff et al. (1992). In the study by Verhoeff et al. (1992), however, a different method was used.

The fungal genera were similar in problem and in reference buildings. The most common genera were the same as in the air samples, but their order of frequency was different. Yeasts and *Aureobasidium* were more common in the dust than in the air, while the frequency of non-sporing isolates was lower in the dust than in the air samples (Table 2).

It seems that with the method used for the dust sampling, a mold problem building cannot be distinguished from a normal building when the exposure level is rather low. The reason for the similarity in concentrations and composition of fungal flora in house dust of problem and reference buildings may be that the majority of the viable fungi collected with a vacuum cleaner originate from outdoor and from brief concentration peaks from normal sources (Lehtonen et al., 1993).

Surface Samples

Surface samples were taken to obtain qualitative information on the genera of the fungi that are attached to the surfaces of the buildings. In the fall, there were several genera in air samples which were not found on surfaces (Table 2). The reason for this may be the effect of the outdoor air on the fungal flora in indoor air.

In the winter, the order of frequency of fungal genera in the surface samples was rather similar to that in the air samples, except the genus *Aspergillus*, which was very rare in the surface samples. Thus, the results of the surface samples supported the data on the fungal flora in the air samples in winter. There is no previous information on fungal concentrations in surface samples in home environments. Thus, more information is needed both on the levels and flora on different surfaces in homes.

Conclusions

Airborne concentrations of viable fungi were higher in mold problem than in reference buildings. The composition of fungal genera in mold problem buildings was different from that in reference buildings. The concentrations of some genera were higher in mold problem buildings and there were also certain fungal genera that were detected only in problem buildings. In mold problem buildings, there may be an unusual composition of fungal flora although the concentrations in the air samples are low. The six-stage impactor gave most clearly the difference between mold problem and reference buildings. Sedimentation plate and surface samples supported the results given by impactor samples. In this study, careful analyses of airborne concentration of viable fungi and fungal flora were sufficiently sensitive methods to differentiate a moldy building from a non-problem building for exposure assessment.

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