Microfungal Contamination of Damp Buildings—Examples of Risk Constructions and Risk Materials

Suzanne Gravesen,[1] Peter A. Nielsen,[1] Randi Iversen,[1] and Kristian Fog Nielsen[1,2]

[1]Energy and Indoor Climate Division, Danish Building Research Institute, Hørsholm, Denmark; [2]Department of Biotechnology, Technical University of Denmark, Lyngby, Denmark

To elucidate problems with microfungal infestation in indoor environments, a multidisciplinary collaborative pilot study, supported by a grant from the Danish Ministry of Housing and Urban Affairs, was performed on 72 mold-infected building materials from 23 buildings. Water leakage through roofs, rising damp, and defective plumbing installations were the main reasons for water damage with subsequent infestation of molds. From a score system assessing the bioavailability of the building materials, products most vulnerable to mold attacks were water damaged, aged organic materials containing cellulose, such as wooden materials, jute, wallpaper, and cardboard. The microfungal genera most frequently encountered were *Penicillium* (68%), *Aspergillus* (56%), *Chaetomium* (22%), *Ulocladium*, (21%), *Stachybotrys* (19%) and *Cladosporium* (15%). *Penicillium chrysogenum, Aspergillus versicolor,* and *Stachybotrys chartarum* were the most frequently occurring species. Under field conditions, several trichothecenes were detected in each of three commonly used building materials, heavily contaminated with *S. chartarum.* Under experimental conditions, four out of five isolates of *S. chartarum* produced satratoxin H and G when growing on new and old, very humid gypsum boards. *A. versicolor* produced the carcinogenic mycotoxin sterigmatocystin and 5-methoxysterigmatocystin under the same conditions. *Key words*: allergy, *Aspergillus versicolor,* building materials, mold, mycotoxins, *Penicillium chrysogenum, Stachybotrys chartarum.* — *Environ Health Perspect* 107(suppl 3):505–508 (1999). *http://ehpnet1.niehs.nih.gov/docs/1999/suppl-3/505-508gravesen/abstract.html*

The correlation between dampness and mold growth, house dust mites, and airway problems has been demonstrated and known for several years (*1–5*). Previous studies on indoor molds such as *Alternaria alternata, Aspergillus fumigatus,* and *Cladosporium herbarum* have been aimed mainly at their allergenic effects and characterization of allergens (*6*). Newer investigations have, however, dealt with the health implication of exposure to both the allergens and the metabolic products derived from the molds (*7,8*). Recent studies have revealed that molds growing on building materials produce and liberate several biologically active nonallergenic compounds. Some of these studies demonstrate mold growth on materials with subsequent detection of mycotoxins, e.g., *alternariols, chaetoglobosin, mycophenolic acid, satratoxin,* and *sterigmatocystins*—mycotoxins with potential dermatoxic, immunosuppressive and carcinogenic effects (*9–11*). From documented cases of mold allergies and the recent identification of mycotoxins, it can be concluded that prolonged presence in water-damaged buildings with extended mold growth may result in unwanted health effects (*8,12,13*).

Consequently, a multidisciplinary pilot study granted by the Danish Ministry of Housing and Urban Affairs was conducted with the purposes of identifying constructions and building materials critical for mold contamination, the most frequently encountered molds found on infested building material, and the possible harmful fungal metabolites produced on the these materials.

Materials and Methods

The susceptibility of the buildings to humidification was identified by registration of the state of maintenance of 23 public buildings consisting mainly of schools, kindergartens, and other nonindustrial public buildings. A chart with relevant physical, chemical, and building parameters was filled in during the visual inspection of the buildings registered as water damaged. The chart was expanded to collect background information necessary to identify the types of constructions and materials at risk for water ingress, water leakage, humidification, and subsequent microbial growth.

Collection of infected building materials was done during the visual inspection of the buildings and 72 samples, approximately 10 × 20 cm, were collected and placed in aroma-tight bags for later chemical and mycological analyses.

For identification of factors regarded as critical for the establishment and subsequent growth of microfungi on the materials, i.e., the bioavailability of the material, a score system was set up with and an index describing the different conditions for the collected materials. The chart described the following parameters:

- surface texture of a material
- state of maintenance
- age
- load (wear and tear on the material)
- availability for cleaning
- cleanliness

Each parameter scored 0 or 1.

Because knowledge is limited regarding a detailed evaluation of the parameters listed in the chart, the materials were given an index from 0 to 6, with 0 representing a low risk and 6 a high risk for microfungal growth. For example, an old (score 1) wooden floor with cracks and scratches (score 1), a worn-out coat of lacquer (score 1), poor maintenance (score 1), clean floor (score 0), and good cleaning availability (score 0) would give an index of (1+1+1+1+0+0) = 4.

For detection and identification of microfungi, samples from building materials with heavy microbial growth, visible to the naked eye, were taken by means of 5-cm contact plates with V8 agar as growth medium (*14*).

The plates were inspected after 3 days and again after 1 week. The colonies were counted and identified, if possible to the species level after current taxonomical standard criteria (*15*). Species from taxonomically difficult genera such as *Aspergillus* and *Penicillium* were further cultivated on Czapek yeast autolysate agar (CYA), yeast extract sucrose agar, (YES), malt extract agar (MEA), oat meal agar (OAT), and creatine sucrose agar (CREA)

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Address correspondence to S. Gravesen, Energy and Indoor Climate Division, Danish Building Research Institute, Dr. Neergaards Vej 15, DK-2970 Hørsholm, Denmark. Telephone: 45 45 86 55 33. Fax: 45 45 86 75 35. E-mail: sug@sbi.dk

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(*15*) for morphologic and chemotaxonomic criteria (*16*).

For direct identification, to genus level, of fungi growing on the materials, the tape method was applied. A piece of transparent adhesive tape was gently pressed on the infected material and then stained with lacto-fuchsin before phase contrast microscopy (*10,17*).

Detection of mycotoxins was performed partly as laboratory experiments on artificially inoculated materials and partly on field samples as described by Nielsen et al. (*10,11*).

For the artificially inoculated materials (*11*), the following building materials were used: new gypsum boards, new plywood, old gypsum boards, pieces of old pine, acoustic ceiling boards consisting of mineral wool with glass-fiber wall paper and wallpapered gypsum boards. Materials were artificially infested with pure cultures of *Aspergillus versicolor* (five isolates), *Stachybotrys chartarum* (five isolates), and *Trichoderma* spp. (eight isolates), isolated from infested materials used in Danish buildings.

Mycotoxin Analyses

Trichothecenes were hydrolyzed to their parent alcohols and derivatizated to the heptafluorobutyrlated ester. Extracts from *Trichoderma* were also analyzed without the hydrolysis step. The hepatafluorobuturylated derivatives were detected using gas chromatography ion trap mass spectrometry and negative ion chemical ionization. Standards of T-2 toxin, HT-2 toxin, diacetoxysrcipenol, fusarenon-X, deoxynivalenol, nivalenol, verrucarol, and trichodermol were available, as described by Nielsen et al. (*10,11*).

Sterigmatocystins were detected using high-performance liquid chromatography diode array detection on a C_{18} column with a water–acetonitrile gradient system (*10*). Extracts were also analyzed for sterigmatocystin by thin-layer chromatography spraying with $AlCl_3$ staining (*10*).

Results

Identification of Construction Types and Materials Susceptible to Humidification

For the majority of the 72 material samples investigated, the period of humidification was more than 6 months. For 37 of the materials, the main reason for water damage with subsequent infestation of molds was water leakage through roofs. In one-third of the buildings investigated, the water ingress resulted from various defects in flat roofs. Half the roofs suffered from water ingress because of different types of defective junction details and construction failures in incomplete junctions between roofs that had been built together. Rising damp (12 samples) and defective plumbing installations (9 samples) were further causes of mold growth, although with minor impact compared with the defective roof constructions. Other minor reasons for humidification of materials (14 samples) were penetration of cleaning water and condensation.

The susceptibility to fungal intestation of the materials was expressed in the index showing a score from 4 to 6 for 62 of the samples, which indicates a poor resistance to mold infestations (*9*). The humidification of materials had lasted more than 6 months altogether, indicating that the water ingress was not a result of a single accident but was caused by long-term lack of remediation.

Description and Evaluation of Materials and Constructions at Risk for Mold Infestation

Table 1 indicates the degree of bioavailability in the different materials groups. In this study, building materials most vulnerable to mold attacks were water-damaged, aged organic materials containing cellulose, such as wooden materials, jute, wallpaper, and cardboard. Other groups of materials having a high degree of bioavailability were linoleum and insulation materials for plumbing installations (canvas). The deposition of dust and dirt, together with long-lasting penetration of water, may also lead to fungal growth on inorganic material such as mineral wool. Generally, it can be concluded that leaking roofs were responsible for mold growth in all of the material groups examined.

Identification of the Isolated Molds

Table 2 shows that *Penicillium, Aspergillus,* and *Chaetomium* are the genera most frequently isolated from the building materials. *S. chartarum* is the species most often identified on the material samples, followed by *A. versicolor. Penicillium chrysogenum* is number three. A substantial number of isolated *Penicillia* are, however, not identified to the species level, making *P. chrysogenum* a possible candidate for ranking as number one on the list of species.

As seen in Table 3, trichothecenes were detected on all four building materials naturally infested with *Stachybotrys.* On the artificially infested materials, four of five cultures of *S. chartarum* were capable of producing trichothecene mycotoxins on damp building materials from the three heavily contaminated materials investigated. Furthermore, trichodermol was detected, probably originating from a low toxigenic strain of *S. chartarum.*

Under experimental conditions, each of five isolates of *A. versicolor* produced sterigmatocystin (Table 3). Two of the isolates produced sterigmatocystins, constituting about 1% of the biomass scraped off the material.

Table 4 lists the number of findings of the different genera on each material group. *S. chartarum* was most often identified on wood, insulation materials for installations (canvas), and gypsum boards. *A. versicolor* was not connected to any specific group of materials but had a broad range of favorite substrates such as damp floor joists, wet, dirty mineral wool, and moist wallpaper.

Table 1. Bioavailability of materials (n = 72) for mold contamination.[a]

Material	Number of materials of each group/bioavailability index			
	3[b]	4	5	6
Wood	1	1	9	5
Linoleum	0	2	5	1
Insulation for installations	0	0	1	7
Gypsum	1	3	2	2
Mineral fiber	0	3	2	2
Wallpaper	1	2	2	0
Plaster	1	1	2	1
Glass-fiber wallpaper	0	3	1	0
Aluminum foil	0	2	1	0
Other	0	4	3	1

[a]Range = 0 (low risk) to 6 (high risk). [b]None of the materials had an index lower than 3.

Discussion

Studies from the most recent years have demonstrated that microfungi produce a substantial number of biologically very active substances other than allergens (*10,18–21*). A new paradigm for the significance of exposure to microfungi in the indoor climate has been developed since mycotoxins of the trichothecene type have been detected from airborne spores, dust, and infected buildings (*20,22,23*). Furthermore, it has been experimentally documented that the toxic effects of mycotoxin T-2 toxin were 10 to 20 times stronger by inhalation than by ingestion (*24*).

As the buildings investigated in this study were specially selected and known to have problems, they do not provide

Table 2. Frequency of microfungal genera and species identified from the infested materials (n = 72).

Genus	Frequency (%)[a]	Species	Number[b]	Total
Penicillium	68	*Penicillium* spp.	35	
		P. chrysogenum	12	
		P. palitans	1	
		P. flavigenum	1	49
Aspergillus	56	*Aspergillus* spp.	20	
		A. versicolor	7	
		A. terreus	1	
		A. ustus	3	
		A. fumigatus	1	
		A. niger	3	
		A. sydowii	2	
		A. ochraceus	2	
		A. candidus	1	40
Chaetomium	22	*Chaetomium* spp.	16	16
Ulocladium	21	*Ulocladium* spp.	7	
		U. oudemansii	6	
		U. chartarum	2	15
Stachybotrys	19	*S. chartarum*	14	14
Cladosporium	15	*Cladosporium* spp.	3	
		C. cladosporioides	2	
		C. sphaerospermum	3	
		C. herbarum	3	11
Acremonium	14	*Acremonium* spp.	10	10
Mucor	14	*Mucor* spp.	2	
		M. plumbeus	3	
		M. spinosus	5	10
Paecilomyces	10	*Paecilomyces* spp.	4	
		P. lilacinus	2	
		P. variotii	1	7
Alternaria	8	*Alternaria* spp.	2	
		A. alternata	4	6
Verticillium	8	*Verticillium* spp.	5	
		V. lateritium	1	6
Trichoderma	7	*Trichoderma* spp.	5	5

[a]Frequency is the percentage of infested materials with presence of the genus in relation to the 72 materials. [b]Number is the number of materials infested with the species in question.

Table 3. Mycotoxins detected on the artificially and naturally infested building materials analyzed.

Mycotoxin	Infestation	No. of samples	No. positive	Concentration range
Sterigmatocystin[a,b]	Artificial	23	19	1–23 μg/cm^2
5-Methoxysterigmatocystin[a,b]	Artificial	23	14	1–8 μg/cm^2
Macrocyclic trichothecenes[a,c] (verrucarol type)	Artificial	13	10	20–140 ng/cm^2
Trichodermol type[a]	Artificial	13	3	Not quantified
Macrocyclic trichothecenes[c,d] (verrucarol type)	Natural	4	4	2–15 ng/cm^2
Trichodermol type[d]	Natural	4	2	Not quantified

[a]Data from Nielsen et al. (*10*). [b]Detection limit 8 ng. [c]Detection limit 100 pg. [d]Data from Nielsen et al. (*11*).

sufficient data to recommend certain building materials to inhibit or avoid mold infestation in case of leakage and humidification for a prolonged period of time. Such recommendations will be published as one of the outcomes of the Danish Mold Programme, 1998–2001.

The main reasons for water ingress, which caused damage to the buildings, were leakage through flat roofs, rising damp, and defective plumbing installations.

The most important factor for mold growth was water activity (a_w). Water activity of 0.96 corresponding to a relative humidity of 96% (at steady state) yielded significantly poorer growth of *S. chartarum* compared with an a_w = 0.98% (*9*). This indicates the importance of using dry gypsum boards in a new building and keeping the boards dry to prevent microbial growth.

Compared to the main reason for the dampness of materials (leaking roofs), rising damp and defective plumbing installations were minor causes of mold growth in the cases investigated.

The susceptibility to fungal growth or the potential for microbial growth is an expression of the interaction between the material itself and the different influences of the environment. The evaluation of the materials examined in this study demonstrated a high degree of susceptibility to fungal growth, indicating a low resistance to mold infestation. Because the surface of a material damaged and exposed to various degradation processes as it ages, the actual age of a material plays an important role for microbial infestation.

In addition to the parameters included in the evaluation chart, the content of biologically degradable components is essential. A material can be classified as organic or inorganic depending on the amount of biodegradable components; cellulose is an important component.

Table 4. Most frequent mold genera from the material groups investigated.

	Number of mold findings/material group											
Material group	*Penicillium*	*Aspergillus*	*Chaetomium*	*Ulocladium*	*Stachybotrys*	*Cladosporium*	*Acremonium*	*Mucor*	*Paecilomyces*	*Alternaria*	*Verticillium*	*Trichoderma*
Wood	10	11	1	2	3	3	4	3	1	2	1	1
Linoleum	6	8	1	1	0	0	1	1	2	0	2	0
Pipe insulation	3	4	5	1	3	1	1	0	0	0	0	0
Gypsum	6	4	4	5	4	1	1	1	1	2	1	0
Mineral fiber	5	0	3	1	0	2	0	0	1	0	0	1
Wallpaper	5	2	0	1	0	2	0	1	0	1	0	0
Plaster	3	1	0	1	2	0	0	1	1	0	0	1
Glass-fiber wallpaper	3	1	0	2	1	0	0	0	0	0	0	0
Aluminum foil	3	0	0	1	1	1	1	2	0	0	1	1
Other	5	9	2	0	0	1	2	1	1	1	1	1
Genera isolated (%)	68	56	22	21	19	15	14	14	10	8	8	7

Concerning possible adverse health reactions, the investigated building materials infested with *S. chartarum* and *A. versicolor* consistently demonstrated the presence of the trichothecene mycotoxins and the carcinogenic mycotoxin sterigmatocystin, respectively (*10,11,20,22,23*).

A building-associated fungal flora (funga) was identified, if possible to species level. The microfungal genera most frequently isolated from the 72 samples of building materials were *Penicillium* (68%), *Aspergillus* (56%), *Chaetomium* (22%), *Ulocladium* (21%), *Stachybotrys* (19%), *Cladosporium* (15%), *Acremonium* (14%), *Mucor* (14%), *Paecilomyces* (10%), *Alternaria (8%), Verticillium* (8%), and *Trichoderma* (7%). These are all known to cause different types of inhalation allergy (*25*). The species most frequently encountered were *S. chartarum, P. chrysogenum,* and *A. versicolor.*

A. alternata, C. herbarum, and *Ulocladium chartarum* are important inhalation allergens also frequently recorded outdoors (*6,25*). Heavy exposure to spores from *A. fumigatus* and *Paecilomyces variotii* may cause allergic alveolitis (hypersensitivity pneumonitis) (*6,25*).

Furthermore, it is experimentally documented that other microfungi frequently found in this study such as *Alternaria* spp., *Chaetomium* spp., *Penicillium brevicompactum, Penicillium polonicum,* and *Aspergillus niger* produce mycotoxins such as alternariol, alternariol-monomethyl ether, chaetoglobosins A and C, verrucosidin, verrucofortine, mycophenolic acid, naphtho-γ-pyrones, and many unknown secondary metabolites (*26,27*).

Under field conditions the toxic macrocyclic trichothecenes and trichodermol were detected by scraping fungal material from each of four materials investigated, which were heavily contaminated with *S. chartarum.* This is the first time that trichothcenes have been detected in Danish buildings. Materials infested with *A. versicolor* produced the cancerogenic mycotoxin sterigmatocystin, and 5-methoxy-sterigmatocystin, constituting a potential health hazard. This is the first time this mycotoxin has been detected from building materials. The production of sterigmatocystin from two of the isolates was 1% of the total biomass removed, which is considered a large amount.

For the assessment of the condition of a mold-infested building, to establish standards for remediation, including choice of safe materials and constructions, and to recommend methods for removal and prevention of mold growth, more medical, biologic, and technical knowledge must be gained. Such recommendations will, however, be published as part of the outcomes of the Danish Mold Programme 1998–2001.

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