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Associations between Fungal Species and Water-Damaged Building Materials▽

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Fungal growth in damp or water-damaged buildings worldwide is an increasing problem, which has adverse effects on both the occupants and the buildings. Air sampling alone in moldy buildings does not reveal the full diversity of fungal species growing on building materials. One aim of this study was to estimate the qualitative and quantitative diversity of fungi growing on damp or water-damaged building materials. Another was to determine if associations exist between the most commonly found fungal species and different types of materials. More than 5,300 surface samples were taken by means of V8 contact plates from materials with visible fungal growth. Fungal identifications and information on building material components were analyzed using multivariate statistic methods to determine associations between fungi and material components. The results confirmed that *Penicillium chrysogenum* and *Aspergillus versicolor* are the most common fungal species in water-damaged buildings. The results also showed *Chaetomium* spp., *Acremonium* spp., and *Ulocladium* spp. to be very common on damp building materials. Analyses show that associated mycobiotas exist on different building materials. Associations were found between (i) *Acremonium* spp., *Penicillium chrysogenum*, *Stachybotrys* spp., *Ulocladium* spp., and gypsum and wallpaper, (ii) *Arthrinium phaeospermum*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *Trichoderma* spp., yeasts, and different types of wood and plywood, and (iii) *Aspergillus fumigatus*, *Aspergillus melleus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Chaetomium* spp., *Mucor racemosus*, *Mucor spinosus*, and concrete and other floor-related materials. These results can be used to develop new and resistant building materials and relevant allergen extracts and to help focus research on relevant mycotoxins, microbial volatile organic compounds (MVOCs), and microparticles released into the indoor environment.

Most water damage indoors is due to natural disaster (e.g., flooding) or human error (e.g., disrepair). Water can seep into a building as a result of melting snow, heavy rain, or sewer system overflow. Water vapor can be produced by human activities like cooking, laundering, or showering and then condense on cold surfaces like outer walls, windows, or furniture. Damp or water-damaged building materials are at high risk of fungal growth (mold growth), possibly resulting in health problems for the occupants and the deterioration of the buildings. The water activity (a_w) ($a_w \times 100$ = % relative humidity at equilibrium) of a building material is the determining factor for fungal growth and varies with the temperature and the type of material (27). The longer a material's a_w is over 0.75, the greater the risk of fungal growth (49), though different fungi have different a_w preferences (11). Some filamentous fungi can grow on a material when the a_w is as low as 0.78 (26), while others can survive 3 weeks at an a_w of 0.45 (30). The severity of indoor dampness varies with the climate, but WHO (52) estimates that in Australia, Europe, India, Japan, and North America, dampness is a problem in 10 to 50% of the buildings, and Sivasubramani et al. (41) estimate that fungal growth is a problem in 15 to 40% of North American and Northern European homes.

The negative health effects of damp building materials and fungal growth in homes, institutions, and workplaces have been reported in many publications, including the WHO guidelines *Dampness and Mould* (52), which concluded that there is sufficient epidemiological evidence to show that occupants of damp or moldy buildings are at increased risk of respiratory problems, respiratory infections, and the exacerbation of asthma. The causality between fungal exposure and development of type I allergy has been proven (18), but clinical evidence linking specific fungal spores, hyphal fragments, and/or metabolites to particular health complaints is lacking. The symptoms reported by occupants in moldy buildings are many and diverse (20, 23), as are the fungal species found on moldy building materials (14, 19). The fact that some people are hypersensitive to fungi while others do not react at all further complicates the issue.

Detection and species identification of all fungi present in a moldy building are the first step toward resolving the cause and effect of building-related illness (sick building syndrome), so the choice of sampling methods is essential. Air and dust samples have been taken in order to associate fungal exposure and health problems (e.g., 10, 17, 48), but no conclusive links have been found. This may be because spore liberation from a surface is sporadic (41) and spore distribution in the air is random (21). Toxic fungi (e.g., *Stachybotrys* spp. and *Chaetomium* spp.) growing on damp building materials do not readily become airborne and/or lose their culturability soon after liberation (21, 29, 45, 47) and may therefore not be detected during air or dust sampling. Correct species identification of the fungi is also important, since new research has indicated that species-specific metabolites, like atranone C produced by

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Stachybotrys chlorohalonata (37), are cytotoxic or immunotoxic or induce inflammatory responses when inhaled (24, 33, 34). The purpose of this study was therefore to estimate the qualitative and quantitative diversity of fungi growing on damp or water-damaged building materials. The study was based on more than 5,300 surface samples taken by means of V8 contact plates from building materials with visible fungal growth in Denmark and Greenland. The aim was to determine if there exists an association between the most common fungi found and particular types of water-damaged building materials.

MATERIALS AND METHODS

Sample collection and treatment. Samples from building materials with visible fungal growth were taken by means of 65-mm contact plates (VWR International) containing V8 agar with antibiotics (200 ml Campbell's original V8 100% vegetable juice, 3 g $CaCO_3$ [Merck], 20 g agar [VWR International]) and 800 ml water. Penicillin (100,000 IU/liter [Sigma]) and streptomycin (1 g/liter [Sigma]) were added after autoclaving (12). The plates were subjected to fungal analysis at the Mycological Laboratory (ML) at the Danish Technological Institute (DTI). Samples were collected from June 2005 to February 2008 and originated from private residences (houses, apartments, and holiday cottages) and private businesses (shops and offices) as well as from public buildings (kindergartens, schools, and offices) from all parts of Denmark and Greenland. Samples were taken from buildings where either professional building inspectors had reported visible fungal growth or water damage or occupants had contacted DTI with self-reported fungal or health problems. Several samples may have been taken from the same building, but only one sample was taken from each damage site. Approximately 75% of all samples were taken on site by the building inspectors by means of contact plates and mailed overnight to ML. The remaining 25% were moldy building materials sent to ML by the occupant after thorough instruction. The materials were then sampled by ML by means of contact plates.

Fungal identification. Identification of fungi in a sample was done directly on the V8 contact plates after 7 days of incubation at 26°C in darkness. Whenever possible, fungi were identified to species using direct microscopy and identified according to the methods of Domsch et al. (7), de Hoog et al. (6), and Samson et al. (36). Fungi present in the sample were determined qualitatively (taxon present) and quantitatively (number of colonies).

Since different *Penicillium* species can be difficult to identify on V8 medium, special attention was given to this genus in the spring of 2010. DTI randomly selected 80 V8 contact plates with *Penicillium* growth and delivered them to the Center for Microbial Biotechnology (CMB) at the Technical University of Denmark (DTU). At CMB, all different *Penicillium* colonies on each plate were isolated, resulting in 120 *Penicillium* cultures. After transfer to Czapek yeast extract agar (CYA) for purity control, the isolates were inoculated onto CYA, malt extract agar (MEA), yeast extract sucrose agar (YES), and creatine sucrose agar (CREA) and identified to species level after 7 days at 25°C in the dark by methods reported by Samson et al. (37).

Data compilation. The samples were evaluated on the basis of the reliability of information on material type and fungal identification. Each sample contained information on type of building (e.g., private home), type of water-damaged construction (e.g., wallpaper on plaster, outer wall), qualitative fungal analysis (e.g., "*Aspergillus niger* [dominant], *Chaetomium* sp."), and quantitative fungal analysis (e.g., "30 *Aspergillus versicolor*, 1 *Ulocladium* sp."). The information of "type of water-damaged material" was divided into categories. If an entry contained two or more components, it was split into component categories (e.g., "painted wallpaper on plaster" into "paint," "wallpaper" and "plaster").

The sample set containing qualitative data was transformed into a binary matrix consisting of 5,532 samples in rows and 51 different component categories and 57 fungi in columns. Fungi and component categories that constituted less than 0.5% of the total 5,532 samples were deleted in order to minimize analytical noise (e.g., Karlit ceiling tiles, *Botrytis cinerea*, *Doratomyces* spp., or *Epicoccum nigrum*). Samples with growth of dry or wet rot fungi (*Serpula lacrymans* or *Coniophora puteana*, respectively) were also deleted. This resulted in a qualitative (binary) matrix (matrix A) with 5,353 valid samples where the association between 30 building material components and 42 fungi was unambiguous. A similar process was repeated on the original sample set to extract the samples with quantitative data, resulting in a matrix (matrix B) with 4,241 samples, 25 building material components, and 41 fungi.

Multivariate statistics. Matrices A and B were analyzed by principal component analysis (PCA) using the program Unscrambler v. 9.2 (CAMO Process A/S, Oslo, Norway). PCA is a bilinear modeling method giving an interpretable overview of the main information. All variables (components and fungi) were standardized (x − average/sdev), thus giving all the variables the same chance to influence the estimation of the components. In PCA, proximities among the objects were judged using Euclidean distances and among the variables using covariance (or correlation) since the variables have been standardized. The information carried by the original variables was projected onto a smaller number of underlying ("latent") variables called principal components.

The data in matrices A and B were then converted into two contingency tables of observed occurrences, where either the fungal count or the number of colonies for each fungus was summarized for each material. This resulted in two tables, contingency table A, based on the qualitative data (5,353 samples summarized into table A [42 rows with fungi and 30 columns with materials]), and contingency table B, based on the quantitative data (4,241 samples summarized in table B [41 rows with fungi and 25 columns with materials]). From the contingency tables of observed occurrences, predicted values were calculated for a particular fungus on a particular material: (sum of counts or colonies for fungus A on all materials × sum of counts or colonies of all fungi on material B)/(sum of counts or colonies of all fungi on all materials). For example, 7,452 *Ulocladium* colonies were counted in total, 19,100 fungal colonies were counted on all wallpaper samples, and 366,304 fungal colonies were counted in total, giving a predicted number of *Ulocladium* colonies on wallpaper of (7,452 × 19,100)/366,304 = 388, compared with the observed number of 1,208 *Ulocladium* colonies counted on all wallpaper. Contingency tables A and B were then analyzed by correspondence analysis (CA) using the program NTSYS version 2.21c (Exeter Software, Setauket, NY) (15). Chi-square distances were used to judge proximities both for the row and for the column variables.

The data in matrix A were also converted into a fungal species distance matrix, where the count for each of the 42 fungi was summarized on the other 41 fungi. This was done to analyze if any of the fungal species cooccurred independently of material preferences. This resulted in matrix C, a qualitative 42-by-42 symmetric matrix, which was then analyzed by principal coordinate (PCO) analysis using NTSYS v. 2.21c. Matrix C was double-centered, and an eigenvector analysis was performed. The correlation coefficient was used, and a minimum spanning tree analysis was superimposed upon the operational taxonomic units (OTUs) in the PCO score plot (42).

TABLE 1. Qualitative (qual) and quantitative (quan) frequencies of building material components with fungal growth from water-damaged buildings

Material component	Frequency (%)[a]	
	qual (n = 5,353)	quan (n = 4,241)
Plaster	23.5	22.1
Concrete	19.0	24.1
Wood	18.4	19.0
Wallpaper	12.6	6.3
Gypsum	7.7	8.1
Paint	5.2	5.8
Mineral wool	3.8	3.3
Glass fiber	3.6	3.6
Plywood	3.0	4.5
Brick	2.1	2.5
Chipboard	2.0	2.5
Linoleum	2.0	3.5

[a] An additional 18 building materials occurred in a <2% frequency each. A sample may contain more than one material component.

RESULTS

Building materials. Table 1 shows the building material components that were most often affected by fungal growth. As can be seen, plaster and concrete were the material components most likely to support fungal growth of the total material components. Together with wood, wallpaper, and gypsum, they constitute ca. 80% of materials and construction parts damaged by dampness, condensation, or liquid water. The other 18 building materials that occurred in fewer than 2% of cases

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TABLE 2. Qualitative (qual) and quantitative (quan) frequencies of fungal species and genera on water-damaged building materials

Fungus	Frequency (%)[a]	
	qual (n = 5,353)	quan (n = 287,169)
Penicillium spp.	69.5	39.7
Aspergillus versicolor	26.5	15.6
Chaetomium spp.	16.5	3.1
Acremonium spp.	14.9	7.8
Ulocladium spp.	8.0	2.1
Cladosporium sphaerospermum	7.4	4.9
Mucor plumbeus (syn. *M. spinosus*)	7.2	0.3
Trichoderma spp.	6.7	0.4
Cladosporium herbarum	6.6	1.5
Alternaria tenuissima	6.5	0.7
Sporothrix spp.	6.4	3.3
Aspergillus niger	6.1	0.5
Yeasts	5.1	2.6
Rhodotorula mucilaginosa	5.1	2.3
Aspergillus ochraceus	5.0	0.9
Penicillium chrysogenum[b] (syn. *P. notatum*)	4.5	2.5
Rhizopus stolonifer (syn. *R. nigricans*)	4.1	0.1
Stachybotrys spp.	3.9	1.9
Aspergillus fumigatus	3.8	0.2
Aspergillus spp.	3.6	1.2
Mucor spp.	3.3	0.2
Mycelia Sterilia	3.1	—[c]
Aspergillus wentii	3.1	1.3
Calcarisporium arbuscula	2.7	1.2
Scopulariopsis brevicaulis	2.1	0.5
Fusarium spp.	2.0	0.4
Mucor racemosus	2.0	0.1

[a] An additional 15 fungi occurred in fewer than 2% of the samples. A sample may contain more than one fungus.
[b] Underestimated, as several of the *Penicillium* spp. may also be *Penicillium chrysogenum*.
[c] Mycelia Sterilia was not quantified in metrix B.

were Masonite, cardboard, gas concrete, glue, wood-wool cement, bitumen, paper, vapor barriers, carpets, cork, medium-density fiberboard (MDF), vinyl, felt, grout, filler, Eternit, textiles, and tar-treated materials.

Fungi. The raw data showed that 45 fungal genera or species in total were identified on the samples of water-damaged building materials. Table 2 shows the qualitative and the quantitative presence of fungi on 5,353 and 4,241 samples, respectively. As can be seen, *Penicillium* was the most dominant fungal genus (3,720 counts and 114,143 colonies) on water-damaged building materials. The most dominant fungal species was *Aspergillus versicolor* (1,421 counts and 44,665 colonies). Together with *Chaetomium* spp., *Acremonium* spp., *Ulocladium* spp., and *Cladosporium sphaerospermum*, they constituted the most frequently detected fungi on damp or water-damaged building materials. The other 15 fungi that occurred in fewer than 1% of cases were *Phoma* spp., *Paecilomyces lilacinus*, *Aspergillus ustus*, *Arthrinium phaeospermum*, *Aspergillus melleus*, *Alternaria* spp., *Scopulariopsis brumptii*, *Verticillium albo-atrum*, *Aspergillus flavus*, *Paecilomyces variotii*, *Aspergillus sydowii*, *Absidia* spp., *Gliocladium* spp., *Guehomyces pullulans* (syn. *Trichosporon pullulans*), and *Aureobasidium pullulans*.

The isolation and identification of 120 penicillia from 80 water-damaged building materials (not the same samples described above) showed that between 70 and 75% of all *Penicillium* isolates were identified as *Penicillium chrysogenum*, while *Penicillium brevicompactum*, *Penicillium corylophilum*, *Penicillium crustosum*, *Penicillium olsonii*, *Penicillium palitans*, and *Penicillium solitum* constituted the last 25 to 30% and were found in almost equal amounts.

Associations between fungi and building materials. The result of a principal component analysis (PCA) of the qualitative (binary) data (matrix A, 5,353 samples × 72 variables [42 fungi and 30 material components]) is shown in Fig. 1. The result of the PCA of the quantitative data (matrix B, 4,241 samples × 66 variables [41 fungi and 25 material components]) gave a very similar result and is not shown. The first four PCA axes described 3%, 2%, 2%, and 2% of the variation in both matrix A and matrix B. By plotting the first two principal component axes (PC1 against PC2), the interrelationships between all variables (fungi and material components) can be seen. The plot in Fig. 1 shows the qualitative associations between the different fungi, between the different components of building material, and between fungi and material. The more often two fungal species occurred in the same sample, the closer they are together in the plot: *Alternaria tenuissima*, *Cladosporium herbarum*, *Rhodotorula mucilaginosa*, and other yeasts, together with *Aureobasidium pullulans*, *Fusarium* spp., *Trichoderma* spp., and *Arthrinium phaeospermum*, are often found together on different types of water-damaged wood and therefore lie close together. The same is seen for the different building material components: plaster, wallpaper, and painted surfaces cooccur in the plot in Fig. 1, because most Danish houses have brick walls leveled with plaster, coated with wallpaper, and then painted. As can be seen, *Acremonium* spp., *Penicillium chrysogenum*, *Stachybotrys* spp., and *Ulocladium* spp. often occur together and are highly associated with water-damaged walls with painted wallpaper or glass fiber. On the other hand, *Chaetomium* spp., *Penicillium* spp., and different *Aspergillus* species were often found on water-damaged concrete. Figure 1 also shows that *Aspergillus versicolor*, *Calcarisporium arbuscula*, and *Sporothrix* spp. are placed opposite wood (negative correlated) in the plot, meaning that *Aspergillus versicolor*, *Calcarisporium arbuscula*, and *Sporothrix* spp. occurred rarely on wood. The same negative correlation can be seen for wallpaper and *Aspergillus niger*, concrete and *Cladosporium sphaerospermum*, and plywood and *Aspergillus ochraceus*. Fungi or components lying close to the centroid in the plot occur infrequently (<4%) and have very loose or little association with each other.

Matrices A and B were converted to contingency tables A and B based on the qualitative data (table A, 42 rows with fungi and 30 columns with materials), and quantitative data (table B, 41 rows with fungi and 25 columns with materials), respectively. Table 3 is an extract of contingency tables A and B and shows the distribution of each of 17 fungi on 7 different materials. The table gives the qualitative and quantitative frequencies and percentage distributions of fungi on different building materials, where the sum of all counts or colonies of one fungal species on all 30 or 25 materials constitutes 100%. Some fungi are overrepresented on some materials (row values in bold) compared to the statistically predicted value, if the fungi were randomly or evenly distributed on all materials. For example, *Sporothrix* spp. have a strong association with plaster; i.e., 39% (162 counts) of all material samples with *Sporothrix*

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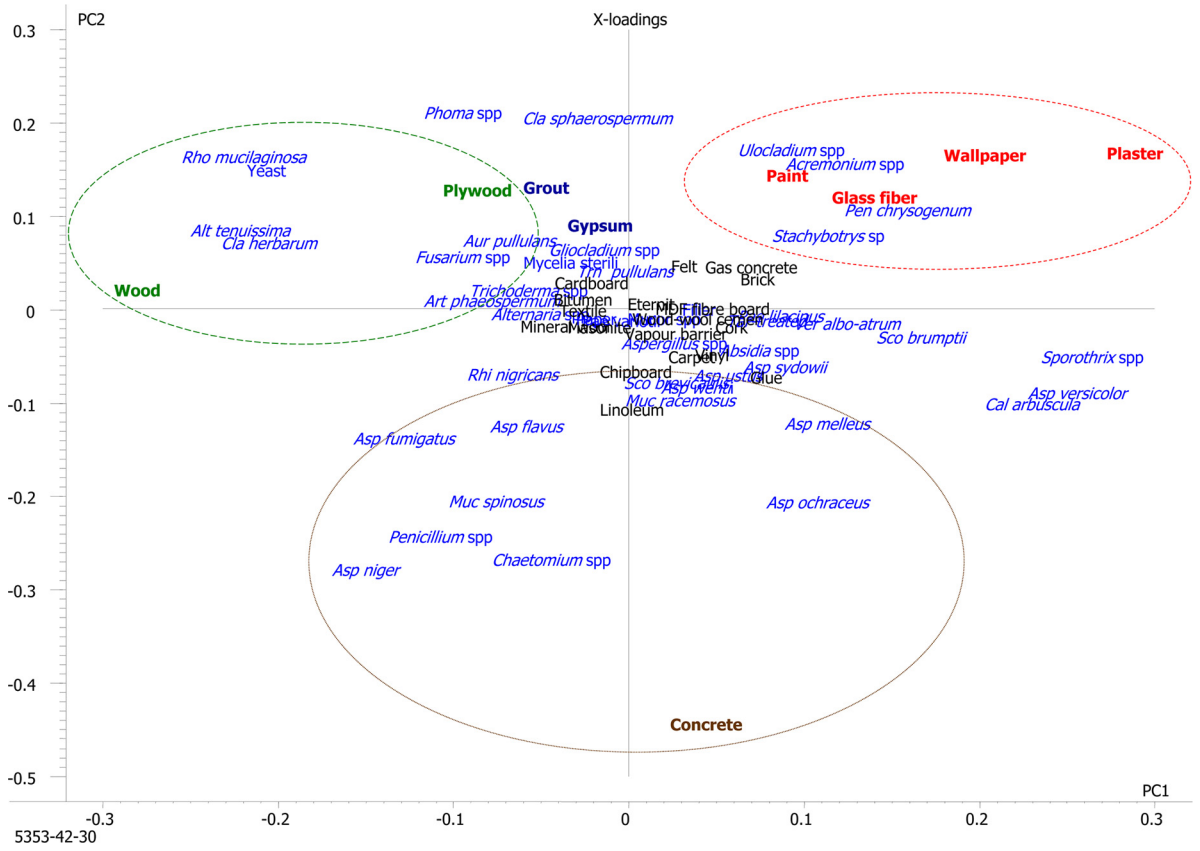

FIG. 1. Loadings plot from the principal component analysis (PCA) based on the qualitative matrix A [5,353 samples × (30 materials and 42 fungi)]. The plot shows associations between building materials and fungi (e.g., between wood and *Alternaria tenuissima*, *Cladosporium herbarum*, *Rhodotorula mucilaginosa*, and yeasts). Fungi and building materials encircled are particularly associated. Fungi or components close to the centroid have little or no association with each other, occur infrequently (<4%), and have little or no influence on the PCA model. Axes are principal components, PC 1 and PC 2, with loading values.

spp. were plaster and 37% (4,118 colonies) of all the *Sporothrix* spp. colonies counted were found on plaster samples. However, had *Sporothrix* spp. been randomly distributed, the statistically predictive values would have been 81 counts and 1,865 colonies, respectively. *Sporothrix* spp. also have an association with concrete; 18% of all concrete samples were colonized with *Sporothrix* spp., and 21% of all *Sporothrix* spp. colonies originated from concrete samples. However, *Sporothrix* spp. are rarely found on plywood (0 and 2%, respectively). Similar strong associations were found between *Stachybotrys* spp. and gypsum, wallpaper, and glass fiber and between *Ulocladium* spp. and wallpaper, plaster, and gypsum. *Phoma* spp. also had a preference for glass fiber. On water-damaged wood, *Alternaria tenuissima*, *Cladosporium herbarum*, *Trichoderma* spp., and yeasts were the associated fungal species, while *Mucor racemosus*, *Aspergillus ochraceus*, and *Aspergillus niger* were associated with wet concrete. It can also be seen that members of the most dominant fungal genus in water-damaged buildings, *Penicillium*, were found to be evenly distributed on all the examined building materials and without any pronounced preference. Likewise, some fungal species are underrepresented on some materials. For example, *Aspergillus versicolor* had a low association with plywood; i.e., 1% (20 counts) of all material samples with *Aspergillus versicolor* were plywood and 1% (776 colonies) of all the *Aspergillus versicolor* colonies counted were found on plywood samples. However, had *Aspergillus versicolor* been randomly distributed, the statistically predictive values would have been 41 counts and 4,307 colonies, respectively. Table 3 also shows that *Aspergillus niger*, *Aspergillus ochraceus*, and *Mucor racemosus* were not associated with wallpaper and that *Cladosporium sphaerospermum* and yeasts were uncommon on concrete.

Table 4 is also based on contingency tables A and B but shows the distribution of 17 fungal species on each of the 7 different materials. The table gives the qualitative and quantitative occurrences and percentage distributions of fungi on different building materials, where the column sum of all counts or colonies of all the 42 fungal species on one material constitutes 100%. As can be seen, *Penicillium* spp. is the dominant genus and can be found on all types of materials with almost the same frequency (27 to 30% quantitatively and 27 to 46% quantitatively) corresponding to the overall occurrence seen in Table 2. *Aspergillus versicolor* and *Acremonium* spp. are also very dominant and found evenly on most materials, except in the case of *Aspergillus versicolor*, where the distribution on plywood is underrepresented (5 and 6%) compared to its overall occurrence.

The result of a correspondence analysis (CA) based on the

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TABLE 3. Qualitative (qual) and quantitative (quan) distribution (%) of associated fungi on different building materials[a]

Fungus	Distribution (%)													
	Concrete		Glass fiber		Gypsum		Plaster		Plywood		Wallpaper		Wood	
	qual	quan	qual	quan	qual	quan	qual	quan	qual	quan	qual	quan	qual	quan
Acremonium spp.	13	14	3	3	8	**8**	**25**	**22**	3	5	8	6	14	15
Aspergillus niger	**32**	**35**	2	0	7	5	11	10	1	0	4	1	18	**23**
Aspergillus ochraceus	**33**	**52**	2	**4**	4	3	20	6	1	0	5	5	12	8
Aspergillus versicolor	**20**	**26**	3	3	4	4	**25**	**21**	1	1	**11**	**7**	11	10
Aureobasidium pullulans	3	0	0	**9**	6	0	14	11	**9**	6	3	0	**46**	**37**
Chaetomium spp.	**25**	**35**	2	2	6	**9**	15	11	1	2	10	3	17	18
Cladosporium herbarum	11	15	1	1	5	5	14	15	**5**	**9**	6	3	**24**	**27**
Cladosporium sphaerospermum	8	10	4	3	3	4	22	**24**	**6**	**11**	12	6	17	16
Mucor racemosus	**36**	**54**	1	0	3	3	17	14	0	0	5	1	15	10
Paecilomyces variotii	13	8	0	1	13	15	10	8	**19**	**15**	2	3	**27**	17
Penicillium spp.	19	26	3	2	6	6	20	16	2	3	10	6	17	18
Penicillium chrysogenum	12	18	2	2	**11**	**14**	21	18	3	4	**16**	5	13	15
Phoma spp.	9	12	**9**	**12**	4	1	15	9	2	4	12	6	12	11
Sporothrix spp.	18	21	3	2	3	4	**39**	**37**	0	2	7	4	7	7
Stachybotrys spp.	10	4	2	**10**	**25**	**39**	22	13	1	0	10	**8**	9	7
Trichoderma spp.	14	16	2	2	**9**	**9**	15	13	4	**8**	6	2	**25**	**30**
Ulocladium spp.	9	3	**5**	2	7	**15**	21	**34**	1	1	**29**	**16**	12	9
Yeasts	8	7	2	3	8	5	10	11	**7**	**9**	5	2	**28**	**39**

[a] Values in bold give the materials on which the fungus is overrepresented (the associated mycobiota). Row sums are ≤100%.

quantitative data (contingency table B: a 41-by-25 table based on matrix B) is shown as a biplot in Fig. 2. The first four CA axes described 20.74%, 17.85%, 14.85%, and 9.88% of the variation in the data for contingency table B. The result of the CA of the qualitative data (contingency table A: a 42-by-30 table based on matrix A) gave a very similar result, with the first four CA axes describing 21.54%, 17.06%, 12.02%, and 8.37% of the variation in contingency table A, and is not shown. Figure 2 shows the quantitative associations between building material and fungi. As can be seen, wallpaper/glass fiber, gypsum, *Stachybotrys* spp., and *Ulocladium* spp. are strongly associated. Likewise, plywood is closely associated with *Arthrinium phaeospermum*, *Rhodotorula mucilaginosa*, and *Trichoderma* spp., while *Aspergillus ochraceus*, *Aspergillus sydowii*, and *Mucor racemosus* are closely associated with each other and loosely associated with *Aspergillus fumigatus* and *Mucor* spp. and concrete, glue, and cork. Being quite close to the centroid of the plot, *Penicillium* spp. is associated with most of the materials, while *Aspergillus versicolor* is more associated with gas concrete, cork, vinyl, and glue than with wallpaper, grout/tile wood, or plywood.

Two-dimensional plots of both PCA and CA can sometimes make variables (i.e., fungi) appear more associated than they really are. A minimum spanning tree can be a good control for determining whether particular variables (i.e., fungi) are indeed as close as their similar score or loading values suggest.

TABLE 4. Qualitative (qual) and quantitative (quan) distribution (%) of dominant fungi on particular building materials[a]

Fungus	Distribution (%)													
	Concrete		Glass fiber		Gypsum		Plaster		Plywood		Wallpaper		Wood	
	qual	quan	qual	quan	qual	quan	qual	quan	qual	quan	qual	quan	qual	quan
Acremonium spp.	4	**5**	**8**	**9**	**7**	9	**8**	**10**	**8**	**10**	5	**8**	5	**7**
Aspergillus niger	4	1	2	0	3	0	1	0	1	0	1	0	3	1
Aspergillus ochraceus	3	2	2	1	1	0	2	0	1	0	1	1	1	0
Aspergillus versicolor	**12**	**19**	**12**	**19**	6	**10**	**14**	**19**	5	6	**13**	**20**	**7**	**10**
Aureobasidium pullulans	0	0	0	1	0	0	0	0	1	0	0	0	1	0
Chaetomium spp.	**9**	**5**	5	2	6	4	5	2	3	1	7	1	**7**	3
Cladosporium herbarum	2	1	1	1	2	1	2	1	5	3	2	1	4	2
Cladosporium sphaerospermum	1	2	4	5	2	3	3	7	**7**	**13**	4	5	3	5
Mucor racemosus	1	0	0	0	0	0	1	0	0	0	0	0	1	0
Paecilomyces variotii	0	0	0	0	1	1	0	0	2	2	0	0	1	1
Penicillium spp.	**28**	**46**	**26**	**27**	**27**	**34**	**27**	**35**	**31**	**41**	**30**	**39**	**28**	**44**
Penicillium chrysogenum	1	2	2	2	3	5	2	2	1	2	3	2	1	2
Phoma spp.	0	0	3	3	1	0	1	0	1	1	1	1	1	1
Sporothrix spp.	3	3	3	2	1	2	5	7	0	1	2	3	1	1
Stachybotrys spp.	1	0	1	7	**6**	**12**	2	1	1	0	2	3	1	1
Trichoderma spp.	2	0	2	0	4	1	2	0	4	1	2	0	4	1
Ulocladium spp.	2	0	6	2	4	5	3	4	2	1	**10**	6	2	1
Yeasts	1	1	1	2	3	2	1	2	6	6	1	1	4	6

[a] Values in bold give the three most dominant fungi. Column sums are ≤100%.

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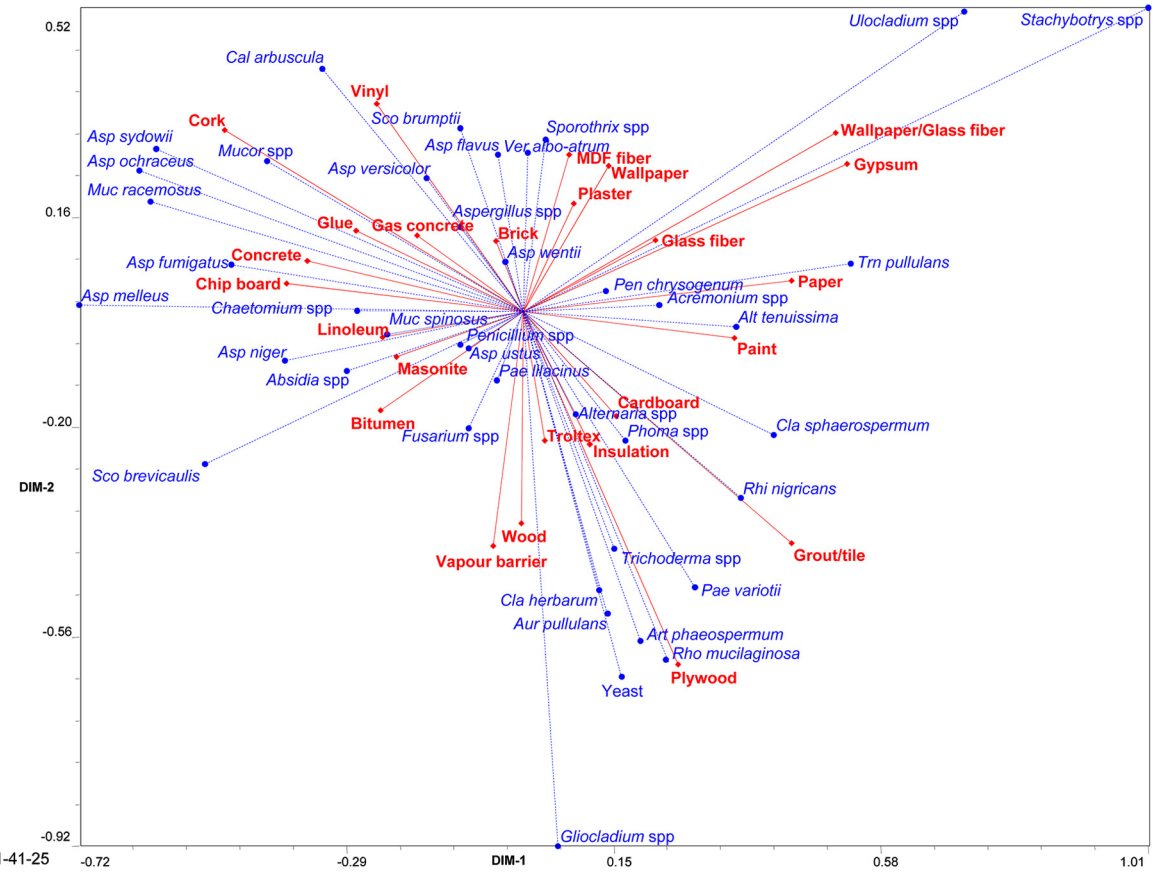

FIG. 2. Biplot from the correspondence analysis (CA) based on the quantitative sum table B (matrix B [4,241 samples × (25 materials and 41 fungi)] summarized in a 41-by-25 table). The biplot shows the association between building material and fungal identity (e.g., between gypsum, glass fiber, and wallpaper and *Ulocladium* spp. and *Stachybotrys* spp.). The dotted lines show the distance of any fungus to the centroid, i.e., fungi farthest away from the centroid deviate the most from what would be expected based on the whole data table. Axes are correspondence components, DIM 1 and DIM 2, with score and loading values.

To test the associations in Fig. 1 and 2, a principal coordinate (PCO) analysis with an overlain minimum spanning tree of matrix C (matrix C, 42 fungi by 42 fungi) was made. The PCO analysis described 42.55%, 23.58%, 8.36%, and 7.22% of the variation in the data on the first four axes, so these four axes described a larger proportion of the variance than expected using the broken-stick model (10.30%, 7.92%, 6.73%, and 5.94%). The PCO analysis showed cooccurrence and strong association between (i) *Acremonium* spp., *Penicillium chrysogenum*, *Stachybotrys* spp., and *Ulocladium* spp., (ii) *Trichoderma* spp., *Alternaria tenuissima*, *Cladosporium herbarum*, *Trichosporon pullulans*, and *Fusarium* spp., (iii) yeasts, *Gliocladium* spp., *Arthrinium phaeospermum*, and *Aureobasidium pullulans*, (iv) *Aspergillus sydowii*, *Aspergillus ochraceus*, *Mucor racemosus*, *Aspergillus melleus*, *Aspergillus niger*, *Mucor spinosus*, *Aspergillus fumigatus*, *Chaetomium* spp., and *Scopulariopsis brevicaulis*, and (v) *Aspergillus versicolor*, *Calcarisporium arbuscula*, and *Scopulariopsis brumptii*.

Comparison of Tables 1 and 3 shows that some building materials are more prone to fungal growth after water damage and that some materials have a high fungal load (Table 3, high total column value), while others support only little fungal growth (Table 3, low total column value). For example, wallpaper often becomes fungus ridden (Table 1, 12.6%) with a high fungal load, whereas glass fiber showed fungal growth less often (Table 1, 3.6%) and had a low fungal load. Comparisons of Tables 3 and 4 show that some fungi have a high occurrence but no association with particular materials (e.g., *Penicillium* spp.), while other fungi have a moderate occurrence and a specific association to particular materials (e.g., *Stachybotrys* spp. and gypsum and *Ulocladium* spp. and wallpaper). Others again have a low occurrence but a tight association with a particular material (e.g., *Aureobasidium pullulans* [blue stain fungus] and wood and *Phoma* spp. and glass fiber).

DISCUSSION

Sampling methods. There exists no single sampling method that can detect all fungal species in a moldy building, because all methods (e.g., air or surface sampling with either spore counting or cultivation) are, in one way or another, biased. Air sampling is unreliable because it favors fungi that produce large quantities of small, dry spores, such as *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. (37) and discriminates against fungi that produce small amounts of spores, large spores, or spores in slime, such as *Acremonium* spp., *Chaeto-*

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mium spp., *Stachybotrys* spp., *Trichoderma* spp., and *Ulocladium* spp. Besides, fungal diversity is different in outdoor air (22), in indoor air (12), and in house dust (13, 53) compared with each other and with moldy building materials (14). Air sampling alone may give an incorrect picture of the fungal diversity actually present in a moldy building. In this study, surface sampling with contact plates containing V8 medium with antibiotics was used. Other agar media (e.g., water agar, MEA, or DG18) (35, 47) have been recommended in older literature, but new research (37) has shown that important toxigenic fungi like *Chaetomium* spp., *Stachybotrys* spp. and *Trichoderma* spp. do not grow or sporulate well on these media. V8, on the other hand, has been shown to support good growth and sporulation of most indoor fungi (3, 12, 22, 37), and V8 allows direct genus identification of indoor fungi and species identification of most *Alternaria*, *Aspergillus*, and *Cladosporium* species and zygomycetes. A disadvantage with V8 is that it does not allow growth of dust fungi such as *Eurotium* spp. or *Wallemia* spp. (37) or identification to species level of *Penicillium*. A disadvantage with all sampling methods based on cultivation is that they detect only viable fungal spores. In the case of *Stachybotrys* spp., detection is essential, because dead spores coated in toxins might still be present on moldy materials. This can be overcome by using Sellotape (Scotch tape) to take samples directly from the surface of moldy building materials. This method complements contact plates or swabs and detects the nonviable and nonculturable fungi (37, 47). The tape preparation method is cheap and quick and can be used on the sampling site prior to the V8 contact plates. Surface sampling using DNA detection would be ideal, but accurate DNA detection and identification of many filamentous fungi are not yet possible (37). None of these sampling methods would detect hyphal fragments, MVOCs, or metabolites that might affect occupants living or working in these environments.

Fungal diversity. Most studies on indoor fungi deal either with surveys of a few fungal species sampled on surfaces (e.g., reference 4) or with many fungal genera detected using air or dilution sampling (e.g., references 5 and 19, respectively). This study deals with many fungal genera (Table 2) on many surface components (Table 1). Our findings (Table 2) do not corroborate the findings reported in the WHO guidelines (52). In this study *Chaetomium* spp., *Acremonium* spp., *Sporothrix* spp., *Calcarisporium* spp., and *Scopulariopsis* spp. were detected together with *Arthrinium* spp., *Aureobasidium* spp., and *Gliocladium* spp. These genera were not listed in the WHO guidelines (52). WHO reports that *Eurotium* spp. and *Wallemia sebi* are common, but these dust fungi were not detected in this study because V8 was used. *Epicoccum* spp. and *Phialophora* spp. were found in this study but so rarely (<0.5%) that they were deleted prior to analyses. Our results, however, correspond well to the findings of Gravesen et al. (14), except for *Stachybotrys* spp. and *Ulocladium* spp., where our study found 3.9% *Stachybotrys* spp. and 8.0% *Ulocladium* spp. compared to 19 and 21%, respectively, in the work by Gravesen et al. (14). One reason could be that Gravesen et al. (14) used tape preparations on the moldy materials as a supplement to the V8 medium, which ensures that nonviable *Stachybotrys* spp. and *Ulocladium* spp. spores were also detected. Another reason could be that Gravesen et al. (14) examined more gypsum samples (11.1%), with which *Stachybotrys* spp. and to some extent *Ulocladium* spp. are associated, than we did (7.7%).

Identification to species level is always recommended, in order to know the fungal diversity (mycobiota) before building renovation commences. Large amounts of alien spores from outside sources can be introduced to a building under renovation, and knowledge of the original mycobiota can be used as a control measure of renovation quality. Identification to species level is also important from a health perspective, since several fungal genera contain species capable of producing species-specific metabolites, mycotoxins (9, 25, 28, 46, 51), and allergens (40, 50). Some fungal genera, however, are notoriously difficult to identify to species level without further cultivation, especially *Penicillium*. Identification of a set of penicillia randomly sampled from other indoor samples showed that *Penicillium chrysogenum* was the most common *Penicillium*, constituting more than 70% of the cultivated penicillia. Using this approximation *Penicillium chrysogenum* would be the most common fungal species in moldy buildings with ca. 50%, being detected twice as often as *Aspergillus versicolor*. Using the same estimation *Penicillium brevicompactum*, *Penicillium citreonigrum*, *Penicillium corylophilum*, *Penicillium crustosum*, *Penicillium olsonii*, *Penicillium palitans*, and *Penicillium solitum* would constitute 4 to 5% each. Several other *Penicillium* spp. have been associated with indoor environments, such as *Penicillium citrinum*, *Penicillium digitatum*, *Penicillium expansum*, *Penicillium glabrum*, *Penicillium italicum*, and *Penicillium roqueforti*, but only when air sampling has been used (e.g., 8, 39). Most of these *Penicillium* species are associated with foods, plants, and herbs (8, 37) and may be more associated with the presence of moldy food in the building than the moldy building materials themselves.

Other fungal genera can be difficult to identify to species directly on V8. Newer literature, however, suggests that the most common *Chaetomium* and *Acremonium* species found on water-damaged building materials are *Chaetomium globosum* and *Acremonium strictum*, respectively (37). *Ulocladium* spp. are also common, and studies have shown that several species (*Ulocladium alternariae*, *Ulocladium atrum*, and *Ulocladium oudemansii*) can be detected on water-damaged building materials (1). *Alternaria tenuissima*, on the other hand, might often be confused with *Ulocladium chartarum*, because both species produce spores in unbranched chains. The most common *Trichoderma* species in water-damaged buildings belongs to the species complex of *Trichoderma harzianum* (37), whereas the only *Stachybotrys* species found are *Stachybotrys chartarum* and *Stachybotrys chlorohalonata* (2).

The findings presented in this study, that *Penicillium chrysogenum*, *Aspergillus versicolor*, and *Chaetomium globosum* are the three most common fungal species on water-damaged building materials, correspond very well with the findings of Polizzi et al. (32). They repeatedly detected the mycotoxins, roquefortine C, sterigmatocystin, and chaetoglobosin A in air, dust, fungal biomass, and wallpaper samples. Roquefortine C, sterigmatocystin, and chaetoglobosin A are the major metabolites produced by *Penicillium chrysogenum*, *Aspergillus versicolor*, and *Chaetomium globosum*, respectively (37). Polizzi et al. (32) also detected roridin E in air samples and ochratoxin A and aflatoxins in air, dust, and fungal biomass samples but were not able to detect the producing fungal species. These toxins

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are produced by *Stachybotrys* spp., *Aspergillus niger*, *Aspergillus ochraceus*, and *Aspergillus flavus* (37), which are also quite common in moldy buildings (Table 2).

Associations between fungi and building materials. This study, which is the largest of its kind, shows that there exists an associated mycobiota on different building materials as there exists an associated mycobiota on different food types (37). The association between *Acremonium* spp., *Penicillium chrysogenum*, *Stachybotrys* spp., and *Ulocladium* spp. on gypsum and wallpaper was indicated by both ordination methods (PCA and CA) and the PCO/minimum spanning tree (Fig. 1 and 2 and Table 3). Production of neutral cellulases has been found in these fungi (31, 43) and may be a common ability of many indoor fungi that have found their niche on damp gypsum or plaster walls clad with wallpaper. A second strong association was seen between *Arthrinium phaeospermum*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *Trichoderma* spp., and yeasts on different types of wood and plywood in all three analyses (PCA, CA, and PCO). *Alternaria tenuissima*, *Fusarium* spp., *Gliocladium* spp., *Rhodotorula mucilaginosa*, and *Trichosporon pullulans* were associated with the group in two out of three analyses. These fungi are also known for their production of neutral cellulases (43) but differ from fungi on damp walls in that they need higher water activity (38). The third association was seen between *Aspergillus fumigatus*, *Aspergillus melleus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Chaetomium* spp., *Mucor racemosus*, and *Mucor spinosus* on concrete and other floor-related materials, such as linoleum, cork, and the glue used to secure them. Concrete is mostly used to cast foundations, floors, and other horizontal structures and will hold soil, dirt, and dust better than vertical surfaces. These *Aspergillus*, *Chaetomium*, and *Mucor* species are common in dust (13) and in hypersaline water and soil (16, 44). They may be introduced along with dirt and may tolerate alkaline conditions in the concrete, beginning to grow when the concrete gets wet.

The results presented here can aid the building profession in choosing materials which are less susceptible to fungal growth (e.g., glass fiber instead of wallpaper) and by manufacturers of building materials to develop nontoxic, fungus- or water-resistant materials (e.g., coating of gypsum board against *Stachybotrys* spp.). Health authorities can use the results to facilitate the development of standardized allergen extracts to test for type I allergy to specific indoor fungi: *Acremonium strictum*, *Aspergillus versicolor*, *Chaetomium globosum*, *Stachybotrys chartarum*, *Stachybotrys chlorohalonata*, and *Ulocladium alternariae*. The results can also be used by the scientific community to focus research on the physiology and ecology of toxigenic species and their potential production of mycotoxins, MVOCs, and microparticles on building materials and to aid in the development of new and better detection and identification methods for fungal growth on building materials.

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