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Levels of microbial agents in floor dust during remediation of a water-damaged office building

Abstract We examined the effects of remediation on loads of culturable fungi in floor dust collected from a large water-damaged office building during four cross-sectional surveys (2002, 2004, 2005, and 2007, respectively). We created a binary remediation variable for each year for each sampled workstation using information on remediation associated with water damage obtained from building management and used generalized linear mixed-effects models. We found significantly lower levels of culturable total and hydrophilic fungi at remediated workstations than at non-remediated workstations in 2004 and 2005 after completion of major remediation. The remediation effect, however, disappeared in 2007. The fraction of hydrophilic to total fungal concentrations was lowest in 2004, increased in 2005, and was highest in 2007. Our results indicate that the 2003 remediation lowered dust indices of dampness temporarily, but remediation was incomplete, consistent with a building assessment report of water infiltration. This study demonstrates the utility of longitudinal evaluation of microbial indices during remediation of water damage in this building, in which elimination of sources of moisture was not fully addressed. Our findings indicate that the fraction of hydrophilic fungi derived from concentrations of fungal species may be a useful index for assessing the long-term effectiveness of remediation.

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Key words: Mold; Hydrophilic fungi; Endotoxin; Water damage; Remediation; Office building.

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Practical Implications

This study demonstrates the utility of longitudinal evaluation of microbial indices during remediation of water damage in this building, in which elimination of sources of moisture was incomplete. Our findings indicate that the fraction of hydrophilic fungi derived from concentrations of fungal species may be a useful index for assessing the long-term effectiveness of remediation.

Introduction

The National Institute for Occupational Safety and Health (NIOSH) received a Health Hazard Evaluation request from the Administrative and Residual Employees Union representing office workers in a 20-story building in the Northeastern United States in 2001. Current tenants first occupied the building in 1994, and, on average, 1240 occupants had occupied floors 5–20. Building occupants had reported respiratory symptoms that they considered to be building-related within a few months after initial occupancy. We reported a 7.5-fold increase in the incidence of asthma among the workers after building occupancy compared to before occupancy and eight hypersensitivity pneumonitis and six sarcoidosis cases in a 2001 survey (Cox-Ganser et al., 2005). A survey (2002) showed that lower respiratory symptoms in this building population, such as wheezing, chest tightness, attacks of shortness of breath, and attacks of cough that improved when away from work, had significant linear exposure–response relationships to total culturable fungi and endotoxin in floor dust (Park et al., 2006).

The building has had a long history of water incursion since it was constructed in 1985. Water leaks occurred mainly along the inside of the exterior walls, especially around terraces and windows on the upper floors (17–19), and from the roof. The first construction activity related to water incursion began with roof coping and window caulking in 2000. Major remediation work, such as roof replacement and repairs around window openings, was completed in 2002 and 2003. Intermittent water leaks around windows, however, continued to occur.

Even though airborne fungi and bacteria, as either colony or spore counts, are widely used for monitoring remediation effectiveness (Haverinen-Shaughnessy et al., 2008; Huttunen et al., 2008; Kleinheinz et al., 2006; Lignell et al., 2007; Meklin et al., 2005), they have many limitations such as large temporal and spatial variability (Institute of Medicine of the National Academies, 2004). In contrast, measurements of microbial agents in floor dust may reflect recent and past environmental conditions because airborne fungal spores and bacteria released from sources accumulate on floors over a period of time. In a cross-sectional study of exposure–response relations in this building population, we used fungal groups based on the moisture requirement for growth (e.g., hydrophilic and mesophilic fungi) (Park et al., 2008). In this study, we examined the effect of remediation on both qualitative species and quantitative fungal information in floor dust, including proportions of fungi classified as hydrophilic and mesophilic. For the analyses, we used data from an initial cross-sectional survey and three follow-up surveys conducted after completion of major remediation.

Table 1 Number of sampled workstations and floor dust samples by floor and survey

Floor	No. of unique sampled workstations[a]	No. of sampled workstations by survey				No. of sampled workstations by number of repeated samples[b]			
		2002	2004	2005	2007	1	2	3	4
Total	689	338	279	297	150	379	261	33	16
5	24	4	6	20	4	15	8	1	0
6	60	30	23	21	10	38	20	2	0
7	55	30	22	21	11	30	22	2	1
8	39	21	16	19	11	19	14	4	2
9	53	26	22	21	12	32	17	1	3
10	51	28	25	21	10	22	26	2	1
11	40	16	15	21	11	20	18	1	1
12	44	19	14	21	10	28	14	0	2
14	57	30	23	20	10	35	18	4	0
15	54	27	25	20	10	30	21	2	1
16	32	8	7	21	10	20	10	2	0
17	79	58	46	21	14	31	37	10	1
18	50	20	20	21	12	30	18	1	1
19	44	20	14	22	12	25	16	1	2
20	7	1	1	7	3	4	2	0	1

[a]Workstations sampled at least once over the four surveys.

[b]No. of dust samples taken from the same workstation across the four surveys.

Methods

Environmental sampling and analysis

NIOSH conducted a series of cross-sectional health and environmental surveys (September 2001, April 2002, August 2004, August 2005, and August 2007). The 2001 and 2002 surveys examined the associations of microbial exposures with occupants' respiratory illnesses, and the follow-up surveys examined the effects of remediation on the building environment and occupants' health. Detailed information on the epidemiological study design and the results for the initial two surveys has been presented previously (Akpinar-Elci et al., 2008; Cox-Ganser et al., 2005; Park et al., 2006, 2008). For the 2002 and 2004 epidemiological studies, we collected dust samples from workstations of respiratory case and comparison group employees identified from the 2001 health questionnaire study (Park et al., 2006). To assess the levels of microbial agents in floor dust after completion of major remediation in 2003, we collected floor dust samples from 300 employees' workstations stratified by floor in 2005 (Table 1). In 2007, we collected samples from 150 workstations that were randomly selected from the 2005 sampling locations.

We collected floor dust on 6.7-μm-pore size polyethylene filter socks (Midwest Filtration Company, Fairfield, OH, USA) attached to a crevice tool using a Backpack vacuum sampler (Pro-Team Inc., Boise, ID, USA) in the 2002, 2004, and 2007 surveys. In the 2005 survey, we used a 250-ml polyethylene catch bottle attached to a High Volume Surface Sampler (HVS3) (CS_3, Inc., Venice, FL, USA). Two square meters of carpeted floor around an employee's chair was vacuumed for 5 min. For each sampling location, we used a different crevice tool that was pre-cleaned for sampling in the 2002, 2004, and 2007 surveys and a different, pre-cleaned cyclone catch bottle in the 2005 survey. In the 2007 survey, we collected side-by-side samples from nine locations in the building to examine whether the mass of dust collected (mg) and loads of culturable fungi [colony-forming units (CFU)/m^2] and endotoxin [endotoxin units (EU)/m^2] using an HVS3 were different from those obtained using a Backpack vacuum sampler. We found no significant differences (*P*-values > 0.1) in the amount of dust or the loads of culturable fungi and endotoxin between the two methods. Geometric means (GMs) (geometric standard deviations, GSDs) of dust collected using HVS3 and Dustpack were 186 mg/m^2 (3.46) and 141 mg/m^2 (3.53), respectively. GMs (GSDs) of culturable fungi collected using HVS3 and Dustpack were 1293 CFU/m^2 (3.28) and 2775 CFU/m^2 (6.74), respectively. GMs (GSDs) of endotoxin collected using HVS3 and Dustpack were 9232 EU/m^2 (2.44) and 6867 EU/m^2 (2.19), respectively.

We made aliquots for the following priority of analyses because of the limited amount of dust collected: culturable fungi, endotoxin, allergens from cats and dogs, ergosterol, and culturable bacteria. The type and the number of analytes varied by survey, as shown in Table 2. Serially diluted dust sample extracts were cultivated onto MacConkey agar for the selection of Gram-negative bacteria (EMLab P&K, Cherry Hill, NJ, USA). Colonies of Gram-negative bacteria were counted and reported as CFU per gram dust. We used the levels of microbial agents per sampled unit area

Table 2 Overall unadjusted building average levels of microbial agents and cat and dog allergens and average fractions of hydrophilic, mesophilic, and other group fungi in floor dust samples across four surveys

	2002		2004		2005		2007	
	N (% <LOD[a])	GM (GSD)	N (% <LOD)	GM (GSD)	N (% <LOD)	GM (GSD)	N (% <LOD)	GM (GSD)
Average levels of microbial measurements								
Total culturable fungi (CFU/m^2)	328 (0.9)	2000 (5.5)	279 (0.4)	4100 (4.0)	296 (0.7)	2400 (5.5)	150 (0.0)	31 900 (4.0)
Ergosterol (ng/m^2)	334 (0.0)	126.2 (3.9)	246 (7.7)	177.7 (3.0)	–	–	143 (0.0)	304.9 (2.1)
Culturable Gram (–) bacteria (CFU/m^2)	–	–	–	–	291 (78.8)	1800 (10.5)	148 (91.2)	28 000 (65.4)
Endotoxin (EU/m^2)	338 (0.0)	2700 (4.8)	276 (0.0)	6100 (5.4)	294 (0.0)	12 800 (5.6)	142 (0.0)	12 000 (2.7)
Cat allergen[b] (μg/m^2)	314 (1.0)	0.7 (2.9)	277 (32.5)	0.4 (3.4)	282 (18.8)	0.7 (4.1)	148 (61.5)	0.3 (6.2)
Dog allergen[c] (μg/m^2)	314 (5.1)	0.6 (3.2)	277 (55.9)	0.3 (3.6)	282 (26.6)	0.5 (4.2)	148 (39.2)	0.4 (3.9)
Arithmetic means of fungal fractions[d]								
Hydrophilic	0.48		0.34		0.40		0.59	
Mesophilic	0.27		0.31		0.33		0.25	
Other	0.25		0.35		0.27		0.16	

[a]Limit of detection (LOD). For the samples below LOD, LOD/2 or LOD/$\sqrt{2}$ was assigned for statistical analyses.
[b]Fel d1 as cat allergen.
[c]Can f1 as dog allergen.
[d]Fungal fraction defined as the concentration (CFU/g) of each group of fungi divided by the concentration (CFU/g) of total fungi in each sample.
–, Not analyzed; GSD, geometric standard deviations; GM, geometric mean.

(microbial load) in our analyses. Other methods were described in detail elsewhere (Park et al., 2006, 2008).

Determination of remediation at employees' workstations

We reviewed 12 unpublished building assessment reports by environmental consultants and newsletters by the building management since 2000 to obtain historical information on water damage and remediation. We defined remediation activity as elimination of sources of water infiltration, such as building exterior (window and balcony) repairs and roof coping or replacement, or replacement of water-damaged materials such as carpet and wallboard. Building exterior repairs around window openings included construction activities, such as brick caulking, window flashing, parapet coping, and plastic barrier repair. We did not include surface cleaning as a type of remediation because a thorough cleaning was made on all the floors of the building in 2004. We considered an employee's workstation as 'remediated' if the workstation was within 15 feet of a remediated section of exterior or interior walls. We considered all workstations on the floor as 'remediated' where an entire floor carpet was replaced. All the workstations on the 20th and 19th floors directly underneath the roof were considered 'remediated' when the roof was replaced or repaired. Based on the definition of remediation above, we created time-dependent binary variables for all sampled workstations for each of the four different time periods based on the NIOSH surveys: prior to the 2002 survey, between the 2002 and 2004 surveys, between the 2004 and 2005 surveys, and between the 2005 and 2007 surveys. To examine the effects of remediation type, we further categorized a binary remediation variable into three levels: no remediation, material replacement only, and structural repairs. Structural repairs included workstations where both structural repairs and material replacement were completed. We created a cumulative remediation variable by the survey time using the binary variables to examine the carryover effects of remediation on the levels of microbial agents.

Statistical analyses

Levels of microbial agents in floor dust were skewed to the right. Accordingly, the data were log-transformed for statistical analyses. For samples without detectable microbial agents, we assigned a value of limit of detection (LOD)/2 or LOD/$\sqrt{2}$, depending on the geometric standard deviation of observed values and the percentage of samples below LOD (Hornung and Reed, 1990).

We categorized fungal species into hydrophilic [requiring water activity (free water available in a substrate), $A_w \geq 0.9$] and mesophilic fungi (requiring $0.8 \leq A_w < 0.9$) (Figure 1), using the same criteria of water activity as used in a previous study (Park et al., 2008). The rest were then classified into other fungi. In the 2005 survey, we used information on fungal genera other than *Aspergillus* because we had species information only on *Aspergillus*. We defined the fractions of hydrophilic and mesophilic fungi in each sample as the concentration (CFU/g) of the specific group of fungi divided by the concentration (CFU/g) of total fungi.

Because major water leaks occurred on the upper floors and remediation was focused on these floors, we grouped occupied floors (5–20th) into two categories – the lower (5–12th) and upper floors (14–20th). We examined the differences in the levels of microbial

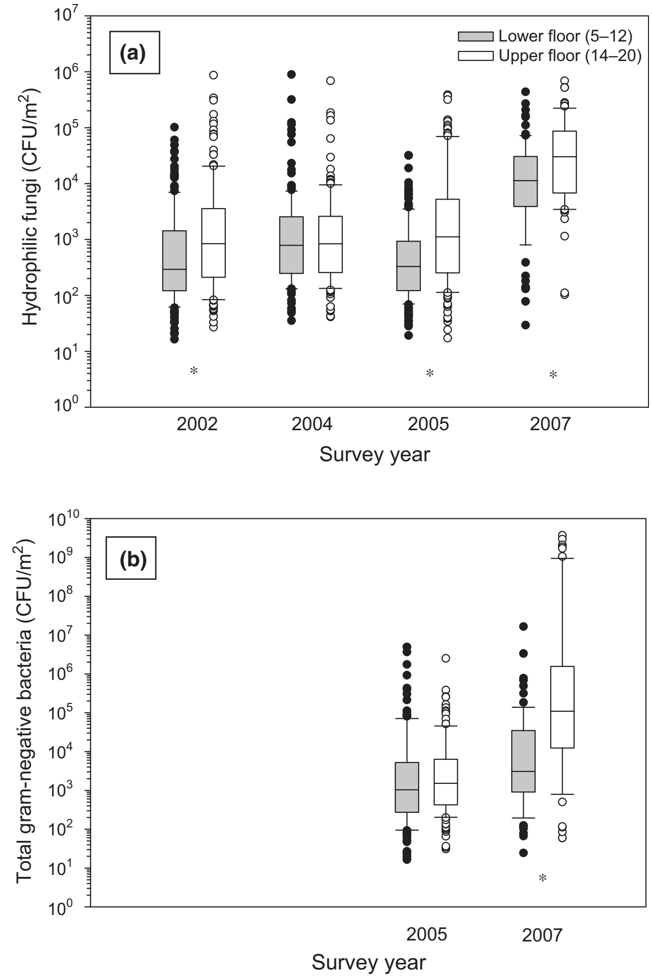

Fig. 1 Observed unadjusted levels of fungi and bacteria on the lower vs. upper floors by survey; (a) Hydrophilic fungi; (b) Gram-negative bacteria (No data available in 2002 and 2004 surveys). Each box plot: an interquartile range (IQR) with median, upper and lower whiskers: upper and lower boundaries (3rd quartile/1st quartile ± 1.5 IQR). *Statistically significant ($P < 0.05$) differences in log means between the lower floors (5–12) and the upper ones (14–20)

agents between the upper and lower floors across the four surveys using a *t*-test. Because we had repeated measurement data from the same workstation over multiple surveys, we used generalized linear mixed-effects models with a compound symmetry covariance structure (same correlation within workstations across surveys) to examine the effects of workstation remediation on floor microbial loads. In brief, a generalized linear mixed-effects model is not only a general form of a multivariate linear regression model that includes both fixed and random variables but also simultaneously accounts for correlation of repeated measurements within the same sampled workstations over time (Singer, 1998). Fixed effects provide estimated population means in the group, while random effects account for individual workstation variability. In the mixed-effects models, natural log-transformed levels of microbial agents were used as outcome variables. Employee workstation was considered a random effect, and survey, building floor, remediation, and a remediation by survey interaction were considered fixed effects. The binary remediation and cumulative remediation variables were considered time-varying covariates in the separate models. The three-level categorical variable for remediation type was used in a mixed-effects model instead of the binary variable to examine the effects of remediation type. For the fraction of hydrophilic fungi, we used a generalized linear mixed-effects model with a logit link function conditional on the binomial distribution of the hydrophilic fraction. We examined the differences in unadjusted means of microbial agents by the remediation across the four surveys using a *t*-test. For sensitivity analysis, we examined the effect of defining remediation as within 30 feet (compared to 15 feet) because most offices are located within 30 feet of the perimeter of the building. All analyses were performed in SAS 9.1 (SAS Institute Inc., Cary, NC, USA). We chose a probability value of $P < 0.05$ for statistical significance.

Results

Environmental sampling locations, although not selected at random in the first two surveys, were approximately evenly distributed on each floor of the building (Table 1). We had a total of 689 different workstations sampled from all four surveys. There were fewer sampling locations on floors 5 and 20 where occupancy was lower compared to other floors. The largest number of sampling locations was on the 17th floor in the 2002 and 2004 surveys because that floor had the largest number of respiratory cases that met the epidemiologic definition used in our previous publications. A majority of sampling locations in 2002 were repeated for sampling in 2004 (60%), but only small fractions of the 2002 locations were selected from random sampling in 2005 (Approximately 15%) and 2007 (Approximately 9%).

Distributions of levels of the microbial agents by survey

In general, GMs of the microbial agents in floor dust showed increasing trends across the four surveys with the lowest levels in 2002 (Table 2). The levels of cat and dog allergens ($\mu g/m^2$) significantly decreased in the 2004 and 2007 surveys compared with the 2002 survey mostly because of an increasing percentage of samples below LOD. The GM of total culturable fungi (CFU/m^2) in floor dust increased by 8–16 times in the 2007 survey compared with those in the 2002, 2004, and 2005 surveys because of the increased levels of hydrophilic and mesophilic fungi, such as *Phoma herbarum*, yeasts, *Aureobasidium pullulans*, *Cladosporium* species, *Alternaria alternata*, and *Epicoccum nigrum*. For ergosterol, the primary sterol in the cell membrane of filamentous fungi and yeasts, the GM in 2007 was more than twice that in 2002.

GMs of endotoxin (12 800 and 12 000 EU/m^2, respectively) in the 2005 and 2007 surveys were about five times higher than that of the 2002 survey (2700 EU/m^2). For Gram-negative bacteria, the difference in GMs (CFU/m^2) in floor dust between 2005 and 2007 was more than one order of magnitude, which was mainly driven by increased levels on floors 14–17 in 2007 (data not shown).

The upper floors where water leaks mainly occurred had significantly ($P < 0.05$) higher levels of hydrophilic fungi than the lower floors except for the 2004 survey (Figure 1). Levels of Gram-negative bacteria on the upper floors were higher than those on the lower floors in 2007.

Change in fungal profiles over the surveys

We recovered a total of 42 genera and 97 fungal species across the four surveys with more diverse species in 2002 and 2004 (63 and 67 species, respectively) than in 2007 (40 species). In 2005, we only identified culturable fungi at the genus level and recovered 22 fungal genera, which were fewer than any other survey. In addition to identified species, *Basidiomycetes* (class of fungi), non-sporulating fungi, yeasts, and unidentifiable species within fungal genera such as *Aspergillus*, *Penicillium*, and *Phoma* were cultured.

In 2002, hydrophilic fungi, such as yeasts (76.2%) and *P. herbarum* (16.5%), and mesophilic fungi, such as *A. pullulans* (53.4%) and *E. nigrum* (36.9%), were not only common fungi but also major contributors to the total fungal concentrations (Figure 2). Other hydrophilic fungi such as *Chaetomium globosum*

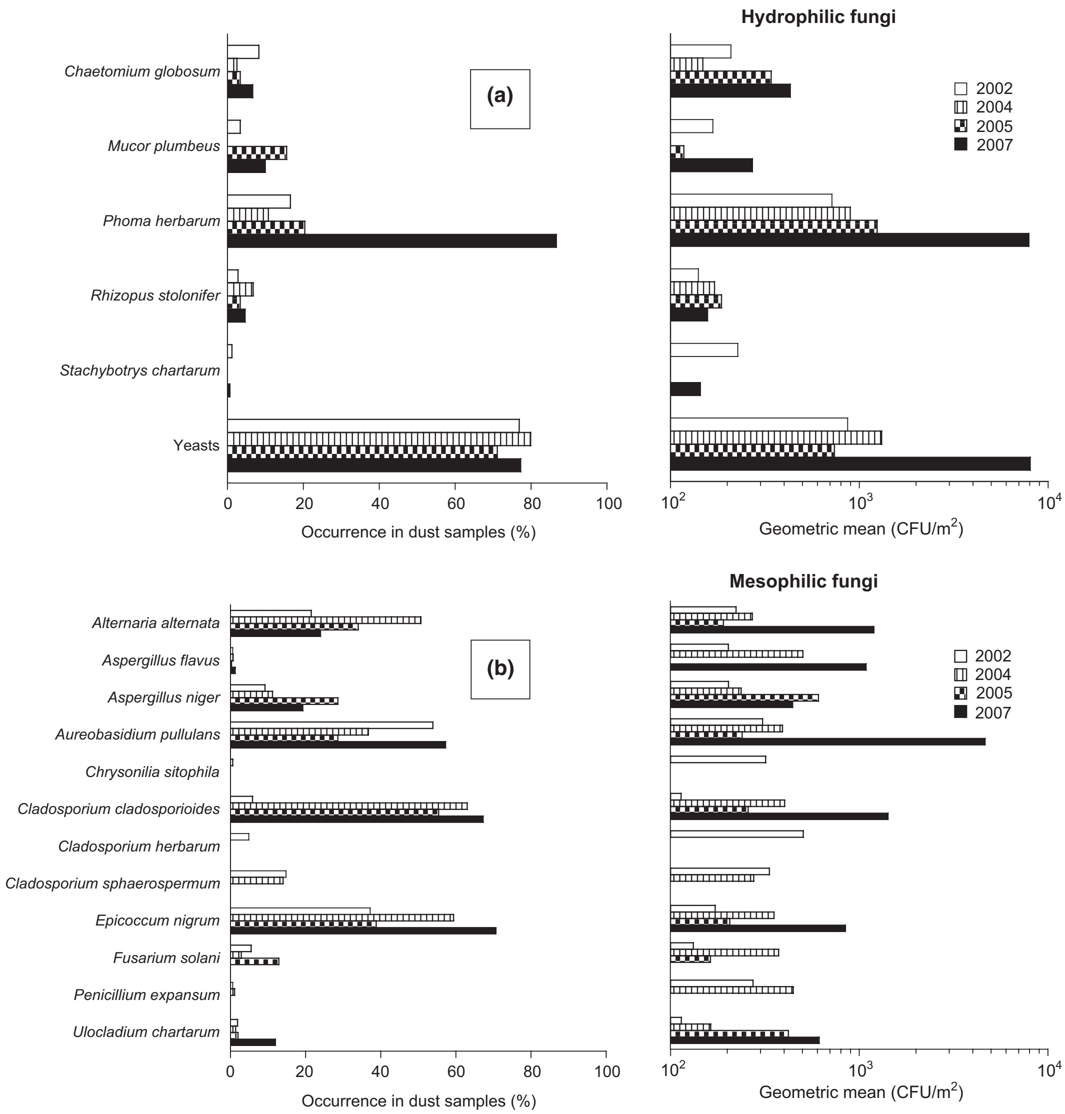

Fig. 2 Percentage of samples and levels of hydrophilic and mesophilic fungi in dust (detectable samples only); (a) Hydrophilic fungi; (b): Mesophilic fungi

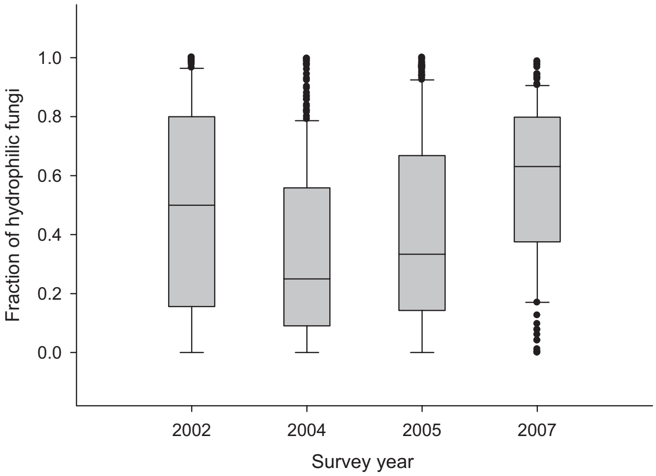

Fig. 3 Box plots for fractions of hydrophilic fungi in total fungi concentrations across four surveys. Each box plot: an interquartile range (IQR) with median, upper and lower whiskers: upper and lower boundaries (3rd quartile/1st quartile ± 1.5 IQR)

(8.2%) and *Stachybotrys chartarum* (1.2%) were also isolated from the floor dust samples. The fraction of hydrophilic to total fungal concentration in 2004 decreased substantially, but the hydrophilic fraction in 2005 increased back toward the 2002 level (Figure 3). In the 2007 survey, the fraction of hydrophilic fungi to total fungi markedly increased and became higher than that in 2002 because of the increased levels of *P. herbarum* (86.7%) and yeasts (77.3%). The levels of *E. nigrum* (70.7%), *Cladosporium cladosporioides* (67.3%), *A. pullulans* (57.3%), and *A. alternata* (24.0%) in the mesophilic fungi group also increased in 2007. The increase in the fraction of hydrophilic fungi occurred on all floors within the building.

Building remediation of water-damaged areas

Table 3 shows the percentage of remediated workstations, including the type of remediation, on all floors for four different time intervals. Detailed information about remediation efforts prior to 2000 was not available. Because damp conditions in this building were mainly owing to water leaks from the roof, terraces, and windows, extensive remediation was focused on the upper floors, mainly floors 17 through 20, throughout the survey years, except for the time interval between 2004 and 2005. Before the 2002 NIOSH survey, construction activities to stop water intrusion began with roof parapet coping and window repairs on the upper floors. The building management replaced water-damaged carpet and wallboard on floors 5 and 16–19. They also cleaned all carpeted floors from November to December 2001 except for the 5th and 17th floors where carpet was planned to be replaced. Building exterior window repairs and roof replacement, as well as replacement of water-damaged materials, had been completed by 2003. There was no construction work in 2004, and a thorough cleaning on all floors, including walls, chairs, partitions, and air vents and diffusers, was completed from February to March 2004. Carpet replacement on the entire 6th floor was the only remediation made during the 2004–2005 interval. The building management also replaced carpet on floors 14–16 from August to December 2005. They found wet wallboard and water-stained ceilings around exterior windows in October 2005

Table 3 Percentage of remediated workstations from unique sampling locations by floor across the surveys

	Percentage of remediated workstations (type of remediation)[a]			
Floor	2000–2002	2002–2004	2004–2005	2005–2007
5	16.7 ($C_{16.7}$)	16.7 ($W_{16.7}$, $E_{16.7}$)	–	–
6	–	11.7 ($W_{11.7}$)	100.0 ($C_{100.0}$)	–
7	–	1.8 ($W_{1.8}$, $E_{0.0}$)	–	3.6 ($W_{3.6}$)
8	–	9.8 ($W_{9.8}$)	–	–
9	–	3.8 ($W_{3.8}$)	–	7.5 ($W_{7.5}$)
10	–	3.9 ($W_{3.9}$)	–	–[b] ($W_{0.0}$)
11	–	–[b] ($W_{0.0}$)	–	5.0 ($W_{5.0}$)
12	–	2.3 ($W_{2.3}$)	–	–[b] ($W_{0.0}$)
14	–	3.5 ($W_{3.5}$)	–	100.0 ($C_{100.0}$, $W_{3.5}$)
15	–	7.4 ($W_{7.4}$)	–	100.0 ($C_{100.0}$, $W_{1.9}$)
16	8.3 ($W_{8.3}$)	22.2 ($W_{22.2}$)	–	100.0 ($C_{100.0}$, $W_{8.3}$, $E_{2.7}$)
17	100.0 ($C_{100.0}$, $W_{43.0}$)	43.0 ($W_{43.0}$, $E_{41.8}$)	–	11.4 ($C_{7.6}$, $W_{3.8}$, $E_{0.0}$)
18	49.0 ($W_{49.0}$)	58.8 ($C_{58.8}$, $W_{35.3}$, $E_{58.8}$)	–	19.6 ($W_{17.6}$, $E_{5.9}$)
19	62.2 ($W_{62.2}$, $R_{60.0}$)	95.6 ($C_{62.2}$, $W_{62.2}$, $E_{93.3}$, $R_{62.2}$)	–	8.8 ($C_{0.0}$, $W_{4.4}$, $R_{4.4}$)
20	100.0 ($R_{100.0}$)	100.0 ($R_{100.0}$)	–	–

[a]Workstations where floor dust was sampled at least once across the four surveys were remediated during given time periods; Remediation included the following activities: carpet replacement (C), wallboard replacement (W), exterior/windows repair (E), and roof repair/replacement (R) (see the Methods). A subscript represents a percentage of remediated workstations for each remediation type from unique sampling locations.

[b]Floors where floor dust was not sampled across the four surveys were remediated.

–, no remediation work associated with water damage based on the review of building assessment reports (see the Methods).

Table 4 Geometric means of microbial agents and mean fractions of hydrophilic fungi in floor dust by survey year and remediation

		Geometric means of microbial agents by survey year and remediation							
		Unadjusted[a]				Adjusted[b]			
	Remediation	2002	2004	2005	2007	2002	2004	2005	2007
Total fungi (CFU/m^2)	Yes	2253	4403	804	50 514	1251	2773	1000	36 326
	No	1978	3984	2618**	27 447**	2265**	4405**	2561**	29 733
Hydrophilic fungi (CFU/m^2)	Yes	685	812	219	24 343	331	461	218	16 624
	No	742	1033	788**	12 333**	837**	1118**	783**	13 244
Ergosterol (ng/m^2)	Yes	206	235	–	340	160	178	–	250
	No	110**	164**	–	293	109**	158	–	314
Endotoxin (EU/m^2)	Yes	6248	4230	11 968	13 227	6007	3671	11 051	11 242
	No	2101**	6634**	12 965	11 614	1938**	6035**	11 500	10 861
Cat allergen (μg/m^2)	Yes	0.71	0.37	0.25	0.30	0.50	0.27	0.33	0.24
	No	0.72	0.43	0.74**	0.26	0.72**	0.42**	0.71**	0.26
Dog allergen (μg/m^2)	Yes	0.51	0.26	0.29	0.50	0.40	0.21	0.28	0.48
	No	0.63	0.28	0.56*	0.39	0.57*	0.25	0.54*	0.37
Hydrophilic fungi fraction[c]	Yes	0.41	0.27	0.32	0.60	0.37	0.24	0.27	0.58
	No	0.50**	0.36**	0.41	0.58	0.49	0.35	0.41	0.58

[a]Statistical differences in unadjusted means (fractions) of microbial agents (hydrophilic fungi) by remediation were determined using *t*-test.
[b]Geometric means of microbial agents estimated using general linear mixed-effects models: a random effect of workstation and fixed effects of survey, floor, remediation, and remediation by survey interaction. Remediated workstations changed over survey in the models. In the 2005 survey, workstations on only the 6th floor were considered 'remediated'.
[c]Defined as concentration of hydrophilic fungi (CFU/g) divided by that of total fungi (CFU/g) in each dust sample; adjusted mean hydrophilic fractions were estimated using a generalized linear mixed-effects model with a logit link function conditional on the binomial distribution of the fraction.
***P*-value < 0.05; *0.05 < *P*-value < 0.1 in comparisons of the remediated and non-remediated workstations for each survey.
–, No data available.

after a wind-driven rain event. They replaced the water-damaged wallboard and repaired the exteriors where water intrusion was suspected around a few windows on floors 16–18 in September 2006. Water leaks from exterior windows, however, have been reported again on the upper floors 17–19 since the 2006 repairs.

Estimated effects of remediation using generalized linear mixed-effects models

Adjusted geometric means of microbial agents and fractions of hydrophilic fungi were estimated from generalized linear mixed-effects models at remediated and non-remediated workstations by survey (Table 4). Unadjusted geometric means (fractions) of microbial agents (hydrophilic fungi) by remediation across the four surveys are also presented in Table 4. In all the models, survey and building floor were important covariates that significantly influenced the levels of microbial agents ($P < 0.0001$). The remediated workstations had lower levels of total and hydrophilic fungi than the non-remediated ones in the 2002 survey. The remediation effect was significant in the 2004 and 2005 surveys, which were conducted after the completion of major remediation work in 2003. However, we did not observe remediation effects on the levels of total and hydrophilic fungi in 2002 and 2004 in the crude analysis without any adjustment. Workstations with structural repairs in 2002 and 2004 tended to have the lowest levels of total fungi, followed by the ones with material replacement only. However, only a small fraction (<5%) of workstations had structural repairs in 2002, 2005, and 2007 and material replacement only in 2004, which made those models with remediation type unstable (data not shown). In 2007, there were no differences in the adjusted means of culturable total and hydrophilic fungi between the remediated and non-remediated workstations. Overall, the adjusted fractions of hydrophilic fungi at the remediated and non-remediated workstations were 0.36 and 0.46, respectively, with a marginal difference ($P = 0.05$). The results of remediation effects on the hydrophilic fraction by survey showed the same pattern as those of fungal concentrations in 2002, 2004, and 2005, but the differences between the remediated and non-remediated workstations were not statistically significant. In 2007, the adjusted mean fractions of hydrophilic fungi also substantially increased in both groups of workstations as observed in the crude analysis and were not different between the two groups.

We did not find significant remediation effects on the levels of ergosterol (measurement of fungal biomass) across the studies. Levels of endotoxin at the remediated workstations became significantly lower than those at the non-remediated ones in 2004, although there was no remediation effect in 2002. Unlike culturable total and hydrophilic fungi, the levels of cat and dog allergens in floor dust tended to be always lower at the remediated workstations than those at the non-remediated except for levels of dog allergen in the 2007 survey. Remediation carry-over effects on the levels of total and hydrophilic fungi and endotoxin continued until 2004 in the models. The carryover effects disappeared in 2005 and 2007 (data not shown). From the sensitivity analyses using a greater distance in defining remediation of workstations, the same patterns and statistical significance as observed in our previous results were found (data not shown).

Discussion

Our findings confirm that the building remediation efforts temporarily lowered microbial indices of water damage but were only partially successful. Over time, the interval remediation efforts did not show a reduction in microbial contamination at the remediated workstations when compared to the non-remediated workstations. In generalized linear mixed-effects models where employee workstation was used as a random effect and survey, building floor, remediation, and a remediation by survey interaction were used as fixed effects, the levels of total and hydrophilic fungi on the upper floors were higher than those on the lower floors. Significant remediation effects on the levels of total and hydrophilic fungi, endotoxin, and cat allergen in 2004 (approximately 6 months after the major remediation) were observed. Effects of structural repairs on lowering levels of total fungi until 2004 were greater than the effects of simple material replacement on measurements. In 2005, environmental monitoring parameters such as the levels of total and hydrophilic fungi and allergens showed that the remediation effect still appeared to exist. The fraction of hydrophilic fungi in 2005, however, was creeping up toward that in 2002 (Figure 3), and the upper floors had higher levels of hydrophilic fungi than the lower floors (Figure 1). In 2007, 3.5 years after the major remediation, the building environment had deteriorated such that environmental measurements between remediated workstations and non-remediated workstations did not differ. The fraction of hydrophilic fungi dramatically increased from that in 2005 and was much higher than that in 2002.

Indeed, after the major remediation in 2003, ongoing water leaks from exterior windows on the upper floors 16–19 had been documented (Silver Petrucelli & Associates, Inc., unpublished data). Water infiltration tests in 2007 demonstrated that the aluminum window head flashing repaired during the major remediation was not correctly installed. Another unpublished report (Turner Building Science, LLC) noted that the building envelope was constructed according to a pre-1987 design: the envelope relied primarily on brick sealants between the window assembly and the brick veneer to keep wind-driven rain out of the structure. This older design does not have continuous drainage planes behind the brick veneer, which eventually leads to the penetration of rainwater into the building. The increased moisture burden in the building in 2007 may have resulted from water infiltration through incorrectly installed window flashings and/or the design of the building envelope.

Dust contents at sampled workstations in 2007 showed increases in the fraction as well as the levels of hydrophilic fungi and Gram-negative bacteria, which require high water content in substrates for growth and survival (American Industrial Hygiene Association, 2005a; Flannigan and Miller, 2001), suggestive of the increased moisture burden in the building. The widespread increase in hydrophilic fraction and the levels in 2007 reflected environmental deterioration distant from water leaks that occurred at the building envelope. The results of sensitivity analysis also show that water damage remediation affected microenvironments at sampled workstations distant from exterior or interior walls. This may suggest that dust sampling locations to examine the effects of water damage should not be limited by proximity to water-damaged or remediated areas.

Measurements of microbial agents in floor dust, especially cultured colony counts and qualitative information on species, may be useful for monitoring and evaluating water damage and remediation within a building. These measurements in dust may reflect recent and past environmental conditions because airborne fungal spores and bacteria released from sources accumulate on floors over a period of time, and they may even grow in carpets under favorable conditions such as high moisture content (Macher, 2001; World Health Organization, 2009). Survival and culturability of fungi and bacteria are more sensitive to changes in moisture than to other environmental factors such as temperature and available nutrients in the building (American Industrial Hygiene Association, 2005a; Flannigan and Miller, 2001). Fungal spores and Gram-negative bacteria quickly lose their viability in dry conditions (American Industrial Hygiene Association, 2005b). In this study, the fraction of hydrophilic fungi in floor dust was lowest in 2004 shortly after the major remediation, but this increased again in 2005 and was highest in 2007. Thus, the examination of changes in both quantitative and qualitative information on culturable fungi (such as the levels of total and hydrophilic fungi, and the fraction and occurrence of hydrophilic and mesophilic fungi) allowed us to document the time-limited benefits of remediation in the building, consistent with consultants' observational reports of failure to permanently interrupt moisture intrusion.

Levels of endotoxin and ergosterol in 2007 did not increase substantially, unlike measurements of culturable microbes. Levels of total culturable fungi and in particular hydrophilic fungi increased between 2005 and 2007. Within hydrophilic fungi, yeasts increased substantially in 2007. Yeasts produce less ergosterol compared with filamentous fungi (Pasanen et al., 1999), and this may account in part for the discrepancy between ergosterol and hydrophilic fungal increases in 2007. Endotoxin is measured as biological potency of lipopolysaccharide originating from Gram-negative bacterial cell walls, and the bioactivity varies by species of Gram-negative bacteria (Sebastian et al., 2005). Although we did not have species information on Gram-negative bacteria in 2005 and 2007 surveys, it is possible that increases in levels of Gram-negative bacterial species were accompanied by a community shift toward species with less potent endotoxin in 2007, which might have resulted in no changes in endotoxin measurements.

Regression models on cat and dog allergen levels showed that remediation tended to decrease the levels of cat and/or dog allergens in floor dust in 2002, 2004, and 2005. The building averages of these allergens did not increase in 2007, unlike those of culturable fungi or bacteria. The levels of cat and dog allergens, which are brought in by people from outside the building, do not increase with internal sources of moisture in the building (Custovic et al., 1998). Thorough vacuuming accompanying replacement of surface material, including carpet replacement, and general housekeeping probably decreased these allergen levels in floor dust.

A potential limitation in this study is that there may have been misclassification in remediation status at sampled workstations because of incomplete remediation information. Incomplete information on remediation most likely affects our 2002 remediation variable because of incomplete historical documentation of remediation before 2002. The effect of misclassification would be to attenuate remediation effects on the levels of microbial agents, but we still found significant remediation effects in the 2002, 2004, and 2005 analyses. The lack of remediation effect in 2007 is not likely attributable to misclassification of remediation status, considering that the building average of culturable total and hydrophilic fungi substantially increased in 2007. This increase suggests that the prior remediation was not permanently successful, and continuing efforts to stop water intrusion were inadequate. In addition, sensitivity analyses did not demonstrate that different definitions of remediated workstations changed the conclusions. Another limitation is that the remediation effect might have been confounded with unmeasured factors that might also have affected the levels of microbial agents in dust, such as indoor relative humidity, temperature, and cleaning efforts. However, inclusion of the floor variable in the models may have captured such unmeasured confounding factors. The absence of information on baseline measurements of microbial contamination before the very first remediation in 1999 or earlier made it impossible to examine whether the remediation decreased the levels of microbial agents from the initial contamination levels. Only minor fractions of the 2002 sampling locations have been followed in 2005 and 2007 surveys, which might have precluded direct comparison between the results of the earlier (2002 and 2004) and later surveys (2005 and 2007). However, a generalized linear mixed-effects model used in the data analysis was able to handle missing data to provide unbiased estimates of remediation effects by survey. We collected floor dust samples in two different months (April of 2002, August of the other survey years), and the survey effect in the models is thus nested within the annual and seasonal effects. We were not able to separate them from each other, and at least part of the observed survey effect could be explained by annual or seasonal variations.

In conclusion, the effect of remediation on the levels of culturable total and hydrophilic fungi and endotoxin at workstations in the studied building was significant in 2004 shortly after the completion of major remediation. The remediation effect, however, gradually diminished over the years after remediation and disappeared in 2007. These findings are consistent with the report of ongoing water incursions after the major remediation. Although the building management attempted to address these issues as they arose, there were aspects of the building design as previously described earlier that made it difficult to keep the building envelope sealed. This study emphasizes that complete building diagnosis, including building inspection for water incursion to ascertain water sources, is essential because incomplete remediation eventually allows persistent water leaks. Recurring microbial proliferation and dissemination even after remediation is likely to adversely affect occupants with building-related illnesses and produce new cases. This study suggests that concomitant examination of species profiles, concentrations, and fractions of hydrophilic fungi in floor dust may be useful for assessing effectiveness of water damage remediation in research intervention studies.

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