

Dustborne fungi in large office buildings

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Abstract

Fungi are ubiquitous in our daily environments. However, their effects on office workers' health are of great interest to many environmental health researchers. Dust has been considered an important reservoir of indoor fungi from which aerosolization and exposure could occur. We have examined the characteristics of dustborne fungal populations recovered from floors and chairs in office buildings. We investigated twenty-one offices in four office buildings in Boston, MA over a year beginning May 1997. We conducted intensive environmental sampling every six weeks to measure culturable dustborne fungi from floors and chairs, surface dust levels and water activity in carpeting. Carbon dioxide, temperature, and relative humidity were monitored continuously. Concentrations of total dustborne fungi recovered from floors were positively related to carbon dioxide ($\beta = 0.00064$; p -value = 0.0002) and temperatures between 20 and 22.5 °C (p -value = 0.0026). Also, total fungal concentrations in floors gradually increased over the year (p -value = 0.0028). Total fungi recovered from chairs varied significantly by season (p -value < 0.0001), highest in September and lowest in March, and were positively correlated with dust loads in floors ($\beta = 0.25$; p -value < 0.0001). We used principal component analysis (PCA) to reduce various observed fungal species to fewer factors. Six groups (PCA factors) were obtained for dustborne fungi recovered from both floors and chairs. The models of the first PCA factors for both floors and chairs were similar to those for total fungal concentrations. The results of this study provide essential information to further evaluate the effects of dustborne fungi on office workers' health.

Key words: dustborne fungi; indoor environmental quality; office environments.

Abbreviations: BRS = building-related symptoms; PCA = principal component analysis.

Introduction

Most environmental fungi are known allergen sources and many of these microorganisms have been associated with allergic reactions, irritation, toxicoses and infections [1–4]. In recent years, increasing attention has been paid to the potential causal relationship between fungal exposures and nonspecific building-related symptoms (BRS), often called the sick building syndrome. BRS refers to a group of work-related symptoms that do not fit a defined clinical illness, and are usually attributed to poor air quality in modern energy-efficient buildings [5–7]. Most commonly reported BRS include headache; irritation of the eyes,

nose, and throat; lethargy; nausea; dizziness; and chest tightness [8–10]. Several epidemiological studies have examined the effects of respiratory exposure to indoor fungi on BRS [5, 11–14], yet few studies have evaluated the role of dustborne fungi in office environments.

Floor dust fungi are commonly measured both as a surrogate for airborne fungal exposure and as a potential source for such exposure [15–19]. Such measurement is considered to represent long-term fungal exposure although little information is available to assure the association between airborne and dustborne fungal concentrations over time [20]. Understanding the representativeness of dust measures for airborne exposure to fungi depends on a thorough knowledge

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of dustborne fungal populations and factors impacting their variability.

We conducted a one-year study to characterize fungal populations in both floor and chair dust. Seasonal variations and the interaction of dustborne fungi with environmental variables are addressed. This study was part of a larger epidemiological study designed to evaluate the role of bioaerosol exposure on building-related symptoms (BRS) and working efficiency.

Materials and methods

Study design

We investigated twenty-one offices in four large office buildings in Boston, Massachusetts over a year beginning May 1997. Ninety-eight occupants with open workstations or low partitions were recruited, and their offices used for this study. Relative humidity, temperature, and carbon dioxide concentrations were monitored continuously at each site. The building managers provided descriptive data and monitoring records for heating, ventilating, and air conditioning systems (HVAC) of the sample buildings. Dust was collected from the floor and chairs at each workstation every six weeks, and, at the same time, surface dust levels and carpet water activity were measured.

Environmental sample collection and handling

Dustborne fungi

Dust samples were collected after working hours and before janitors started vacuuming the floors on Thursday evenings. For each sampling site, a 2-m² area of the floor was vacuumed for 5 minutes using a 2 horse-power portable canister vacuum cleaner (Eureka Mighty Mite II, The Eureka Co., Bloomington, Illinois). The vacuum pulled air and entrained dust through clean tubing into a cellulose thimble (Whatman International Ltd., Maidstone England), which was then sealed in a zip-lock bag for transport. The chairs of the recruited occupants were sampled for 1 minute using the same sampling method. The dust from all the chairs in a sampling site was pooled in a single cellulose thimble.

The collected dust in each thimble was sifted using a 425 μ m sieve, and the resulting fine dust was weighed. This fine dust weight was used as one measurement of dustiness in the statistical analyses. For

cultural analysis, twenty milligrams of the fine dust were suspended in 0.02% Tween 20 in distilled water (20 mg dust/2ml solution). Dust suspensions were diluted 1:10 and 1:100, and 0.1ml of full strength suspension and of each dilution were plated on duplicate malt extract agar (MEA) and dichloran glycerol-18 agar (DG18) plates. Fungal culture plates were incubated at room temperature for 7–10 days before counting and identification. Dilution plates with 10 to 30 colonies for MEA and 20–50 colonies for DG18 were used for counting colonies of fungi. Each discernable fungal colony on the MEA and DG18 plates was counted. All the fungal colonies were identified to the level possible by low power microscopy (generally, to genus), and counts were recorded by colony type.

Surface dust

The foil method, developed by Schneider *et al.* 1996 [21], was used to estimate dust levels on indoor non-textile surfaces. Briefly, a transparent sticky gelatin foil is pressed against the surface of interest using a constant pressure. The amount of dust is estimated by detecting the change in attenuation of light directly through the foil in a laser-based instrument, the BM-Dustdetector. The results are given as a percentage of the surface area covered by dust. At each location samples were taken from three different types of horizontal surfaces of non-textile furniture, including 1) close-to-person, 2) easily accessible and 3) other surfaces, in accordance with the sampling strategy suggested by Schneider *et al.* 1994 [22, 23].

All of the samples were taken within two meters of the continuous monitors (carbon dioxide, temperature, and relative humidity data loggers). For each type of surface, five samples were taken. The average of the five readings was used for data analysis. The surface dust levels were measured after working hours, but before janitors' cleaning and our own dust collection procedures.

Carpet water activity

Carpet water activity was measured immediately before dust collection at each sampling site. The relative humidity within the carpet was used as a measure of A_w ($A_w = RH/100$ within the carpet), assuming no source of water in the carpet other than that in the ambient air. Careful examination was conducted to ensure this assumption. Relative humidity in the carpet was calculated by measuring the temperature and relative humidity just above the carpet surface and the temper-

ature within the carpet material. The carpet RH was then derived using a psychrometric chart.

Temperature, relative humidity and carbon dioxide

Temperature, relative humidity (RH) and carbon dioxide (CO₂) were monitored continuously at each sampling location. Battery-operated data loggers, HOBO-Temp and HOBO-RH, were used to measure temperature and relative humidity levels, respectively (Onset Computer Co., Pocasset MA). Carbon dioxide concentrations were measured using CO₂ transmitters (GMW21 CO₂ transmitter, Vaisala Inc., Woburn MA), which were connected to external, battery-operated HOBO-Volt data loggers for data storage (Onset Computer Co., Pocasset MA). The samplers were placed on the top of the partitions (approximately 2-meter above ground) and within 5-meter of the participants. Hourly average readings were recorded for these variables.

HVAC systems

The four sampling buildings are in busy urban areas. Three buildings [Buildings I (14 stories), II (4 stories) and III (10 stories)] form an inter-connected campus building complex and Building IV (10 stories high) is on a different campus. All four buildings have constant air volume systems. Seven administrative offices selected from Buildings I, II and III were controlled by four different air handling units. Fourteen sampling sites were chosen from Building IV, which is supplied by 2 air handling units and 380 fan coil units. At least 10% outside air is maintained in these four buildings, according to building managers. Specific ventilation rates for outdoor air were not available for each sampling site, and carbon dioxide concentrations are used as a surrogate for outdoor air ventilation rates.

Statistical analysis

SAS (v.6.12, SAS Institute Inc., Cary, NC) and S-Plus (v.5.3, MathSoft, Inc., Seattle, Washington) statistical packages were used for data analysis. Seven out of twenty-one sampling sites were dropped before the end of the study because of low compliance of the participants. To examine the effects of these missing values on the entire data set, the dustborne fungal distributions of the sites with complete data and those with missing data were examined using multiple regressions. There were no statistically significant differences between distributions with and without complete data ($p > 0.1$). Therefore we assume that the missing data did not bias our analysis results.

Principal component analysis (PCA) was used to reduce the many observed fungal types to fewer factors (principal components) that accounted for most of the variance of the original data. A principal component is a linear combination of optimally-weighted observed variables. The first principal component (PCA factor-1) accounts for a maximal amount of variance in the data set and the 2nd one (PCA factor-2) accounts for a maximal variability remaining from the first principal component, and so forth. The number of principal components retained is usually determined by the proportion of total variance accounted for, as well as whether or not the variables that load on a given component share the same conceptual meaning (in this case, biological or physical characteristics). A principal component consists of variables (i.e., fungal taxa) that have factor loadings greater than 0.4 on the component. Factor loading is the correlation between a principal component and an original variable. Rotation is a method of altering the initial components in order to increase interpretability. We used oblique rotation (Promax method) in these analyses, which allowed correlations between principal components [24–27]. In other words, we assumed that the presence of one group of fungi (one principal component) could be associated with the presence of another group of fungi (another principal component).

Total culturable dustborne fungal concentrations and PCA factor-1 for both floors and chairs were correlated with environmental variables using *Generalized additive models* (GAM) in S-Plus. Amounts of floor and chair dust were not included in the floor and chair fungal models, respectively, since these factors were used for calculating dustborne fungal concentrations. We transformed total culturable fungal counts and other right-skewed environmental variables using the logarithm base-10 to approximate normality for modeling. PCA factor-1 values for both floor and chair fungi had close-to-normal distributions and were modeled directly. We coded sampling dates as 1 to 10 for the 10 equally-spaced sampling events. Robust Gaussian distribution was utilized in GAM. We assumed that sampling sites had random deviations from the overall mean and therefore treated sampling site as a random effect in modeling. We used Akaike's information criterion (AIC) to determine the degrees of freedom of the random site effects for floor and chair fungi. Regression coefficients are shown for linear correlations in GAM results. If linear correlation was not significant, Loess smoothers were used to model non-parametric variables. The spans for Loess smoothers

were determined using Akaike's information criterion (AIC). Nonlinear relationships between fungal concentrations and variables (shown as Loess smoothers) are presented graphically, with regression lines and approximate 95% point-wise confidence intervals. Serial correlation (autocorrelation) resulting from repeated measurements at each sampling site was examined using partial autocorrelation functions in S-Plus. Since the results showed that the within-site autocorrelation was not statistically significant, we did not correct for autocorrelation in GAM results.

Results

Characterizing fungal populations

Media comparison

In order to determine how to use the fungal data in our models, we compared fungal populations recovered on two types of culture media, MEA and DG18. For both floors and chairs, average culturable dustborne fungal concentrations for each sampling at each site were calculated from MEA and DG18 plates separately. Differences in fungal concentrations between the two media were calculated by subtracting the concentrations on DG18 from those on MEA, and the significance of the differences tested using the Wilcoxon signed-rank test (Table 1). Observations with no recoveries on either medium were not included. We also compared frequencies of recovery for common taxa for the two media. The overall recovery frequency of a taxon is the percent of total samples with one or more colonies of the given taxon. Only fungal taxa recovered on more than 2% of either floor or chair samples are listed.

Median differences between recoveries on MEA and DG18 for total culturable dustborne fungi were small (<10% of median concentrations for total culturable dustborne fungi), although the total for chair fungi was statistically significantly higher on DG18 than on MEA. A few prevalent taxa, including non-sporulating fungi, *Penicillium*, *Cladosporium* and *Aspergillus*, as well as *Ulocladium*, a less common taxon, had consistently higher recoveries on DG18 than on MEA. *Fusarium*, *Epicoccum*, *Trichoderma*, *Curvularia* and *Drechslera* were more frequently recovered on MEA than on DG18 for both floor and chair samples. Overall, MEA recovered a slightly greater variety of taxa than DG18 for samples from both floors and chairs (28 vs. 25 taxa in floors and 30

vs. 28 taxa in chairs for MEA and DG18 respectively). Some taxa were recovered on only one medium, but the recovery frequencies were very low (<2%), except for *Chaetomium*. Since differences between the two media were variable and small, we combined the results from the two media for our quantification of culturable dustborne fungal concentrations.

Principal component analysis (PCA)

Overall, thirty-three fungal taxa were recovered from the floor and thirty-eight taxa from the chair samples. We used PCA to identify important subsets of these taxa collected from dust. Taxa with less than 5% recovery in the total sample set were excluded from the PCA to avoid potential distorted groupings, resulting in 20 taxa for floor and 21 taxa for chair dust. We tested several mathematical transformations (e.g., logarithm, 3rd-root and 4th-root) of the culturable fungal concentrations for totals and for each taxon to approximate normality. None of the transformations were ideal. However, groupings derived from 4th-root transformed data were judged as likely to arise from similar environmental conditions and their distributions were the least skewed. Also, the 4th-root transformation did not require a change in the values of fungal concentrations as would have been the case for log-transformed data to avoid transformation of 0 values. Thus, the results derived from 4th-root transformation were used for further analyses.

Table 2 lists the PCA factors for culturable dustborne fungi, as well as the cumulative percent variance explained by these factors. Fungal taxa listed under a given factor had high correlations with the factor (factor loading >0.4) and were ordered accordingly (from highest to lowest factor loading). Six factors were retained for culturable dustborne fungi in both floors and chairs, which accounted for 55% and 52% of the total variance, respectively. PCA factor scores were calculated for each factor based on linear composites of the optimally-weighted original variables. We examined correlations of PCA factor-1 and of total culturable fungal concentrations with other environmental variables using GAM models. The remaining PCA factors were not included in the modeling because they contributed little to overall variance and did not have significant correlations with environmental variables of interest in preliminary analyses.

Table 1. The overall fungal recovery frequencies on DG18 and MEA from floor dust and chair dust

Fungal taxon	Dustborne fungi in floors (CFU/m ²)			Dustborne fungi in chairs (CFU/Chair)		
	Rec. Freq. (%) (DG18/MEA)	Highest medium (Wilcoxon test)	Median difference (Med. of MEA–Med. of DG18)	Rec. Freq. (%) (DG18/MEA)	Highest medium (Wilcoxon test)	Median difference (Med. of MEA–Med. of DG18)
Total ^a	100/100	NS ^b	–186.2	99.4/100	DG18	–1042.0
Non-sporulating	98.9/86.7	DG18	–202.5	98.3/79.9	DG18	–426.3
Yeast	95.6/96.1	NS	0.0	98.9/95.5	NS	–107.0
<i>Aureobasidium</i>	87.3/80.6	NS	–17.4	93.9/91.6	NS	–51.4
<i>Penicillium</i>	87.3/73.3	DG18	–42.6	90.1/70.4	DG18	–151.8
<i>Cladosporium</i>	85.6/77.2	DG18	–68.7	93.4/78.8	DG18	–97.8
<i>Aspergillus</i>	72.4/56.1	DG18	–81.9	95.0/78.2	DG18	–262.3
<i>Alternaria</i>	64.1/53.9	NS	–20.2	88.4/77.7	DG18	–86.4
<i>Coelomycetes</i>	39.2/40.0	MEA	17.4	43.6/34.6	NS	0.0
<i>Zygomycete</i> ^c	28.2/29.4	NS	–40.8	47.5/60.9	NS	27.0
Unknown ^d	27.6/25.6	MEA	40.8	39.2/35.8	MEA	63.8
<i>Ulocladium</i>	15.5/5.0	DG18	–80.3	56.4/21.8	DG18	–78.4
<i>Pithomyces</i>	14.9/15.0	NS	–5.5	31.5/37.4	NS	25.7
<i>Fusarium</i>	9.4/21.1	MEA	82.9	4.4/11.7	MEA	75.3
<i>Paecilomyces</i>	8.8/5.6	NS	–87.7	12.7/7.8	NS	–36.8
<i>Epicoccum</i>	7.7/17.8	MEA	64.3	27.1/41.3	MEA	69.8
<i>Botrytis</i>	4.4/2.2	NS	–40.6	11.0/6.7	NS	–36.5
<i>Trichoderma</i>	2.8/14.4	MEA	84.7	3.9/15.1	MEA	66.9
<i>Curvularia</i>	2.8/8.3	MEA	119.8	3.9/11.2	MEA	66.4
<i>Drechslera</i>	2.8/7.2	MEA	86.7	1.1/10.6	MEA	89.4
<i>Wallenia</i>	2.2/0.6	NS	–106.8	6.6/0.0	(DG18)	–
<i>Nigrospora</i>	1.1/3.9	NS	46.9	6.6/5.6	NS	–32.1
<i>Monilia</i>	1.1/0.0	(DG18) ^e	–	0.6/2.2	MEA	67.3
<i>Arthrinium</i>	0.6/1.7	NS	168.0	0.0/2.2	(MEA)	–
<i>Chaetomium</i>	0.0/12.2	(MEA)	–	0.0/4.5	(MEA)	–

^aAll taxa combined.^bThere is no significant difference between two media using Wilcoxon signed-rank test.^c*Zygomycetes* includes *Rhizopus*, *Mucor*, and non-sporulating zygomycetes.^dUnknown are colonies that were either overgrown by other colonies or very rarely seen and not readily identified.^eMedium in parenthesis indicates that the corresponding taxon was only recovered on that medium in the entire sample set.

Table 2. Fungal groupings derived from principal component analysis for fungi in floor dust and chair dust

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
(Floor Fungi) ^{0.25}	Yeast	<i>Pithomyces</i>	<i>Paecilomyces</i>	<i>Aspergillus</i>	<i>Zygomycetes</i>	<i>Drechslera</i>
	<i>Coelomycetes</i>	<i>Epicoccum</i>	<i>Curvularia</i>	<i>Penicillium</i>	Unknown	<i>Botrytis</i>
	<i>Aureobasidium</i>	<i>Alternaria</i>	<i>Ulocladium</i>			
	Non-sporulating (<i>Cladosporium</i>)	<i>Cladosporium</i> <i>Fusarium</i>				
Cumulative % of variance	18.19	27.95	35.12	42.09	48.63	54.80
(Chair Fungi) ^{0.25}	Non-sporulating	<i>Aspergillus</i>	<i>Pithomyces</i>	Unknown	<i>Botrytis</i>	<i>Fusarium</i>
	<i>Alternaria</i>	<i>Zygomycetes</i>	<i>Nigrospora</i>	– <i>Paecilomyces</i>	<i>Penicillium</i>	<i>Wallemia</i>
	<i>Cladosporium</i>		– <i>Trichoderma</i>	– <i>Drechslera</i>	<i>Ulocladium</i>	
	Yeast					
	<i>Aureobasidium</i> <i>Epicoccum</i>					
Cumulative % of variance	17.85	26.72	34.41	40.80	46.94	52.31

Fungi that had factor loading >0.4 on 2 factors are listed under both factors and are shown in parenthesis under the factor with the weaker correlation.

Fungus preceded by a negative sign had a high negative correlation with the corresponding factor.

Summary statistics

Table 3 summarizes the environmental measurements. The fungal data and dust measurements are right-skewed. The distributions of culturable floor and chair fungal concentrations did not show an obvious seasonal variation over the course of the study period. Temperature and water activity varied significantly by sampling date. Temperature was slightly higher in winter than in summer. Conversely, water activity was highest in summer and lowest in winter. Other environmental variables, including surface dust, dust loads in floors and chairs, and CO₂ concentrations did not show significant temporal variation.

Total culturable dustborne fungi in floors and PCA factor-1 for floor fungi

Table 4 and Figures 1–4 show the model results for total culturable floor fungal concentrations and the PCA factor-1 for floor fungi (Floor Factor-1). Floor Factor-1 consists of yeast, *Coelomycetes*, *Aureobasidium*, non-sporulating and *Cladosporium*. The model results of total culturable floor fungal concentrations and Floor Factor-1 are very similar. Both floor fungal measurements were positively associated with CO₂ concentrations and with temperatures from 20 °C to 22.5 °C. Also, the two measures increased throughout the sampling period. No correlations were found

for water activities, surface dust levels and amount of dust in chairs.

Total culturable dustborne fungi in chairs and PCA factor-1 for chair fungi

Concentrations of total culturable fungi in chair dust (CFU/chair) were related to amount of floor dust (in grams) and sampling date (Table 5 and Figure 5). Chair fungal concentrations were strongly seasonal, with highest levels in summer and early fall, and lowest in winter. The PCA factor-1 for chair fungi (Chair Factor-1) includes non-sporulating, *Alternaria*, *Cladosporium*, yeast, *Aureobasidium* and *Epicoccum*, which had very similar model results (Table 5 and Figure 6) when compared to total culturable chair fungi. Chair Factor-1 was positively related to amount of dust in floors and had highest levels in September and lowest in March.

Discussion

The aim of this study was to assess dustborne fungal distributions in large office buildings across space and time and to identify environmental variables that correlate with fungal populations. Understanding the characteristics of dustborne fungal populations in floors and in chairs will enable our further exploration

Table 3. Distribution of the environmental variables

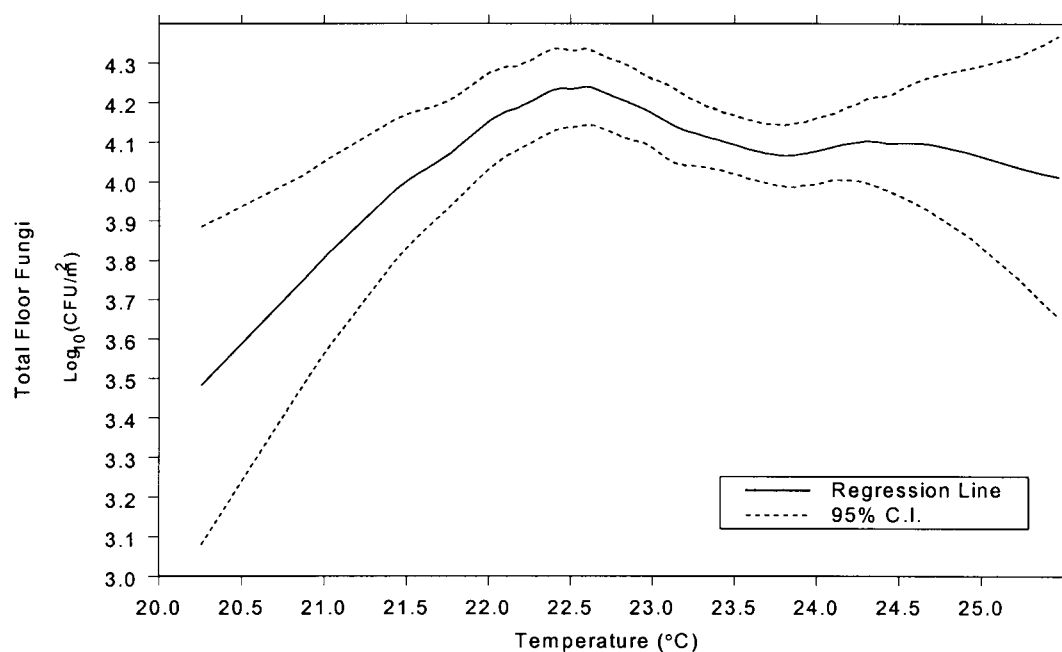
Environmental parameters	Unit	Mean	SD	Med.	Min.	Max.
Dustborne fungi in floors	Total CFU/m ²	41232	147219	11725	298	1495689
Dustborne fungi in chairs	Total CFU/chair	29764	86352	15468	946	1068214
Temperature	°C	23.29	0.98	23.43	18.66	25.47
CO ₂	PPM	689.44	184.01	670.40	379.50	1344.67
Water activity		0.53	0.11	0.53	0.23	0.90
Surface dust	%	3.83	2.61	2.85	0.51	12.58
Floor dust	g/m ²	0.20	0.19	0.15	0.02	0.94
Chair dust	g/chair	0.14	0.10	0.12	0.01	0.62

Table 4. GAM results for total culturable floor fungi and PCA factor-1 for floor fungi

Total culturable floor fungi				PCA factor-1 for floor fungi			
Variables	Coefficient	SE	p-value	Variables	Coefficient	SE	p-value
Intercept	3.6297	0.1223	<0.0001	Intercept	-0.6780	0.2307	0.0037
CO ₂	0.00064	0.00017	0.0002	CO ₂	0.00094	0.00032	0.0041
lo(Temp, 0.6) ^a	(Figure 1)	–	0.0026	lo(Temp, 0.7)	(Figure 3)	–	0.0043
lo(Sampling Date, 0.65)	(Figure 2)	–	0.0028	lo(Sampling Date, 0.4)	(Figure 4)	–	<0.0001
Random (Site, df = 20) ^b	–	–	0.0380	Random (Site, df = 20)	–	–	0.0027

^alo(Temp, 0.6) is a Loess smooth function of temperature, with a span of 0.6. Other non-parametric variables use the same expressions.

^bSampling site is a random effect, with 20 degree of freedom.

Figure 1. Relationship between temperature and total culturable floor fungi (CFU/m²).

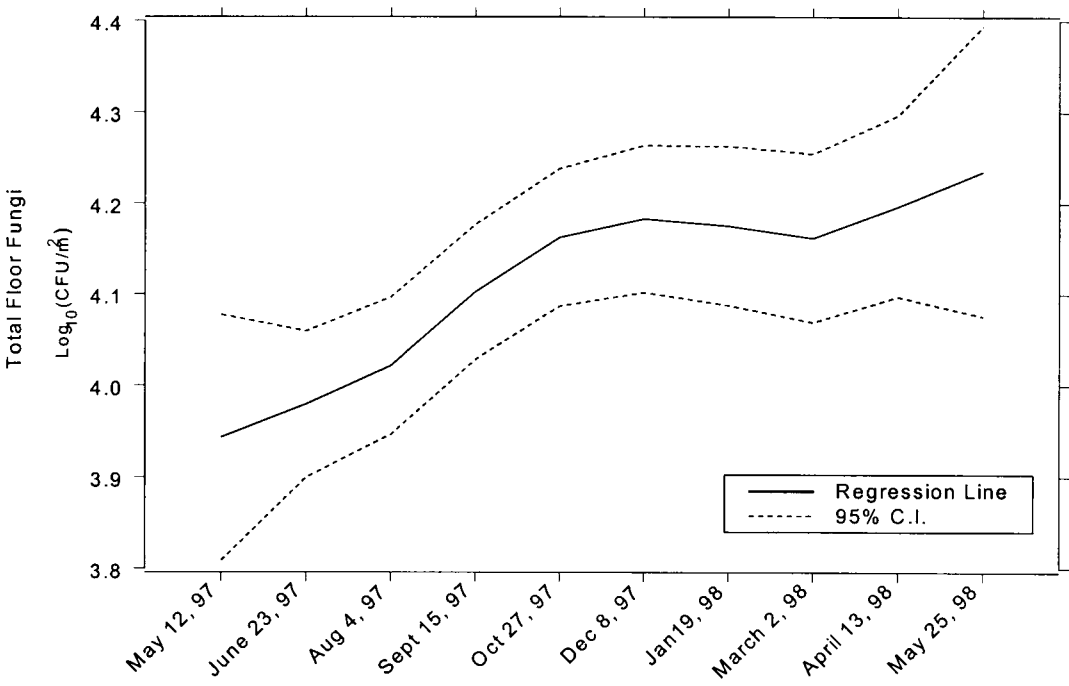


Figure 2. Relationship between sampling dates and total culturable floor fungi (CFU/m²).

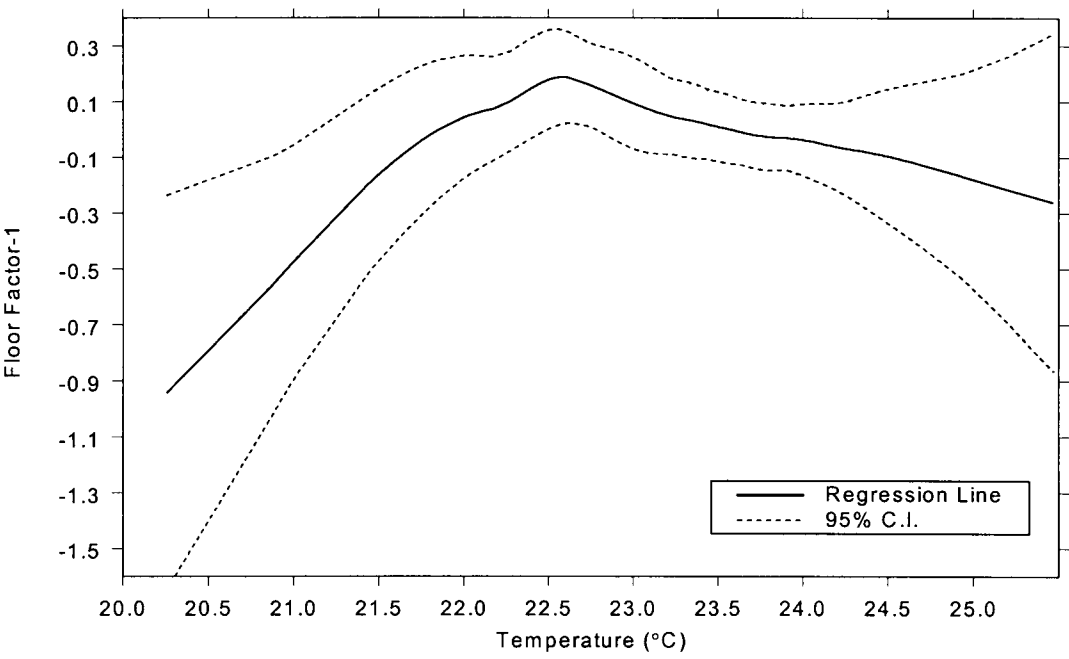


Figure 3. Relationship between temperature and PCA factor-1 for floor fungi.

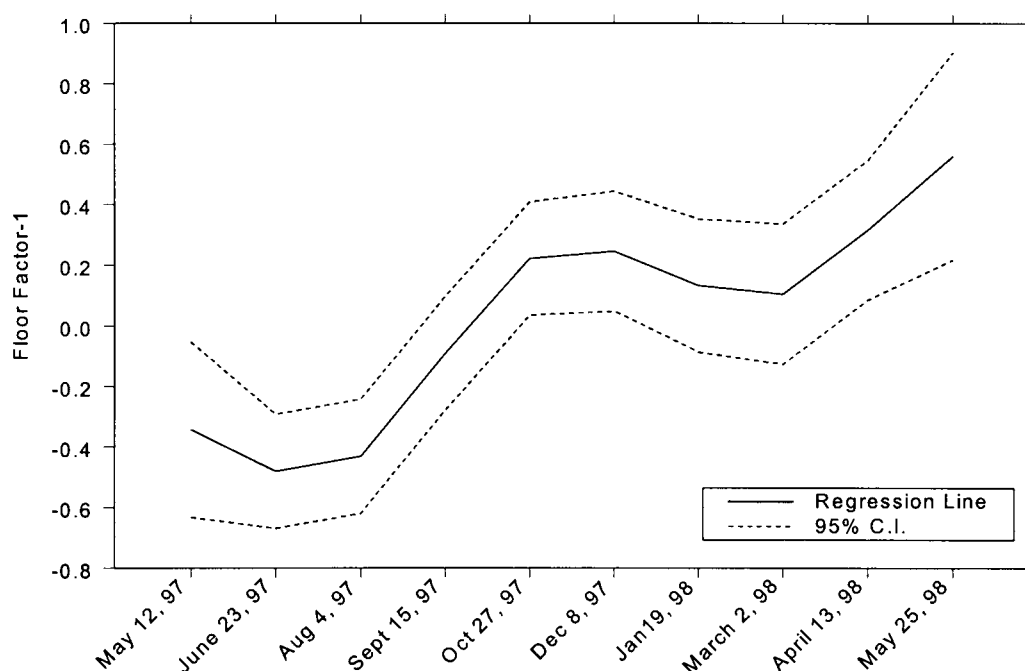


Figure 4. Relationship between sampling dates and PCA factor-1 for floor fungi.

Table 5. GAM results for total culturable chair fungi and PCA factor-1 for Chair fungi

Total culturable chair fungi				PCA factor-1 for chair fungi			
Variables	Coefficient	SE	p-value	Variables	Coefficient	SE	p-value
Intercept	4.4155	0.05679	<0.0001	Intercept	0.6776	0.1386	<0.0001
Floor dust*	0.2502	0.06118	<0.0001	Floor dust*	0.8059	0.1482	<0.0001
lo(Sampling Date, 0.5)	(Figure 5)	—	<0.0001	lo(Sampling Date, 0.5)	(Figure 6)	—	<0.0001
Random (Site, df = 21)	—	—	<0.0001	Random (Site, df = 21)	—	—	<0.0001

*Log10-transformed floor dust was used in modeling to approximate normality.

of the relationship between BRS and dustborne fungi in future analyses.

Several studies have examined the differences in culturable fungal recoveries on MEA and on DG18. Smid *et al.* compared the performance of four air sampling devices and three culture media in seven occupational settings (a flour mill, two locations of an animal feed production facility, two locations of a municipal archive building, and two microbiological laboratories) [28]. No significant difference was found between recoveries on MEA and DG18. In the study conducted by Verhoeff *et al.*, four culture media including MEA and DG18 were evaluated, in combination with five air sampling devices [29]. Airborne culturable fungi were collected from living room or bedroom in 11 houses. Both MEA and DG18 had the

highest total fungal concentrations and number of species isolated. Ren *et al.* collected airborne culturable fungi from infant bedrooms and from main living areas in 1000 homes and found significantly higher total concentrations and yeast levels on MEA but higher concentrations of *Aspergillus* and *Cladosporium* on DG18 [30]. Wu *et al.* sampled airborne culturable fungi at 17 sites in a hospital environment [31]. They found higher total fungal concentrations and a greater variety of taxa on DG18 than on MEA. Thus, whether or not MEA and DG18 recover the same or different fungal populations from a reservoir varies by study. In none of the reviewed studies were differences between the two media large. In view of these data, we used both MEA and DG18. Our results agree with most of the above studies in that we found few meaningful

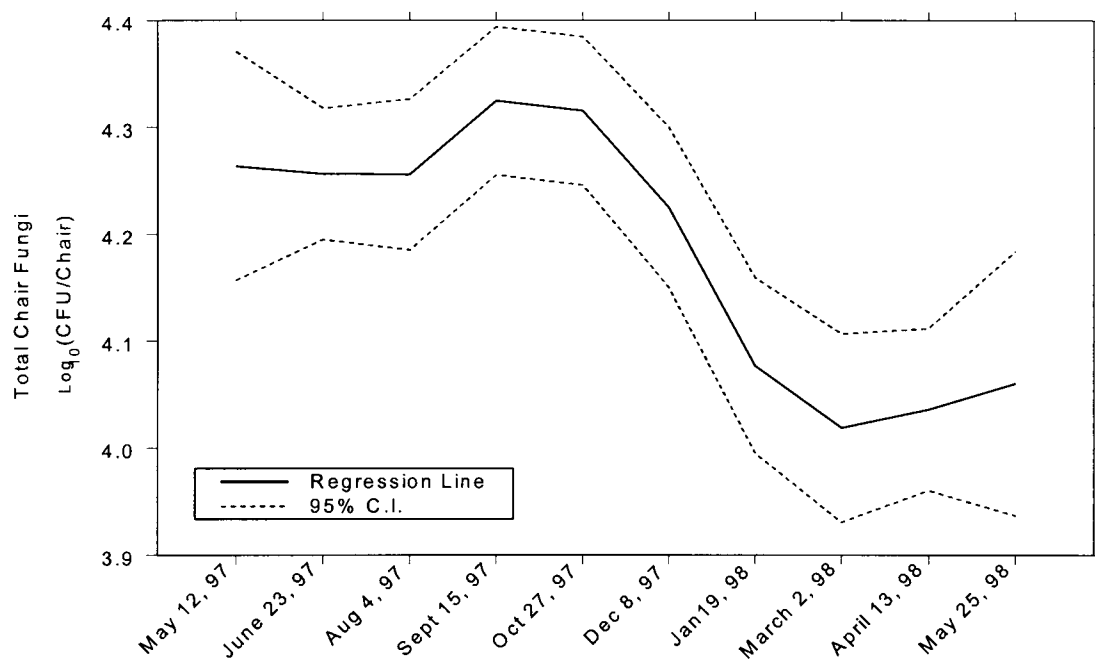


Figure 5. Relationship between sampling dates and total culturable chair fungi (CFU/Chair).

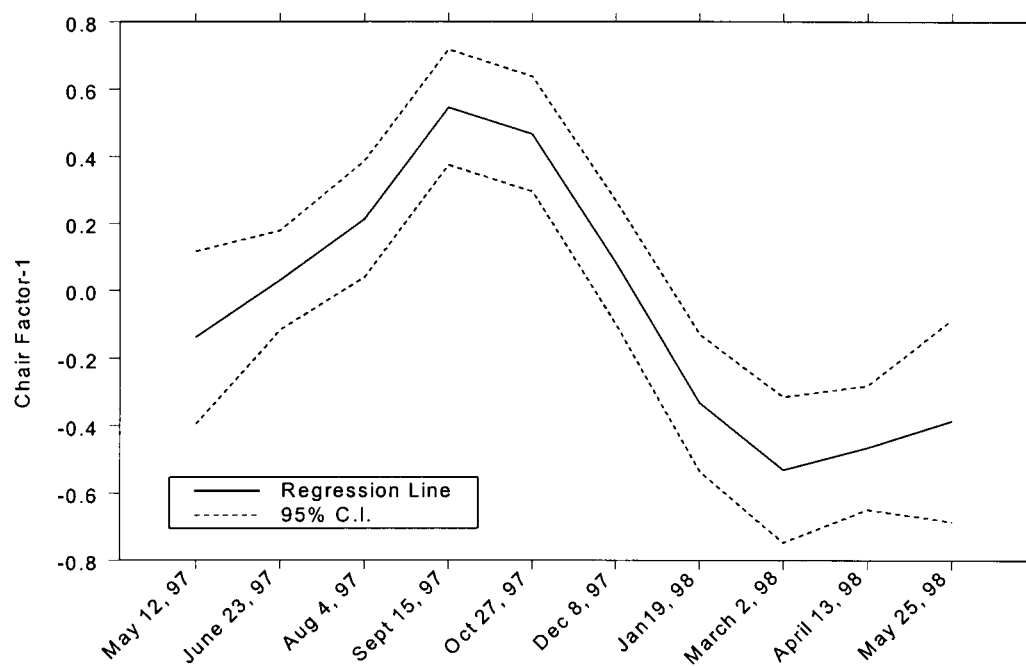


Figure 6. Relationship between sampling dates and PCA factor-1 for chair fungi.

differences between recoveries on the two media. We were thus able to justify combining all data from both media.

Plate loading (total colony counts per plate) is an important factor that is rarely considered in determining recovery frequencies on culture plates. Overloaded plates lead to competition for nutrients, interspecies inhibition, merging of same-species colonies, or overgrowth by one or more other fungi. Hence, an overloaded plate may underestimate total fungal counts and affect taxon characterization. DG18 contains dichloran, a pesticide that limits fungal growth, as a partial solution to this problem. To minimize these effects, whenever possible we chose dilutions for data collection with enough colonies to satisfy statistical requirements, but not so many that plates were overloaded.

We used principal component analysis (PCA) to identify subsets of fungal taxa that shared similar prevalence patterns. Each derived subgroup, a PCA factor, may have resulted from common environmental requirements or have settled in dust from similar sources or through similar mechanisms. Six factors were identified in dustborne fungal populations recovered from both floors and chairs (Table 2). However, the groupings were very different in floor and chair dust. The first factor for floor fungi (Floor Factor-1) includes *Aureobasidium*, *Coelomycetes*, yeast, non-sporulating, and *Cladosporium*. We commonly see *Aureobasidium*, *Coelomycetes* and yeast in dust, but much less commonly in air. This is logical for yeasts, many of which are extreme xerophiles [32]. For *Coelomycetes* and *Aureobasidium*, we must speculate either that they survive longer than common airborne fungi, or that they actually grow in the dust. These hypotheses have yet to be tested. *Cladosporium* is routinely the most abundant airborne fungus indoors and out [2, 33] and we assume its presence in Factor-1 represents residual settled spores. This is reinforced by the dominance of *Cladosporium* in Factor 2. Although present in Floor Factor-1, *Cladosporium* was most closely related to the Factor-2 group. It is of note that the species of *Cladosporium* of Floor Factor-1 could have been different from the ones of Floor Factor-2. Certainly the *Cladosporium* species that commonly grow on indoor substrates (e.g., *C. sphaerospermum*) are different from those common on in outdoor air (e.g., *C. herbarum*, *C. cladosporioides*).

The PCA factor-2 for floor fungi (Floor Factor-2) includes *Pithomyces*, *Epicoccum*, *Alternaria* and *Cladosporium*, which are primarily outdoor fungal taxa

with melanized cell walls that grow on aboveground plant materials [2]. These are among the most prevalent airborne fungi in outdoor air [2] and are likely to have settled in dust from outdoor air. *Fusarium* species, also part of Floor Factor-2, are common soil fungi [34] and are frequently recovered from outdoor air. They are also found in very wet indoor environments (e.g., reservoirs of humidifiers) [32, 35].

Floor Factor-3 includes *Curvularia*, *Ulocladium*, and *Paecilomyces*. *Curvularia* and *Ulocladium*, similar to the common outdoor fungi in Floor Factor-2 but much less prevalent, have dark-pigmented cell walls [36]. They are considered outdoor fungi, although *Ulocladium* is often part of fungal populations on wet wallboard and ceiling tile. Other than the fact that they were uncommon in our data set, it is not clear that these three fungi share characteristics or requirements different from those in Factor 2. *Paecilomyces* species, although rarely abundant, are also infrequently a component of the outdoor aerosol [34].

Penicillium and *Aspergillus*, components of Floor Factor-4, are both common soil fungi without cell wall melanin and are the most prevalent genera in indoor environments [32, 37]. These two large genera are taxonomically similar and are generally alike in airborne behavior and spore size [38]. Both readily colonize indoor materials and can occupy a wide variety of environments. When *Penicillium* and/or *Aspergillus* are dominant in the indoor dust or aerosol, the environment is usually considered to be supporting indoor growth.

Floor Factor-5 includes *Zygomycetes* and Unknown. These *Zygomycetes* are mainly *Mucor* and *Rhizopus* species, which are common soil fungi with rapid mycelial growth [34, 39]. Unknown is a group of unidentified fungi, including mainly masked colonies resulting from overgrowth, often due to *Zygomycetes*.

Floor Factor-6 consists of *Drechslera* and *Botrytis*. *Drechslera* is a large genus that was rarely recovered in our environments, possibly because of culture medium selectivity. *Botrytis* commonly grows on senescing plant leaves and in rhizospheres [34]. The environmental relationship between these two taxa remains obscure.

The PCA factor-1 for chair fungi (Chair Factor-1) is most similar to Floor Factor-2, indicating that most of the chair fungi are likely to be derived from outdoor air. These fungi probably settled on chairs directly from air or were brought in and deposited from occupant clothing. Other chair PCA factors did not have similar taxon groupings compared to floor PCA

factors. The different compositions of the floor and chair dust may partially account for this observation. The dust collected from floors appeared to be mostly sand and was probably tracked in on shoes. Chair dust appeared to be mostly skin scales and fibers from clothing.

Su *et al.* conducted a study using factor analysis to investigate correlations among airborne microorganisms in home environments [40]. Their first factor included *Cladosporium*, *Alternaria*, Yeast, *Aureobasidium* and *Epicoccum*. The second factor included *Alternaria*, *Aureobasidium*, *Epicoccum*, *Pithomyces* and Unknowns. Unknowns often included non-sporulating colonies of basidiomycetes. Their first two factors included all the dominant outdoor plant-associated fungi, similar to the first two Floor Factors derived in our data set. Their third factor consisted of *Penicillium*, *Aspergillus* and Unknowns, similar to our Floor Factor-4. Similar fungal groupings of the most dominant indoor fungi in these two studies suggest possible interactions between airborne and dustborne fungal populations in floors. More studies are needed to examine the correlations between airborne and dustborne fungi in total and by specific taxon.

Most reported studies of floor dust fungi use a different unit (CFU/g) for fungal concentrations compared to ours (CFU/m²). We considered an area measure more relevant for exposure assessment than a mass measure. However, our concentrations per gram for total culturable floor fungi (mean = 355,756 CFU/g; median = 81,143 CFU/g) are similar to those reported in other studies [19, 20]. There are no reported chair fungal concentrations available that can be contrasted to our results (mean = 205,441 CFU/g; median = 132,766 CFU/g). Overall, fungal concentrations in chairs were fairly consistent. This is possibly because our vacuuming every six-weeks did not decrease the total culturable fungal concentrations in chairs significantly, or there were sufficient amounts of fungi deposited in chairs between two consecutive samplings to offset the loss due to vacuuming. Although fungi could have reproduced in the chairs, such growth should have produced a more uniform population than was found.

Total culturable dustborne fungal counts and PCA factor-1 for both floors and chairs were correlated with other environmental variables. Total culturable floor fungi and Floor Factor-1 shared similar model results and were associated with temperature, CO₂ concentration and sampling date (Table 4 and Figures 1–4).

Indoor temperatures throughout our study were in a range known to support fungal growth [41]. However, our study showed that temperatures between 22 °C and 23 °C were optimal for recovery of total culturable fungi from floor dust, although it should be noted that more than 75% of our temperature measurements were within this range.

Water availability is considered the most important factor for fungal growth and the optimum water activity is above 0.90 for many fungal taxa [41]. However, an association was not found between water activity and fungal concentrations in floors. It is likely that dormant fungal spores in floor dust are not affected by lower-than-optimum water activities in office environments. It is also possible that our method for assessing water activity was not a good measure for available water in floor carpets. We assumed the water content of floor carpets reached equilibrium with the air immediately surrounding the carpet fibers. Therefore, the absolute humidity of these two adjacent environments should be the same, and carpet water activity could be derived by measuring carpet temperature and absolute humidity in the surrounding air (derived by air relative humidity and temperature). If the assumption was not always true during the study, the measurements could be incorrect, although obvious water spill or leakage was not observed at sampling. The relationship between temperature and floor fungi we observed might, in fact, be due to a correlation between temperature and actual water content in floor carpets.

CO₂ concentrations were positively related to both total culturable floor fungal counts and Floor Factor-1. For a change in CO₂ concentrations of 261 ppm (the interquartile range), we estimated that total culturable floor fungal concentrations would have increased by 47%. The correlation between floor fungal concentrations and CO₂ concentration may be related to the positive association between CO₂ levels and number of people or human activities in the sampling sites. Floor fungal concentrations gradually increased over the course of the study, even though regular vacuuming and cleaning were conducted in the sampling sites. This might suggest that usual cleaning approaches, such as vacuuming and occasional shampooing, are not sufficient to decrease total fungal concentrations in carpets.

Both total culturable chair fungi and Chair Factor-1 had positive relationships with floor dust mass. The amount of floor dust was probably a surrogate for overall office cleanliness. However, surface dust

levels, also considered as a possible surrogate for office cleanliness, were not associated with any chair fungal measurements. Surface dust was mostly settled from dust suspended in air and the levels did not vary significantly over time. Thus, it is likely that surface dust levels were not directly affected by overall office house-keeping work, but, rather, by more complicated environment factors, including air movements and human activities.

There was significant seasonal variation for both total culturable chair fungi and Chair Factor-1, controlling for other variables (highest levels in September and lowest levels in March) (Tables 5, Figures 5–6). This would support the conclusion that chair fungi are shed from occupants' clothing, and that the clothing fungi are deposited from outdoors. In the Boston area, outdoor spore concentrations are highest in September, and usually very low in March. Also, occupants wear outer clothing that is removed before sitting in March but not usually in September. Comparing the models for floor and chair fungi, the fungal levels in the two reservoirs were controlled by very different sets of environmental factors and their contributions to BRS might differ as well.

This study provides data on temporal variations and compositions of dustborne fungal populations in office buildings, for both floors and chairs. It is clear that the biological characteristics of the two reservoirs, floor dust and chair dust, are distinct. The different groupings (PCA factors) of dustborne fungi in floors and in chairs indicate that these fungal groups have deposited in dust by various mechanisms and/or have grown under different environmental conditions. Therefore, it is essential to examine the possible health effects of fungal measurements separately for floors and chairs as well as for different fungal groupings (PCA factors). Future analyses will look at the interrelationships between airborne fungal concentrations and dustborne fungal concentrations in floors and chairs and examine these measures' impacts on office occupants' symptoms.

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