

Populations and Determinants of Airborne Fungi in Large Office Buildings

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Bioaerosol concentrations in office environments and their roles in causing building-related symptoms have drawn much attention in recent years. Most bioaerosol studies have been cross-sectional. We conducted a longitudinal study to examine the characteristics of airborne fungal populations and correlations with other environmental parameters in office environments. We investigated four office buildings in Boston, Massachusetts, during 1 year beginning May 1997, recruiting 21 offices with open workstations. We conducted intensive bioaerosol sampling every 6 weeks resulting in 10 sets of measurement events at each workstation, and recorded relative humidity, temperature, and CO₂ concentrations continuously. We used principal component analysis (PCA) to identify groups of culturable fungal taxa that covaried in air. Four major groupings (PCA factors) were derived where the fungal taxa in the same groupings shared similar ecological requirements. Total airborne fungal concentrations varied significantly by season (highest in summer, lowest in winter) and were positively correlated with relative humidity and negatively related to CO₂ concentrations. The first and second PCA factors had similar correlations with environmental variables compared with total fungi. The results of this study provide essential information on the variability within airborne fungal populations in office environments over time. These data also provide background against which cross-sectional data can be compared to facilitate interpretation. More studies are needed to correlate airborne fungi and occupants' health, controlling for seasonal effects and other important environmental factors. *Key words:* airborne culturable fungi, indoor air quality, longitudinal exposure assessment, office environments, principal component analysis (PCA). *Environ Health Perspect* 110:777–782 (2002). [Online 14 June 2002]

<http://ehpnet1.niehs.nih.gov/docs/2002/110p777-782chao/abstract.html>

There are essentially no fungus-free environments in our daily lives. Fungi prosper in conditions within the human comfort range, and certain fungi can also survive or even flourish at low (–5°C) or high (60°C) temperatures, limited water activities (0.70), low (pH 1) or high (pH 9) pH, and very low oxygen content (1). Fungal spores are abundant in outdoor air, and exposure to fungi and their metabolites occurs commonly in indoor environments such as offices, schools, and homes (2–6).

Sources for indoor airborne fungi can be outdoor air and indoor reservoirs (7–9). Fungal spores in outdoor air are a major source for indoor fungi during the growing seasons (e.g., spring and summer) for naturally ventilated buildings (10). Although outdoor fungi do not penetrate easily into large buildings with complex ventilation systems, the outdoor aerosol still may dominate indoors (11). Accumulated dust is also a potential indoor source of bioaerosols. Microorganisms (e.g., fungi and bacteria) can accumulate and grow on nonliving material in dust. Materials (e.g., wallpaper, carpeting, ventilation duct surfaces) can also become bioaerosol sources if water content can support the growth of microorganisms (7,12).

Fungal exposures have been documented to cause allergic diseases (e.g., allergic rhinitis and asthma, hypersensitivity pneumonitis), toxicoses (e.g., aflatoxicoses,

ergotism), irritation (e.g., mucous membrane or skin irritation), and infections (e.g., histoplasmosis, blastomycosis) (13–17) and have been blamed for nonspecific building-related symptoms (BRSs) (4,18). BRS refers to symptoms that cannot be associated with an identifiable cause but that appear to be building related, including headache, irritation of the eyes, nose, and throat, lethargy, nausea, dizziness, and chest tightness (19–24). The role of fungi in BRS has not yet been fully studied, and the few available findings are contradictory (4,18,25,26). These contradictory findings may be related, in part, to limited sampling protocols that fail to clearly document the structure of fungal populations in buildings.

We conducted a longitudinal exposure assessment study using repeated bioaerosol sampling protocols and related our findings to simultaneously collected environmental measures. This study was part of a larger epidemiologic study designed to evaluate the role of bioaerosol exposure on BRS and working efficiency.

Materials and Methods

Study design. We investigated four office buildings in Boston, Massachusetts, during 1 year beginning May 1997. We recruited 21 offices with open workstations (low partitions) in the four buildings and conducted intensive environmental sampling every 6

weeks at each workstation, producing 10 sampling events for each site. Each 6-week sampling event lasted for 1 week. During the sampling week, we collected air samples for evaluation of fungi in the mornings and in the afternoons on Tuesday and Thursday. We recorded relative humidity (RH), temperature, and carbon dioxide concentrations continuously at each site. Building managers provided descriptive data and monitoring records for heating, ventilating, and air conditioning systems (HVAC) of the sample buildings.

Environmental sample collection and handling. Airborne fungi. We collected air samples near occupants' breathing zone (approximately 1 m above ground), using duplicate single-stage Andersen N-6 samplers (Graseby Andersen Co., Smyrna, GA), operating at 28.3 L/min. We collected sequential 2-minute samples on two types of media, malt extract agar (MEA) and dichloran glycerol-18 agar (DG18), to culture "mesophilic" and "xerophilic" fungi, respectively. We collected a total of 16 culture plates [4 samplings per week × 2 plates for each culture medium (duplicates) × 2 culture media] at each sampling site per sampling event. We incubated fungal culture plates at room temperature for 7–10 days before identifying colonies. We counted every discernible fungal colony on each plate. Two or more colonies on a given impaction point were counted separately, and a notation made so that positive hole counts could be determined. We identified all the fungal colonies to the level possible by low-power microscopy (generally, to genus) and recorded counts by colony type.

Temperature, RH, and carbon dioxide. We continuously recorded temperature, RH, and carbon dioxide (CO₂) levels in all sampling locations, using battery-operated data loggers, HOBO-Temp, and HOBO-RH (Onset Computer Co., Pocasset, MA), to

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We thank M. Muilenberg and E.-C.J. Lee for their assistance in the intensive field and laboratory work.

This project was supported by the U.S. Environmental Protection Agency (R824797) as part of the grant "Bioaerosols, Health and Productivity in Large Office Buildings."

Received 5 September 2001; accepted 30 January 2002.

measure temperature and RH levels, respectively. We measured carbon dioxide concentrations 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). For these three parameters, we recorded hourly average readings and downloaded data once a month.

HVAC systems. The four sampling buildings are located in busy urban areas. Buildings I, II, and III are 14, 4, and 10 stories, respectively, forming an interconnected campus building complex. The seven administrative offices selected from these three buildings are controlled by four different air handling units, all of which are constant air volume systems. We selected 14 sampling sites from building IV, which is a 10-story office building on a different campus. The building is supplied by two air handling units and 380 fan coil units. Temperature sensors are installed in almost every room and are monitored by the computer system in the management office.

At least 10% outside air is nominally supplied to these four buildings. However, specific outdoor air supply amounts were not available for each sampling site, and we used carbon dioxide concentrations as a surrogate for outdoor air supply.

Statistical analysis. We performed statistical analysis using SAS (version 6.12; SAS Institute, Inc., Cary, NC) and S-Plus (version 5.3; MathSoft, Inc., Seattle, WA) statistical packages.

We recruited 21 sampling sites in the beginning of the study. We dropped seven sites before the end of the study because of low compliance of the participants in the epidemiologic study. To examine the effects on the entire data set of the resulting missing values, we examined the bioaerosol distributions of the sites with complete data and those with missing data using multiple regression. We included a categorical “data set” variable (data with complete sampling sites and data with incomplete sampling sites) and sampling date in the regression model to predict airborne fungal concentrations. The results suggested no statistically significant differences ($p > 0.50$) between the distributions of sites with and without complete data sets. Thus, we assume that the missing data are not significant and can be ignored.

We used principal component analysis (PCA) to reduce a myriad of observed fungal categories to fewer factors. PCA is a variable reduction procedure that can identify important subsets (i.e., principal components) of the original set of variables. A principal component can be defined as a linear combination of optimally weighted observed variables,

which explains a relatively high amount of variation in the data set. The first principal component accounts for the largest portion of the variability of the original data set, the second principal component accounts for the second largest, and so on. A principal component consists of variables (i.e., fungal taxa) with > 0.4 factor loading on this component. Factor loadings on each principal component are the correlations between the component and the original variables. Rotation is a method of altering the initial components to achieve more interpretability. We used oblique rotation (Promax method) for our analysis, which allowed correlations between factors and also allowed better separation into ecologically plausible groups (27–30).

The relationships between airborne fungi and environmental factors may not be linear; hence, we employed generalized additive models (GAMs) (31). GAMs allow the outcome to depend nonparametrically on smooth functions of some or all of the predictors, and have been widely used in environmental studies (32,33). We used Loess smoothers in a GAM if the regression functions were nonlinear, and tested the existence of nonlinearity using likelihood ratio tests between a model with a smooth and a linear function of the predictor. We used Akaike’s information criterion (31) to determine the spans for Loess smoothers and degrees of freedom for random effects. We examined serial correlation (autocorrelation) resulting from repeated measurements at each sampling site using partial autocorrelation functions. The results indicated that the within-site autocorrelation was not statistically significant. Therefore, GAMs are appropriate in predicting fungal levels without accounting for

autocorrelation. We applied log transformations to total fungal counts and other right-skewed environmental variables. We present the nonlinear relationships between fungal concentrations and variables (shown as Loess smoothers) in graphs and show regression coefficients for linear correlations.

Results

Positive hole conversion. To collect airborne fungal samples using single-stage Andersen N-6 samplers (Graseby Andersen), the colony count per culture plate is usually adjusted for possible multiple impactions using the positive hole conversion table (34). The positive hole conversion is based on the theory that as the number of particles impacting on a given plate increases, the probability of the next particle going into an “empty hole” decreases. To determine whether the colony counts should be adjusted for possible multiple impacts, we compared total colony counts and positive hole counts with conversion. Linear correlations between total counts and positive hole counts with conversion were more than 0.99 for both DG18 and MEA, and only 1.17% and 0.96% of samples, respectively, had more than 20 impaction points with colonies, thus requiring conversion. The β coefficients for linear regressions were 1.20 and 1.23 for DG18 and MEA, respectively. The results suggest that positive hole conversion is not likely to have a statistically significant effect on this data set. Therefore, we used actual fungal colony counts, and we did not convert them for the following analyses.

Characterizing fungal populations. Media comparison. We used the Wilcoxon signed-rank test to examine whether fungal

Table 1. Comparison of media.

Fungal taxon	Recovery frequency (%) (DG18/MEA)	Highest medium	Median difference ^a (CFU/m ³)
Total ^b	98.9/98.9	MEA	4.42
Nonsporulating	80.2/86.8	MEA	2.21
<i>Penicillium</i>	73.1/67.6	NS	-2.21
<i>Cladosporium</i>	66.5/61.0	NS	0.00
Yeast	58.2/72.0	MEA	2.21
<i>Aspergillus</i>	53.3/45.6	DG18	-1.10
<i>Aureobasidium</i>	23.1/18.7	NS	-2.21
<i>Alternaria</i>	14.8/8.2	DG18	-2.21
<i>Wallemia</i>	9.9/2.2	DG18	-2.21
<i>Paecilomyces</i>	6.0/7.1	NS	2.21
Unknown ^c	4.9/6.6	NS	2.21
<i>Zygomycetes</i> ^d	4.4/6.6	NS	2.21
<i>Botrytis</i>	3.8/1.1	NS	-2.21
<i>Coelomycetes</i>	3.3/3.3	NS	0.00
<i>Ulocladium</i>	2.7/0.5	NS	-2.21
<i>Pithomyces</i>	2.2/0.0	(DG18)	—
<i>Epicoccum</i>	0.5/3.3	MEA	2.21
<i>Sporobolomyces</i>	0.0/2.7	(MEA)	—

NS, no significant difference. Media in parentheses indicate that the corresponding taxon was recovered only on that medium in the entire sample set.

^aMedian of MEA—median of DG18. ^bAll taxa combined. ^cUnknown are colonies that were either overgrown by other colonies or very rarely seen and not readily identified. ^dIncludes *Rhizopus*, *Mucor*, and nonsporulating zygomycetes.

concentrations, in total and by taxon, were different on the two culture media. We calculated separately the average airborne fungal concentrations per sampling per site from MEA and DG18 culture plates and calculated differences between paired MEA and DG18 by subtracting the concentrations of DG18 from those on MEA. We excluded the observations with zero recoveries on both media from the test.

Table 1 summarizes the results and lists the overall recovery frequencies for total fungi and those fungal taxa recovered on >2% of samples on at least one culture medium. Total counts were slightly higher on MEA than on DG18. The median difference for individual taxa was very small (2.2 CFU/m³ maximum), although some differences were sufficiently consistent to reach statistical significance. MEA recovered a slightly greater variety of taxa than did DG18 (29 and 21 taxa on MEA and DG18, respectively), but the recovery frequencies were fairly small (< 3% of total samples) for those fungi recovered on only one medium. Given the absence of an overall systematic advantage for either medium, we combined data from the two media for further analysis.

PCA. We used PCA to identify groups of fungal taxa abundant in air, using average airborne fungal concentrations per sampling week per site for the analysis. We observed 32 taxa in airborne fungal samples, and included in the PCA 12 taxa that were recovered on > 5% of total samples. Variables must be normally distributed to meet underlying assumptions of the PCA procedure. We conducted analyses to select the best transformation to approximate normality. All the transformations were less than ideal. However, fungal groupings derived from fourth-root and log-transformed data shared similar biologic characteristics, and their distributions were least skewed. We used results of fourth-root transformation for further analyses because the transformation process

Table 2. Fungal groupings derived from PCA for airborne fungi.

Airborne fungi ^{i0.25}	Cumulative percent of variance
Factor 1	22.32
<i>Alternaria</i>	
<i>Aspergillus</i>	
<i>Cladosporium</i>	
<i>Penicillium</i>	
Unknown	
Factor 2	33.71
Yeast	
Nonsporulating	
Factor 3	43.93
<i>Aureobasidium</i>	
<i>Coelomycetes</i>	
<i>Zygomycetes</i>	
Factor 4	53.27
<i>Paecilomyces</i>	
<i>Wallemia</i>	

did not require a change in the values of fungal concentrations (compared with having to add a small value to log-transformed data to eliminate transformation of zero values).

Table 2 lists the PCA factors (groupings) and the cumulative percentage variance explained by these factors using fourth-root transformation. Fungal taxa listed under a given factor had high correlations with the factor (factor loading > 0.4). We calculated PCA factor scores for each factor based on linear composites of the optimally weighted original variables, and evaluated both factor scores derived from fourth-root transformation and total fungal concentrations for correlations with other environmental variables.

Summary statistics. Table 3 summarizes the environmental measurements, totaling 179 measurements for each parameter. Airborne fungal concentrations are the means per sampling week per site. For RH, temperature, and carbon dioxide, we used averages during working hours (0900–1700 hr) per sampling week per site. Figure 1 shows box plots of the distributions of total airborne fungal concentrations across sampling dates. Airborne fungal concentrations, RH, and temperature varied significantly by season. Airborne fungal concentrations and

RH were highest in summer and lowest in winter. Temperature, on the contrary, was slightly higher in winter than in summer. We did not find significant seasonal variation in CO₂ concentrations.

Modeling fungal concentrations. We evaluated total airborne fungi (CFU/m³) and the PCA factors for airborne fungi for relationships with other environmental parameters using GAM. We transformed total airborne fungal concentrations using the logarithm base-10 to approximate normality, because it resulted in the best approximation of normality. Also, we found no zero values in total fungal counts, as in individual taxa, so log₁₀ transformation could be used without adding a constant. The PCA factor scores had close-to-normal distributions and were not transformed. We coded sampling dates as 1–10 for the 10 equally spaced sampling events. We assumed that each sampling site had a random deviation from the overall mean effect, so we treated sampling site as a random effect in GAM. Plots show nonparametric relationships in the models, indicating regression lines (solid lines) and approximate 95% pointwise confidence intervals (broken lines). For linear correlations, we present only regression coefficients and *p*-values.

Table 3. Distribution of environmental variables.

Environmental variables	Mean ± SD	Median	Minimum	Maximum
Airborne fungi (total CFU/m ³)	42.05 ± 69.60	21.53	1.10	618.37
Relative humidity (%)	33.12 ± 13.31	32.62	9.13	59.65
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

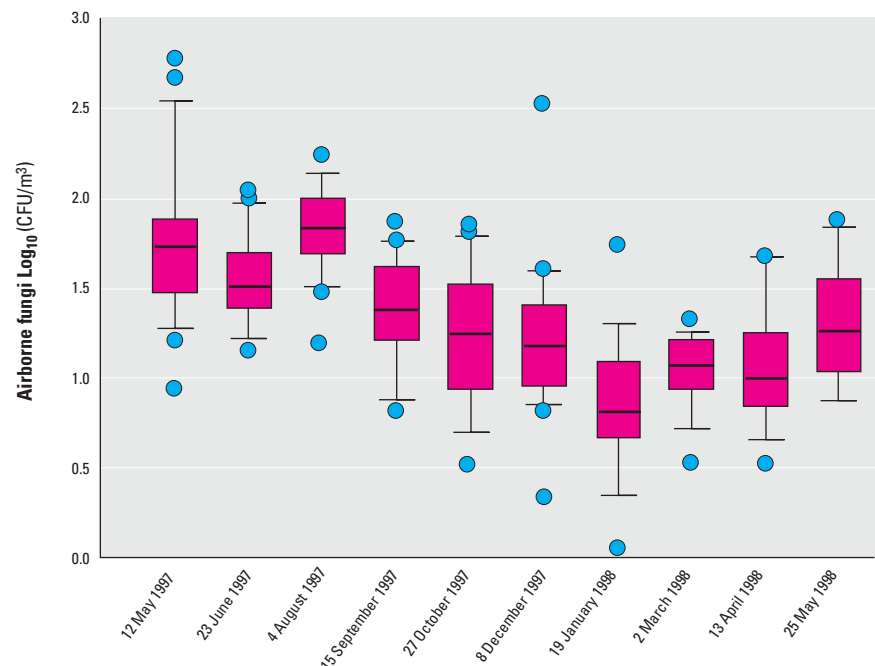


Figure 1. Airborne fungal distribution over time. The box plots show medians; 10th, 25th, 75th, and 90th percentiles; and outliers.

Total airborne fungi. Table 4 shows the model results for total airborne fungi. Coefficients are not available for the variables that had nonlinear relationships with total airborne fungi. Total airborne fungal concentrations had a linear negative correlation with CO₂ levels. Figures 2 and 3 show the nonlinear partial regression plots between total airborne fungi and RH and sampling dates, respectively. Each partial regression plot was controlled for all other covariates in the model. Total airborne fungi were positively correlated with RH < 30% and > 40%, and decreased from first sampling date (12 May 1997) throughout the subsequent summer and winter sampling dates, then began to increase in April.

PCA factors. **First PCA factor for airborne fungi (Factor 1).** Factor 1 consisted of *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, and Unknown. Factor 1 was negatively related to CO₂ levels (Table 4) and had a strong positive correlation with RH > 35% (Figure 4). The factor also had a significant seasonal variation, highest in August and lowest in March (Figure 5).

Second PCA factor for airborne fungi (Factor 2). Factor 2 included yeast and non-sporulating fungi. Table 4 and Figure 6 show the GAM results. Factor 2 was negatively related to CO₂ concentrations and had a positive linear correlation with RH. The

factor also had significant seasonal variation, highest in summer and lowest in winter.

Third and fourth PCA factors for airborne fungi (Factors 3 and 4). Factor 3 included *Aureobasidium*, *Coelomycetes*, and *Zygomycetes*. Factor 4 consisted of *Paecilomyces* and *Wallemia*. Neither factor was significantly associated with any of the environmental parameters we measured.

Discussion

Estimation of fungal exposures is of increasing importance in assessing indoor air quality. However, major questions remain regarding the representativeness of assessment protocols currently in use. In particular, most protocols involve a single sampling event on a single day (2,4,18,25,35). We monitored airborne fungal concentrations over a year in four office buildings using standardized protocols developed by the U.S. Environmental Protection Agency [the Building Assessment, Survey and Evaluation (BASE) Study] (36). The U.S. EPA study used these protocols to cross-sectionally evaluate a series of randomly selected buildings.

Positive hole conversion has been conventionally applied to airborne fungal samples collected using Andersen culture plate impactors (37,38). The conversion accounts for the possibility of underestimation of concentrations resulting from more than one cell impacting

a single point on the agar. However, multiple impactions not only lead to count underestimation but also tend to cause underestimation of slow-growing taxa. Ideally, the sampler should be used in a mode that minimizes the chances of multiple impactions. In our data set this was the case, and conversion did not significantly change the data set.

Often two media with different water activities are used in environmental surveys to better characterize total fungal populations. In our study, we used MEA and DG18 to recover airborne fungi. The water activity and the nutrients of MEA support luxurious growth and sporulation of most fungi. Also, it is one of the diagnostic media for *Aspergillus* species, often allowing species identification without transfers. DG18, on the other hand, has a lower water activity that encourages the growth of xerophilic fungi (39). DG18 also restricts the growth of fast-growing taxa, facilitating the counting of colonies (40). Most culturable fungi can grow on both media, although a species might grow or sporulate better on one medium than on the other. We compared the fungal concentrations, in total and by taxon, recovered from both media (Table 1). We found no systematically higher recoveries on one medium or the other. Although MEA recovered a greater variety of fungal taxa, a few taxa were found only on DG18 in our air

Table 4. Generalized additive model results for total airborne fungi and first two PCA factors for airborne fungi.

Variables	Total airborne fungi			Factor 1			Factor 2		
	Coefficient	SE	p-Value	Coefficient	SE	p-Value	Coefficient	SE	p-Value
Intercept	1.62	0.08	< 0.001	0.32	0.19	0.094	0.18	0.23	0.44
CO ₂	-0.00038	0.0001	< 0.001	-0.00055	0.0003	0.039	-0.0011	0.0003	< 0.001
lo (RH)	(Figure 2)	—	0.004	(Figure 4)	—	0.003	0.018 ^a	—	< 0.001
lo (Sampling date)	(Figure 3)	—	< 0.001	(Figure 5)	—	< 0.001	(Figure 6)	—	0.008
Random (site) ^b	—	—	< 0.001	—	—	< 0.001	—	—	< 0.001

lo (RH), Loess smooth function of relative humidity, with spans of 0.5 and 0.6 for total and Factor 1 for airborne fungi, respectively; lo (Sampling date), Loess smooth function of sampling date, with spans of 0.6, 0.5 and 0.9 for total, Factor 1, and Factor 2 for airborne fungi, respectively.

^aFactor 2 has a linear relationship with RH. ^bSampling site is a random effect, with 20 degrees of freedom for all three fungal measures.

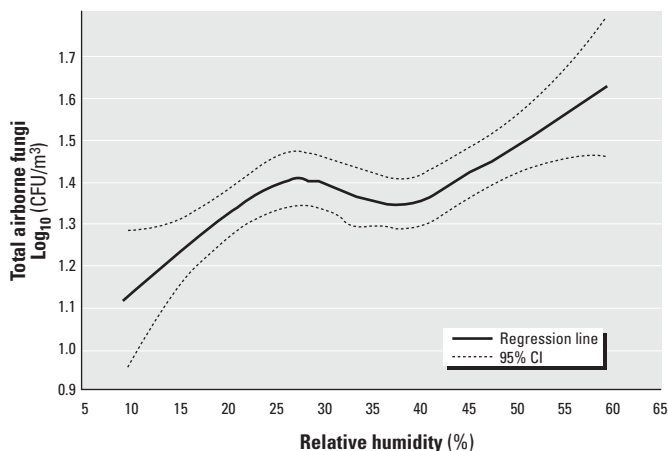


Figure 2. Relationship between RH and total airborne fungi (CFU/m³). CI, confidence interval.

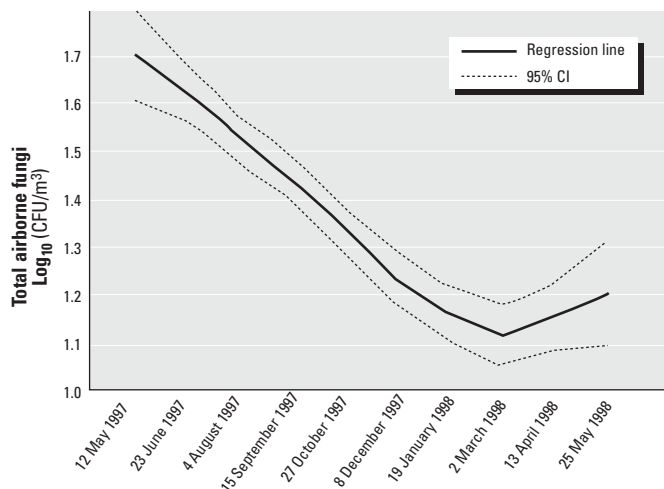


Figure 3. Relationship between sampling dates and total airborne fungi (CFU/m³). CI, confidence interval.

samples. However, because overall, both culture media provided essentially the same information about fungal populations, we combined the two data sets to increase the representativeness of the fungal measures.

Water availability and temperature are critical environmental factors controlling microbial growth indoors (41). Water availability is usually measured as water activity, the amount of water available for fungal growth in a substrate, and the optimum water activity is above 0.90 for most fungi. Different fungal taxa have different optimum temperature ranges for growth, but mostly between 15°C and 30°C. Temperature also influences fungal growth indirectly by interacting with water activity. In this study, we measured RH and temperature at each sampling site to examine the correlations with airborne fungal counts. The level of RH is determined by temperature and absolute water content in air. We observed significant seasonal variation for RH, highest in summer (median = 50%) and lowest in winter

(median = 15%). Clearly, the HVAC systems in the buildings studied did not maintain a constant RH; in winter, the intake of cold and low-absolute-humidity outside air resulted in lower indoor RH (42). Temperature also varied with season, although not as dramatically, and was highest in winter. Although not intuitive, this seasonal temperature pattern is common in climates where both heating and cooling are used.

We measured CO₂ concentration at each sampling location as a surrogate for fresh air supply. CO₂ is inversely related to the amount of outdoor air supply in a sampling site, if the number of occupants and their activity levels remain constant (43). We found no significant temporal variation of CO₂ levels in this study, which suggested the ventilation rates were possibly fixed in the sampling locations throughout the year.

The airborne fungal concentrations in the buildings studied (median = 22 CFU/m³) are considered very low according to commonly used standards/guidelines (44). The maximum

fungal count, 618 CFU/m³, was not indicative of serious indoor contamination (11). Nevertheless, a multi-building study reporting similar airborne fungal levels found that BRSs were positively associated with airborne fungi (18).

Total fungal concentrations were negatively related to CO₂ concentrations (Table 4). Because CO₂ is inversely associated with ventilation, this suggests that outdoor

air might be a source for indoor fungi in those buildings with no strong internal sources. Total airborne fungal concentrations had strong seasonal variation (Figure 1), similar to that of RH. The temporal pattern of total fungal counts was less dramatic in the GAM model after controlling for RH and other variables (Figure 3), likely because the seasonal trend in airborne fungi was related in part to the seasonal variation in RH. Temperature was not significantly associated with total fungal concentrations, perhaps related to the fact that outdoor temperature does not control indoor temperature. Also, temperature ranges in offices were optimum for most fungal growth, and because RH was related to temperature, it is not surprising that temperature did not have an independent correlation with total fungal counts.

The concentration of total fungi is the most frequently used variable for indoor fungal exposure. However, this approach overlooks the potential for environmental correlations and for health effects related to individual fungal species. Although examining the effect of every fungal taxon is ideal, it is impractical in statistical analysis because of infrequent recoveries of many fungal taxa indoors. Low recovery frequencies may result in insufficient power to detect the relationships between fungal concentrations and other environmental variables or health outcomes. Therefore, we conducted PCA to identify groups of fungi sharing similar ecologic characteristics, and used both total fungal counts and PCA factors for exposure assessments in our study.

The first PCA factor for airborne fungi (Factor 1) accounted for 22% of the total data variance and included the most common airborne fungi indoors. Factor 1 had very similar modeling results compared with total airborne fungi, except its seasonal variation was

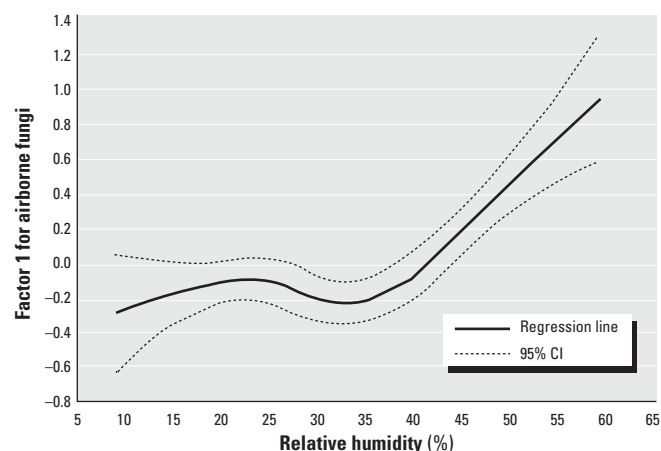


Figure 4. Relationship between RH and first PCA factor for airborne fungi. CI, confidence interval.

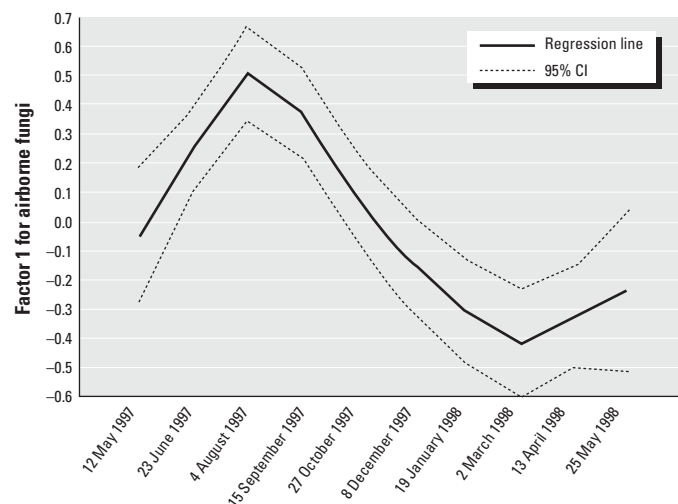


Figure 5. Relationship between sampling dates and first PCA factor for airborne fungi. CI, confidence interval.

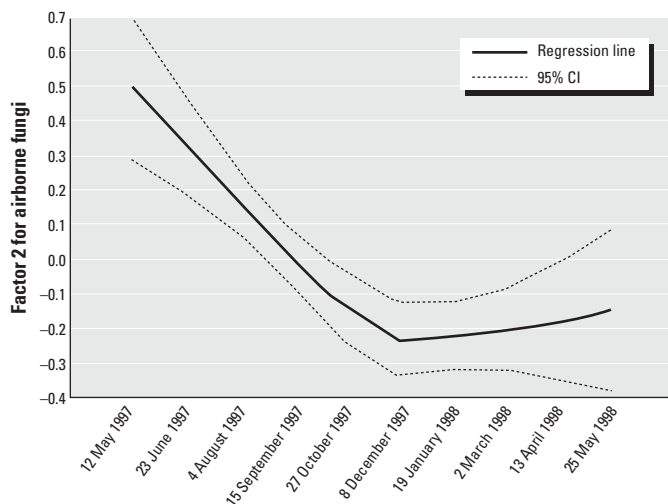


Figure 6. Relationship between sampling dates and second PCA factor for airborne fungi. CI, confidence interval.

more significant. The second PCA factor (Factor 2) accounted for 11% of the total data variance. The factor consisted of yeast and nonsporulating fungi, which were also prevalent components of the indoor aerosol. Factor 2 had similar correlations with environmental parameters, compared with total fungi and factor 1. Factor 3 included *Aureobasidium*, *Coelomyces*, and *Zygomycetes*. These fungal taxa are frequently found in dust. Factor 4 consisted of *Paecilomyces* and *Wallemia*. Neither Factor 3 nor Factor 4 had statistically significant relationships with the environmental variables we measured.

Our study provides important information on airborne fungal concentrations over time, in total and by groups (PCA factors), in relatively clean office environments. Our findings should allow more accurate interpretation of cross-sectional fungal data. Furthermore, the airborne fungal groups derived using PCA allow us to better understand the prevalence and ecology of airborne fungi indoors. Our findings also confirm the correlations between airborne fungi and environmental parameters (i.e., CO₂ and RH) longitudinally. These environmental variables have been associated with perceptions of health of office occupants (22,45). With the models we built, we can further investigate the impact of airborne fungal exposures on office workers' perceptions of health and their working efficiencies, controlling for potential confounders. The longitudinal design of this study facilitated our analyses of these relationships. The standardized sampling protocols used in this study will allow interstudy comparison with the large-scaled cross-sectionally U.S. EPA BASE studies and enhance the generalizability of our study.

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