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Dynamics of Airborne Fungal Populations in a Large Office Building

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Abstract. The increasing concern with bioaerosols in large office buildings prompted this prospective study of airborne fungal concentrations in a newly constructed building on the Gulf coast. We collected volumetric culture plate air samples on 14 occasions over the 18-month period immediately following building occupancy. On each sampling occasion, we collected duplicate samples from three sites on three floors of this six-story building, and an outdoor sample. Fungal concentrations indoors were consistently below those outdoors, and no sample clearly indicated fungal contamination in the building, although visible growth appeared in the ventilation system during the course of the study. We conclude that modern mechanically ventilated buildings prevent the intrusion of most of the outdoor fungal aerosol, and that even relatively extensive air sampling protocols may not sufficiently document the microbial status of buildings.

The role of microorganisms in indoor air quality, especially with respect to large, energy-efficient office buildings has drawn considerable attention [2, 5, 8]. Associations among symptoms and various measures of microbial growth in buildings have been reported [9, 12, 13]. Fungal growth has become a particular concern owing to the rising incidence of asthma [11] and concern over possible exposure to mycotoxins [3]. Many studies of the relationships between symptoms and microbial exposure have used cross-sectional air sampling for culturable microorganisms. However, the relevance of these kinds of sampling data to long-term exposure remains controversial. In addition, little is known about the process of microbial buildup in modern buildings, and few long-term studies document the relationships between indoor and outdoor microbial concentrations in mechanically ventilated buildings. We report here the results with respect to airborne culturable fungi of a longitudinal sampling study in a new, modern multistory building with a computer-controlled climate regulation system.

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Materials and Methods

Sampling plan. Studies were conducted in a newly built six-story office building located on the Texas Gulf Coast in the Houston-Galveston area. Sampling began within a month of building occupancy. From July 1993 to September 1994, samples were collected approximately monthly, and thereafter one set of samples was collected in June of 1995.

On each occasion, air samples were collected sequentially outdoors at the loading dock, indoors on the 1st, 3rd, and 6th floors. Finally, a second outdoor sample from near the loading dock was collected. Indoor samples were collected in the mechanical room and the occupied space of each floor. Mechanical room samples included mixed supply and return air (plenum Andersen samples), unfiltered return air (return Burkard samples), and filtered outdoor air (supply Burkard samples). In the occupied space, Andersen samples were collected at two sites: one in an elevator lobby of each floor and one in an office area.

Sample collection methods. Three Burkard portable culture plate samplers (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, UK) were used to collect samples of supply and return air in the mechanical rooms. Two Andersen two-stage impactors (Graseby Andersen, Atlanta, GA) were used for all other air samples. Each sampler was calibrated with a hot wire anemometer at intervals throughout the period of study, and all were operated at a flow rate of 28 L/min. Five-minute samples were collected at each indoor site, and 3-min samples were collected outdoors. Two samples were collected on each sampling occasion at each site, one on malt extract agar (20 g malt

Table 1. Summary of building characteristics

Floor	AHU[a] Capacity	Outdoor air	# of occupants	Usable square ft.
1	52,300	3700	93	21,755.2
2	49,200	3700	217	27,580.3
3	54,100	3700	139 + 89	27,589.8
4	51,000	3700	276	28,848.7
5	48,000	4000	218	30,527.2
6	53,000	4000	170	29,860.9

[a] AHU, air handling unit.

extract, 20 g dextrose, 15 g agar, 1 g peptone/L distilled water) and one on DG18 (10 g D-glucose, 5 g peptone, 1 g monobasic potassium phosphate, 0.5 g magnesium sulfate ($7H_20$), 15 g agar, 220 g glycerol, 1 ml 0.2% dichloran in ethanol, 100 mg streptomycin sulfate/L distilled water).

Sample analysis methods. Culture plates were transferred to an on-site laboratory and incubated at 25°C for 5 days. Each sporulating colony was identified to genus, and colonies were counted by generic category or as non-sporulating.

Data analysis methods. Counts were converted for multiple impactions with the formula described by Andersen [1A]. For the two-stage Andersen samples, counts for each plate were converted where necessary, then the counts from the two stages were combined. This combined count was used to represent the sampling event. Each combined count was divided by the volume of air sampled to produce data expressed as Colony Forming Units (CFU)/m^3 of air. Graphs were drawn and basic statistical analyses were performed with Microsoft Excel. Count data were transformed to $\log_{10}$ for analysis, and parametric statistics were used to compare data sets.

Results

Description of the building. The six-story study building contains over 166,000 ft^2 of floor space and was occupied by about 1000 workers during the course of this investigation (Table 1). Two air-handling units on each floor provide mechanical ventilation, heating, and cooling. The air-handling units, along with five return air ducts and two supply air ducts, are housed in one mechanical room on each floor. The mechanical room serves as the mixing plenum in which outdoor air is mixed with return air, filtered (25–30% efficiency, 90–95% arrestance pleated cotton filters), and supplied to the occupied space. The climate control system within the building is designed to maintain temperature and relative humidity within the ranges 21–25°C and 37–50% respectively. Air-handling units and supply ducts were lined with fiberglass until August 1994. At that time, all fiberglass was removed from all air-handling units, and from the first 15 meters of the supply ducts on the 3rd floor [1].

Table 2. Summary of Andersen recoveries (CFU/m^3); n = 14 for all sites

Site sampled	Range	Median	Geo. mean	SD
Out, beginning	99–3195	865	516	221
Plenum, 1st floor	21–251	120	115	34
Site 1, 1st floor	67–372	141	140	35
Site 2, 1st floor	14–109	55	49	12
Plenum, 3rd floor	31–202	92	77	19
Site 1, 3rd floor	39–195	83	86	18
Site 2, 3rd floor	10–70	28	21	8
Plenum, 6th floor	46–329	133	122	35
Site 1, 6th floor	21–219	90	81	25
Site 2, 6th floor	17–131	58	55	14
Out, end	106–1113	241	431	123

Fungal concentrations. We have analyzed the Andersen data (outdoors, plenum, and occupied sites) and the Burkard data (supply and return) separately because the samplers differ in efficiency of particle collection. The calculated d_{50} (particle diameter collected at 50% efficiency) for the Andersen sampler is 0.95 µm and for the Burkard sampler is 2.6 µm [7]. DG-18 recovered slightly, but not significantly, more colonies than malt extract agar. For these analyses, we have combined data for the two culture media so that each outdoor data point represents 168 L (6 min) and each indoor point represents 280 L (10 min) of air.

Total fungal concentrations (Andersen data). Log_{10} transformed data were approximately normally distributed, and the overall indoor distribution lay about an order of magnitude below the outdoor distribution. Descriptive statistics for these data are presented in Table 2. Figure 1 displays changes in concentrations over time for outdoor and occupied sites.

Total culturable fungal concentrations at all sites in the occupied space were significantly lower than those outdoors (Student t test, $p < 0.0001$). Concentrations were lower in occupied sites than in associated plenums (paired t-tests by floor, $p < 0.0001$).

Correlations between plenum and outdoor samples ranged from 0.2 to 0.7, and all were highly significant ($p < 0.0001$). Correlations between occupied site and plenum concentrations ranged from 0.2 to 0.8, and four of six relationships were significant ($p < 0.007$). Relationships between outdoor concentrations and those in the occupied space were generally weak but significant and ranged from 0.02 to 0.5 ($p < 0.0001$). Total fungal concentrations in the second outdoor samples were usually higher than in the first, but the two concentrations were not significantly different, and there was a strong linear correlation between the logs of the two concentra-

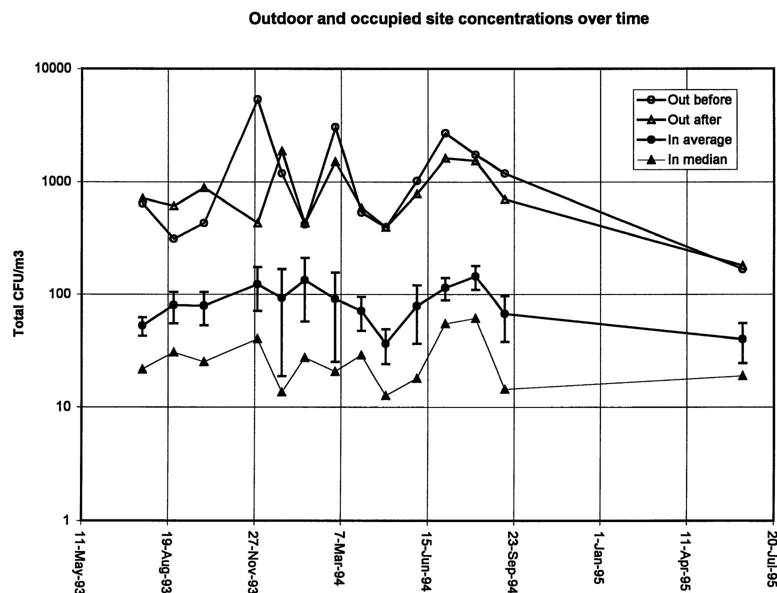


Fig. 1 displays changes in concentrations over time for outdoor and occupied sites.

tions ($R^2 = 0.59$). No distinct seasonal trends were observed, either outdoors or indoors. Levels in occupied sites tended to follow those outdoors but with less dramatic variability from one month to the next.

Fungal taxon concentrations (Andersen data). Changes over time for concentrations of *Cladosporium*, *Penicillium*, and *Aspergillus* are presented in Fig. 2 (a, b, and c). All three taxa tended to be highest outdoors during the fall months. Indoor trends for *Penicillium* and *Aspergillus* generally followed the outdoor pattern. No pattern was apparent for indoor *Cladosporium*. Average outdoor concentrations of these taxa exceeded plenum and occupied site concentrations for all sites. However, some individual occupied space measurements for *Penicillium* (5/84) and for *Aspergillus* (7/84) exceeded those outdoors for the same day. Other taxa were recovered too infrequently to allow this kind of quantitative data analysis.

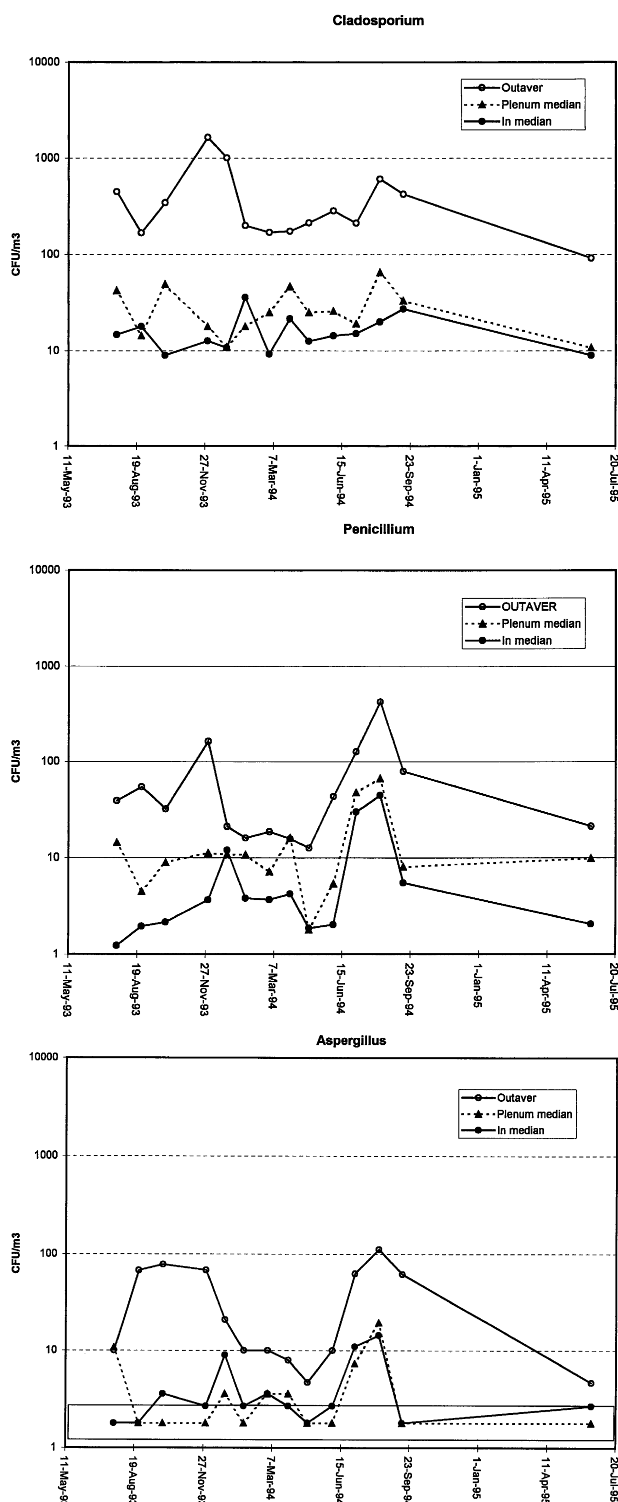
The two most frequently recovered taxa (*Cladosporium* and *Penicillium*) were ranked 1st and 2nd for all three kinds of sites (outdoors, plenums, occupied space) (Table 3). All taxa were more frequently recovered outside than inside except for *Aureobasidium*, which was marginally more frequent indoors. Ranks of taxon frequency were strongly correlated for outdoors, plenum, and indoor sites ($R = 0.89$, Spearman Rank Correlation).

Indoor/outdoor ratios. Indoor/outdoor ratios were calculated by dividing each indoor measurement by the average of the two outdoor measurements for the sampling day. Indoor/outdoor ratios for all fungi and the three most frequently recovered taxa are presented in Table 4. Overall, indoor/outdoor ratios for total concentrations averaged 0.13 for plenum sites and 0.12 for occupied sites. For totals and for *Cladosporium*, indoor/outdoor ratios were strongly dependent on outdoor concentrations ($R^2 > 0.5$, Fig. 3). *Penicillium* ratios were not significantly correlated with *Penicillium* concentrations outdoors, but all ratios approaching 1 occurred at outdoor concentrations less than 100 CFU/m^3.

Fresh air/return air relationships (Burkard data). Fresh air concentrations were nearly always higher than return air concentrations ($p < 0.00001$), and there was only a very weak correlation between the two measures. This relationship held when data from each of the three floors were analyzed separately. Ranks of taxa both by frequency of recovery (i.e., number of positive plates) and total colonies recovered were strongly correlated for fresh and return air samples ($R = 0.89$ and 0.87 respectively).

Discussion

The data we present were collected to document changes in airborne microbial populations over time and represent a relatively comprehensive mycological profile of a large building in a humid climate. We have used range and medians to describe the data so that we could compare sampling sites for which different volumes of air were collected and therefore limits of detection differed. We collected 3-min samples outdoors, and 5-min samples indoors to increase sensitivity at sites where we expected low levels. In retrospect, we should have collected



Figs. 2a,b, and c display changes over time for concentrations of *Cladosporium*, *Penicillium*, and *Aspergillus*.

Table 3. Percentage frequency of recovery, taxa recovered on at least 10% of samples from one site

Taxon	Outside (n = 28)	Plenum (n = 42)	Occupied (n = 84)
Cladosporium	100	95	81
Penicillium	96	83	64
Aspergillus	54	36	38
Curvularia	54	14	39
Alternaria	46	17	30
Rhodotorula	36	7	12
Cryptococcus	29	24	14
Wallemia	21	17	7
Fusarium	18	2	1
Aureobasidium	11	7	14
Acremonium	11	5	2

Table 4. Indoor/outdoor ratios

	Floor	Range	Mean
Total fungi	1	0.02–0.45	0.13
	3	0.01–0.18	0.07
	6	0.02–0.18	0.09
Cladosporium	1	0.04–0.59	0.12
	3	0.00–0.10	0.05
	6	0.01–0.11	0.05
Penicillium	1	0.07–1.04	0.44
	3	0.01–0.56	0.20
	6	0.00–4.60	0.77
Aspergillus	1	0.01–1.80	0.48
	3	0.00–1.30	0.24
	6	0.00–2.47	0.45

duplicate samples outdoors to increase sensitivity proportional to that of the indoor samples. A similar problem is encountered when comparing indoor/outdoor frequency of recovery data. For individual paired samples, recoveries of taxa indoors can be considered only when levels are above the limit of detection for outdoor samples.

The study was not performed in response to complaints, but rather to document the building's air quality over time with respect to its microbiology. As represented by our data, the aeromycology of the building remained relatively constant over the period of the study. These data may also be interpreted from the point of view of the microbial status of the building, and used to assess whether or not an unusual exposure situation existed on the days when we collected samples in this building. Among the interpretation approaches most frequently used are quantitative comparisons with published guidelines or with databases, comparisons between indoor and outdoor recoveries, and the presence of indicator species.

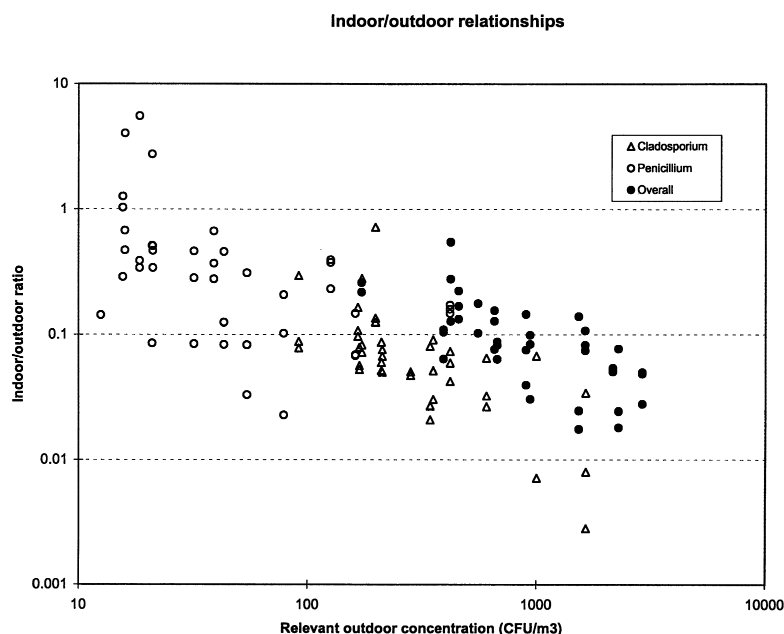


Fig. 3 displays indoor/outdoor ratios for totals and for *Cladosporium* and *Penicillium* ($r^2 > 0.5$).

Comparison with existing guidelines, or baseline data. Concentrations of culturable fungi in this building did not exceed 1000 CFU/m^3, an often-quoted guideline [10], and median and geometric mean levels were always below 200 CFU/m^3. Median values for individual taxa were always below 50 CFU/m^3, and peak levels for individual taxa never exceeded 200 CFU/m^3. For *Cladosporium*, a level of 200 CFU/m^3 is usually considered to represent outdoor intrusion. For *Penicillium* and *Aspergillus*, this level might be considered as indicating indoor growth. Using this (arbitrary) criterion, no subset of the sampling data represented a potentially problematic environment.

Indoor/outdoor relationships. Concentrations of total airborne culturable fungi indoors were consistently below those outdoors, and indoor/outdoor ratios for all sites were <0.5, a commonly accepted cut-point for non-problem buildings. Indoor/outdoor ratios for total fungi and *Cladosporium* were strongly correlated with outdoor concentrations, with all ratios near 0.5 occurring when outdoor concentrations were low. Thus, such ratios must be interpreted with caution, and if they are to be used, criteria for levels of recovery will have to be set.

Average *Penicillium* and *Aspergillus* ratios also were below 0.5. However, indoor concentrations of both of these taxa occasionally exceeded those outdoors. All of these events where ratios exceeded 1 occurred at outdoor concentrations <100 CFU/m^3. At these low levels variability is high, and from our data sets it is not clear that any individual excursion of an indoor level above that outdoors is significant or important.

We also calculated correlation coefficients for the sites, using paired data from each sampling occasion. There were strong correlations among most sites, again indicating that, overall, indoor populations were reflecting those outdoors. However, practically speaking, these correlations are of little value unless large amounts of data are collected from each building under investigation.

Another approach to assessing indoor/outdoor differences is to list taxa from each site by rank and either by eye or statistically comparing the rankings. We have listed frequency of recovery for the predominant taxa in our study, and calculated Spearman Rank order coefficients. The results clearly indicate that the three sets of data (outside, plenum, and occupied space) represent the same populations. However, for this method to be used accurately, the number of samples (i.e., the air volume sampled) must be equal for the sites being compared. If the limit of detection for one is below that of the other, then frequency of recovery cannot be accurately compared. Because all of the dominant taxa in our samples were most frequent outdoors in spite of the overall lower volume of air processed, we can assume that the rank orderings were appropriate. However, if this had not been the case, we would not have been able to judge the accuracy of differences in ranking had they appeared.

We also compared culturable fungi in fresh and return air. Return air was unfiltered air returning from the occupied space, while fresh air had passed through medium-efficiency filters. It appears that the filters were removing only a portion of the outdoor air spora and that the remaining spores were being introduced into the

supply ducts. On the other hand, low levels in the occupied space (return air) indicate that 1) these outdoor spores are lost in transit, and 2) sources within the occupied space are few or of low concentration compared with the outdoor air.

Indicator taxa. An indicator taxon is usually defined as a particular fungus that carries an excess health risk. For example, in a hospital setting the presence of *Aspergillus fumigatus* may be considered to represent an unacceptable exposure situation. No taxa in this category were recovered during our investigations. However, one could consider that the presence of (for example) a sharp peak of *Aspergillus* recoveries at one of our sites could indicate a local source that should be sought and removed. Likewise, the several occasions when *Penicillium* was the dominant organism at one or more sites could prompt similar action. These are qualitative decisions that may guide investigators in specific investigations.

Changes over time. No systematic discernible increase in airborne fungal concentrations occurred over the course of this investigation, although an outbreak of building-related complaints occurred that stimulated an in-depth investigation of potential causes. Because moldy odors were prominent among complaints, the air-handling units and ductwork on the floors with most complaints were inspected. The inspections revealed extensive contamination with *Cladosporium herbarum* and *Penicillium* spp. of the insulation in the air handlers, and some of the ductwork on the third floor. In response to these discoveries, the insulation was removed from all air handlers in the building, and the visibly contaminated insulation was removed from the third floor. The August and September 1994 samples collected for this longitudinal study bracketed these remediation efforts. However, no evidence of any increase in either *Cladosporium* or *Penicillium* recoveries was seen in our air samples, nor were such recoveries made by the team sampling during remediation. Because our sampling was conducted on single days each month, we may have missed releases of culturable spores from these sources. However, the extent of sampling on each day is evidence against this hypothesis. Fungal growth in and near air-handling units may release insufficient living spores for detection, and any symptoms that are attributable to such growth may be related to release of non-living effluents. Reports of moldy odors suggested that, perhaps, fungal volatile organic compounds may have been involved [1, 4]. Documentation that such compounds produce building-related symptoms remains to be provided.

Finally, interpretation of the microbial status of a building from any single sampling occasion that included so few samples is problematic. On each sampling occasion we collected 34 samples indoors and 4 outdoors. Although this is many more than are collected by many investigators, in fact this number is too small to accurately document the potential variability in microbial populations in this building [6]. We are able to draw conclusions from our data because we repeatedly sampled at each site so that we had data from 476 individual indoor samples.

Summary and conclusions. Our data emphasize the fact that modern, mechanically ventilated buildings prevent the intrusion of a large fraction of the outdoor fungal aerosol. It is also clear from our data that even relatively extensive air sampling protocols in large buildings are insufficient to document the microbial status of the buildings, and investigations designed to address complaints or to assure well-functioning clean buildings must include extensive visual inspection.

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