Measurements of Airborne Fungal and Endotoxin Levels in Water-Damaged Buildings

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ABSTRACT

Airborne fungi are often collected during the assessment of water-damaged buildings. Air sampling for endotoxin, a characteristic group of constituents of outer membranes of gram-negative bacteria, is another approach commonly used to characterize microbial conditions in buildings. This study presents results of fungal and endotoxin air samples collected in buildings with microbial complaints. Airborne fungal and endotoxin samples were collected from 11 buildings, including office buildings and residences, between November 1999 and March 2000. One building that did not have water damage history was used as a reference. Two indoor locations and one outdoor location were sampled for each building. The fungal and endotoxin levels outdoors were well correlated with a correlation coefficient of 0.91. All, except one outdoor sample, yielded endotoxin levels less than 1 EU/m^3. An analysis of the association between water damage conditions with the detected total fungal, Penicillium*, and endotoxin levels was performed. The results suggest that a combination of fungal and endotoxin air sampling is a useful tool in evaluating microbiological contamination in most water-damaged buildings selected in this study. For a more comprehensive assessment for microbial contaminations in buildings, air sampling for fungi and endotoxin as well as other sampling strategies are recommended.*

INTRODUCTION

Buildings with a history of water damage are often associated with microbial contamination. Conducting a preliminary walk-through for a problem building is usually the first step in gathering building background information and identifying potential biological contamination sources. Interviews with building operators and tenants for water damage history and indoor environmental complaints and obtaining information on the ventilation system, blueprints, and recent reconstruction work in the buildings are important steps to get further information about building conditions. If potential microbial contamination related to water damage conditions is of concern, sampling activity will be the logical step for identifying the agents in the problem building.

A variety of sampling methods including air, bulk, and surface sampling have been suggested in the literature and practiced in buildings with microbial contaminations (AIHA 1996; ACGIH 1999). Air sampling is commonly conducted to evaluate microbial contamination conditions in water-damaged buildings due to the possibility of respiratory illness from inhalation of microorganisms. Air sampling for culturable fungi can provide sufficient information for fungal species in air using appropriate culture media. Significant levels of gram-negative bacteria are commonly detected in buildings with water-damage conditions. Endotoxin, a heat-stable pyrogen from the outer membranes of gram-negative bacteria, has been associated with respiratory diseases (ACGIH 1999). Airborne endotoxin measurements in residential settings (Rylander et al. 1989; Park et al. 2000) and office buildings (Teeuw et al. 1994; Hines et al. 2000) have been applied in many studies to characterize building conditions. A study showed that airborne countable bacteria are correlated well with airborne endotoxin ($r^2 = 0.64$) (Walters et al. 1994).

This study combines both airborne fungal and endotoxin sampling strategies to evaluate buildings with water-damage conditions.

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MATERIALS AND METHODS

Sampling Plan

A total of eleven buildings are included in this study, sampled between November 1999 and March 2000. These buildings were selected due to microbial complaints. Ten of the eleven buildings had water damage history. One building that did not have water damage history was used as a reference. Two locations inside each building and one outdoor location were selected to gather the most representative samples. Two Andersen air samples and one airborne endotoxin sample were collected at each location inside and outside the buildings. Sampling locations indoors include bedrooms, dining rooms, basements, living rooms, kitchens, office spaces, lobbies, and different levels of buildings from residential settings and office buildings.

Fungal Identification

Airborne fungi were collected onto 2% malt extract agar (MEA) plates using an Andersen single-stage sampler for 5 minutes at a flow rate of 28.3 L/min. Two samples were collected for each location. Fungal colonies were counted and identified after seven days of incubation at 25°C. Fungal identification is based on morphology using reference keys. Results were reported as colony-forming units per cubic meter of air (CFU/m^3).

Determination of Endotoxin Levels

Airborne endotoxin was collected on endotoxin-free polycarbonate filters with a pore size of 0.45 µm. Filter cassettes were custom made for endotoxin use by a contracted manufacturer. Endotoxin sampling was conducted using personal pumps with a flow rate of 2-4 L/min. In general, quantifying endotxin relies on the *Limulus* Amebocyte Lysate (LAL) gelation test method (AIHA 1996). In this study, the kinetic chromogenic LAL method is used to quantify airborne endotoxin levels. Filters were extracted in 5 mL endotoxin-free LAL water for 60 minutes at 20°C in a bath sonicator. The extracts were vortexed for one minute before applying onto microplates. *Limulus* amebocyte lysate and standards were obtained in kit form.[1] Results were reported in endotoxin units per cubic meter of air (EU/m^3). Each sample was analyzed with one duplicate and with spike duplicates. The QA/QC procedure for endotoxin results was performed for each run of the test to meet the method requirements.

Data Analysis

Descriptive analysis was used to describe the relationship between indoor/outdoor fungal and endotoxin levels from water-damaged buildings. Comparison of indoor and outdoor fungal and endotoxin levels within each building was conducted by matching sampling results from total fungal levels, indicator fungal levels, and endotoxin levels detected indoor and outside of each building. It has been a common practice to use outdoor or appropriate background levels as a comparison level for interpretation indoors. In this study, the outdoor sample result from each building is used as a comparison for indoor samples in each building. In each building, we chose the data from sampling points that yielded the highest levels for analysis, which most represent the indoor contamination conditions. Fungal and endotoxin levels in the reference building are not used for comparison with other buildings.

Correlation analysis was used to evaluate the relationship between airborne fungal and endotoxin levels. This analysis tool measures the relationship between two data sets that are scaled to be independent of the unit of measurement. The correlation coefficients ranges from –1 (negative correlated) to +1 (positive correlated).

RESULTS

Building Types and Water Damage Conditions

The building types included in this study were condominium, single-family house, office building, and commercial building. Ten of eleven buildings had water-damage problems. One building with no water-damage history was used as a reference building. Detailed building information, including sampling date, building types, water-damage conditions, and remediation actions taken before sampling are listed in Table 1.

In Table 1, a total of 11 buildings are included. Sampling seasons ranged from late fall to spring (between November and March). Some buildings conducted remediation before sampling and some did not. Most building floors were covered with carpets.

Indoor and Outdoor Airborne Fungal Levels

Indoor fungal levels ranging from 7 to >2,827 (over method quantitation limit) CFU/m^3 and outdoor fungal levels ranging from 28 to >2,827 CFU/m^3 were measured in the 11 buildings. *Aspergillus* species, Basidiomycetes, *Cladosporium* spp., *Penicillium* spp., and *Pithomyces chartarum* were the major fungi detected inside buildings. Basidiomycetes, *Cladosporium*, and *Penicillium* were commonly detected in outdoor samples.

In Table 2, Buildings 2, 6, 9, and 11 show higher fungal levels indoors than outdoors. Elevated indoor fungal levels were observed in Buildings 9 and 11.

Penicillium at elevated levels is indicative of water damage conditions. In this study, *Penicillium* was the dominant fungus detected in indoor sampling collected from Buildings 2, 10, and 11.

Indoor and Outdoor Airborne Endotoxin Levels

Indoor endotoxin levels ranging from 0.16 to 19.97 EU/m^3 and outdoor endotoxin levels ranging from 0.14 to 3.82 EU/m^3 were detected from 11 buildings. Building 4 with no water damage history yielded a mean endotoxin level of 0.3 EU/m^3.

1. Bio Whittaker, Walkersville, Md.

TABLE 1
Building Sampling and Condition Information in This Study

Building ID	Sampling Date	Building Type	Water Damage Condition	Remediation Action Before Sampling
1	November 3, 1999	Condominium	Water-damaged carpet for extended period of time	None
2	November 3, 1999	Condominium	Water-damaged carpet for extended period of time	Damp carpet removed
3	November 22, 1999	Office	Water-damaged carpet	Hot water extraction (9 months prior to test)
4	December 13, 1999	Condominium	No known water damage (control)	N/A
5	January 10, 2000	Single Family	Recently flooded in partially carpeted basement	None
6	February 1, 2000	Single Family	Recently flooded in partially carpeted basement	Poor carpet cleaning
7	February 29, 2000	Bank	Water damage beneath window; moisture through floor from crawlspace	None
8	April 6, 2000	Single Family	Historic water damage on both floors	New carpet recently installed
9	March 29, 2000	Single Family	Water damage in basement	None
10	July 10, 2000	Bank	Crawlspace constant with flow through water stream	None
11	March 2, 2000	Single Family	Flooded basement, first and second floors	New carpet recently installed

TABLE 2
The Highest Fungal and Endotoxin Levels from 11 Buildings, Which Are Used for Evaluating Association with Water-Damaged Conditions

Building ID	Indoor Fungal Levels (CFU/m^3)	Outdoor Fungal Levels (CFU/ m^3)	Indoor Endotoxin Levels (EU/ m^3)	Outdoor Endotoxin Levels (EU/ m^3)
1	184	283	19.97	0.42
2	297	283	0.63	0.42
3	240	1767	0.62	0.64
4	276	516	0.31	0.33
5	219	417	0.7	0.36
6	42	28	1.66	0.18
7	219	290	0.18	0.2
8	735	>2827	3.79	3.82
9	261	99	2.9	0.24
10	872	1590	0.26	0.77
11	>2827	325	0.9	0.14

TABLE 3
Summary Table for Association Between Microbial Measurements and Water-Damaged Conditions in 11 Buildings*

Building ID	Elevated Fungal Levels Indoors Compared to Outdoor Levels	*Penicillium* Dominant in Indoor Samples	Elevated Endotoxin Levels Compared to Outdoor Levels	Possible Reasons for No Association of Water Damage Conditions and Bioaerosol Measurements
1			+	
2		+		
3				Remediation may have removed potential microbial sources
4				No known water damage history
5				*
6			+	
7				*
8				*
9	+		+	
10		+		
11	+	+		

* Note: + denotes a positive association between measurement and water damage conditions.
Note: * will be mentioned in discussion section

In Table 2, Buildings 1, 6, and 9 had indoor endotoxin levels higher than outdoor levels with at least 1 EU/m^3 difference. All other indoor samples yielded endotoxin levels lower than that of outdoor or below 1 EU/m^3 difference.

Correlation Between Airborne Fungal and Endotoxin Levels

The fungal and endotoxin levels outdoors were well correlated with a correlation coefficient of 0.91 ($p<0.05$). All outdoor samples except one yielded endotoxin levels less than 1 EU/m^3.

The correlation between indoor airborne fungal and endotoxin levels was not significant in this study. This may be due to constraints of the indoor environment, including building conditions and materials that distort the relationship between fungal and gram-negative bacteria growth in different stages of water damage.

Association between Water Damage Conditions and Airborne Fungal and Endotoxin Levels

Table 3 summarizes the association between microbial measurement and water damage conditions in 11 buildings of this study. In this table, Buildings 1, 2, 6, 9, 10, and 11 are positively associated with at least one of the bioaerosol measurements, including elevated airborne fungal, endotoxin levels, and one of the indicator fungi—*Penicillium*. Buildings 3, 4, 5, 7, and 8 did not show any indicators of microbial problems based on sampling results in this study.

DISCUSSION

Comparisons of total indoor and outdoor fungal levels have been used as a screening tool for evaluating microbial contamination in indoor environments. Combinations of types and levels of indicator fungi comparisons can avoid ignoring important information. Indoor samples from Building 10 did not show elevated total fungal levels; however, *Penicillium* was the dominant fungus in these samples. The elevated level of *Penicillium* indicates water-damage conditions in the indoor environment of Building 10.

Within each building, outdoor airborne endotoxin levels were used as a comparison for indoor ones. The ACGIH (1999) suggested a relative limit value (RLVs) for endotoxin in the air. In the presence of symptom, an RLV of 10 times of background is proposed. In our study, elevated endotoxin levels were detected in buildings with a difference between indoor and outdoor above 1 EU/m^3. Comparing our criterion with the ACGIH proposed number using outdoor endotoxin level as a background level, it suggested a similar pattern of elevated endotoxin levels from Buildings 1, 6, and 9.

The maximum levels of airborne fungal counts and endotoxin levels were used to evaluate the association with building water-damage conditions. Using maximum concentration from the limited sampling size collected in this study is a better choice than using mean concentration for the study purpose of evaluating sampling strategies.

In Table 3, combination of measurements from total fungal levels, *Penicillium*, and endotoxin levels indicated the microbial problems in six buildings (Buildings 1, 2, 6, 9, 10,

and 11). Based on these findings, six out of ten (60%) water-damaged buildings were positively associated with bioaerosol measurements. In this study, a combination of airborne fungal and endotoxin levels proved to be a useful tool to evaluate microbial issues in most water-damaged buildings.

Buildings 3, 5, 7, and 8 had known water-damage histories but did not show the existence of airborne fungal and endotoxin in samples. Building 3 had been renovated, so major microbial contamination sources may have been eliminated. Sampling results may be affected by sample size, sampling duration, sampling media, bioaerosol viability, and ventilation systems and conditions. Visual inspection of the building envelope should also be included in data interpretation for microbial contamination evaluation. In certain conditions (such as in Buildings 5, 7, and 8), other sampling strategies may be recommended to obtain a more comprehensive assessment of a water-damaged building. Settling dust sampling has been recommended for evaluating historical microbial problems in buildings. However, even a relatively extensive sampling protocol may not sufficiently document microbial status in certain buildings (Burge et al. 2000).

Remediation of water damage conditions is the key to eliminating microbial contamination in buildings. Therefore, after comprehensive evaluation of microbial and moisture sources, a well-designed remediation protocol is the next step to prevent reoccurrence of microbial issues in the indoor environment.

CONCLUSIONS

The results from this study suggest that combinations of fungal and endotoxin air sampling can be useful in evaluating microbiological contamination in most water-damaged buildings, depending on the environmental conditions, sampling protocol, and biological characteristics. Using only one sampling method may not sufficiently document microbial amplification and contamination due to water-damage history in a building. In certain instances, airborne fungal and endotoxin levels failed to reflect microbial contamination related to water-damage conditions. Negative air sample results for fungi or bacteria are often inconclusive (ACGIH 1999), particularly in known water-damaged buildings. For a comprehensive assessment of water-damaged buildings, other microbiological sampling strategies, including settle dust sampling and surface wipe sampling, are necessary in addition to air sampling.

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