

INDOOR BIOAEROSOL BLOOMS ASSOCIATED WITH EXTERIOR CLIMATOLOGICAL MOISTURE EVENTS

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ABSTRACT

Sick Building Syndrome (SBS) is a prevalent problem with a microbial etiology in at least 5% of cases. This project examined background bioaerosol levels in office and non-industrial occupational environments in relation to moisture both inside and exterior to paired sampling locations. Culturable bioaerosols of total mesophilic fungi and bacteria, and selective fungi, were sampled during both wet and dry climatological conditions from typically dry and typically wet locations in 30 buildings. Both categories of bioaerosol were demonstrated to increase (“bloom”) during periods of exterior rainfall. These results indicate that climatological moisture events are capable of producing bioaerosol blooms in regulated building environments in the days immediately following such events. The importance of these findings is discussed relative to SBS investigation methodology.

INTRODUCTION

A recent report by Meyer *et al.* [1] of SBS symptoms associated with serum Ig-E specific to *Penicillium chrysogenum* (synonym *P. notatum*) has provided a significant causal link between a case of SBS and environmental exposure to this ubiquitous, year-round mold [2]. That result comes at a time when others have found a significant correlation between increased *Penicillium* prevalence and SBS in school buildings involved in indoor air quality (IAQ) complaints [3]. The problematic role of gross fungal growth generating office or other non-industrial IAQ issues has been widely reported [4,5,6,7,8,9,10,11]. However, the *P. chrysogenum* finding justifies increased attention to total bioaerosol concentration increases when investigating certain IAQ complaints.

With the identification of a specific Asp f 1 allergen [12] produced by the common indoor fungus *Aspergillus fumigatus*, in addition to other allergens known from similarly widely distributed fungi, the opportunity exists to more conclusively study the importance of these microbial agents or their by-products in SBS episodes. One notable relationship already noted in the case of Asp f 1 has been an increase in mean Asp f 1 leaf extract concentration following heavy rainfall episodes [12]. That finding, considered in the context of the known association between mold-generated IAQ issues and moisture or water intrusion events [6,8,13], supports the possibility of indoor microbial blooms following water intrusion events. Such blooms may in turn produce detrimental constituents in certain SBS episodes of a microbial etiology.

METHODS

Thirty (30) non-problematic buildings were selected for study. Indoor sampling locations were selected to determine the effect environmental factors, most notably moisture, had on total bacteria and fungi growth. Paired samples, from a potentially moist site (wet) and a typically dry location (dry), were collected from all facilities twice, during meteorologically defined “Low” (rain) and “Hi” (no rain) sampling periods (n=120). Hi periods were defined as all days of 0.1 inches or less of precipitation subsequent to at least a consecutive three day (72 hour) period of 0.1 inches or less of precipitation (i.e., dry periods began three days after the most recent rainfall of 0.1 inches or less). Low periods were defined as any 60 hour period commencing 12 hours following at least 0.5 inches of precipitation, inclusive (i.e., 12-72 hours after the beginning of rainfall exceeding 0.5 inches in a 24 hour period). Actual on-site rainfall was verified with instruments atop one of the study buildings, as later confirmed from U.S. National Weather Service data.

Culturable bioaerosol samples were collected with Andersen N6 samplers (Graseby-Anderson, Atlanta, GA). Five minute samples were collected indoors (sampled air volume 0.142 m³). Air was impacted into plastic petri plates containing 27 ml of media. Trypticase Soy Agar (TSA; Becton Dickinson, Cockeysville, MD) was utilized for mesophilic fungi and total bacteria enumeration, and BBL Inhibitory Mold Agar (IMA; Becton Dickinson) was used for selective fungi enumeration. Three minute bioaerosol samples were also collected from outdoor locations each day facilities were sampled.

Plates were grown out in fluorescent light at room temperature (22°C) in a standard microbiological laboratory. Plates were initially read after 48 hours, with final plate counts determined 48-120 hours post-sampling to ensure that all sampled colony forming units were detected prior to plate overgrowth by more rapidly growing species. Plate counts were determined by the positive hole method [14]. Counts were related to volume of air sampled in order to report colony forming units per cubic meter air sampled (CFU/m³).

Environmental factors of room and floor relative humidity (RH), and room and floor temperature were recorded for all areas; dewpoints were calculated from this data for all sample locations using standard formulae [15]. Temperature and relative humidity was assessed with a Testoterm model 6100 sensor (Davis Instruments, Baltimore, MD). Accuracy of the direct reading probe was compared to a calibrated recording hygrometer, with readings within 2% of scale taken as acceptable. Floor temperature was assessed with a Raytek Raynger model ST2 infrared thermometer (Raytek, Santa Cruz, CA).

Bioaerosol data were analyzed as observed and following log transformation. Descriptive point estimates and measures of variability for the bioaerosols were computed by climatological condition and location. Environmental measures of relative humidity and dewpoint were compared across climatological condition and building sampling location dichotomies using two-sample t-tests and ANOVA. Tukey confidence intervals were computed to ensure acceptable overall significance levels when making *a priori* pairwise multiple comparisons.

RESULTS

Results of culturable bioaerosols for total mesophilic fungi or bacteria and selective fungi for all sampling conditions are shown in Table 1. All bioaerosol data were lognormally distributed (W test of Log transformed data, $\alpha=0.05$) for all measurements with the exception of selective fungi samples collected during a Hi (no rain) period from moist locations. In that case results were influenced by one outlier, and a lognormal transformation was accomplished by substitution of the outlying value with the sample set median value. Significant differences ($p=0.030$) were indicated between Hi/Dry (GM=252 CFU/m³) and Low/Wet (GM=494 CFU/m³) conditions for indoor mesophilic fungi and bacteria when means of the logs were tested (two-tailed two sample t-test). Differences for indoor selective fungi between Hi/Dry (GM=106 CFU/m³) and Low/Wet (GM=235 CFU/m³) were significant as well ($p=0.020$).

Table 1. Bioaerosol Sampling Results by Climate Condition and Sampling Location

<u>Mesophilic Fungi and Bacteria</u>						
Climate condition/ sampling location	mean CFU/m ³	median CFU/m ³	SD ^A	GM ^B CFU/m ³	GSD ^C	
Hi/Dry (n=30)	345	272	299.8	252 ^Δ	2.3	
Hi/Wet (n=30)	1330	481	3333.1	530	3.3	
Low/Dry (n=30)	376	262	342.6	261	2.5	
Low/Wet (n=30)	1289	424	2147.5	494 ^Δ	4.5	

Δ Significant difference ($p=0.030$)

<u>Selective Fungi</u>						
Climate condition/ sampling location	mean CFU/m ³	median CFU/m ³	SD ^A	GM ^B CFU/m ³	GSD ^C	
Hi/Dry (n=30)	171	106	173.8	106 [∞]	3.0	
Hi/Wet (n=30)	513	235	644.9	269	3.1	
Low/Dry (n=30)	165	68	301.8	82	3.0	
Low/Wet (n=30)	494	205	659.9	235 [∞]	3.6	

∞ Significant difference ($p=0.020$)

^A Standard Deviation

^B Geometric Mean

^C Geometric Standard Deviation

Outdoor bioaerosol data were collected on all days that indoor bioaerosols were sampled (data not presented). These bioaerosol concentrations were compared (two-tailed two sample t-test) for the two climatological conditions after determining that the data were lognormally distributed. There were no significant differences between the means of logs of either outdoor

mesophilic fungi and bacteria (Hi and Low GM = 1,176 and 941 CFU/m³, respectively; p=0.770), or outdoor selective fungi (Hi and Low GM = 863 and 773 CFU/m³, respectively; p=0.910) by weather condition ($\alpha=0.05$).

Environmental data collected simultaneously with the bioaerosols showed distinct differences between mid-room level relative humidity and mid-room level dewpoint according to climatological condition (Table 2). Differences between Hi/-- and Low/-- categorical data were highly significant (p<0.001) for both relative humidity and dewpoint (two-tailed two sample t-test). Differences in Hi/Dry and Low/Wet RH and dewpoint measurements taken directly at floor-level were likewise significantly different (data not presented). As might have been anticipated from the mixing characteristics of large HVAC systems, there was a strong correlation (0.967) between mid-level and floor-level RH values, as well as between mid-level dewpoint and floor-level dewpoint (0.854) temperatures.

Table 2. Environmental Measures by Climatological Condition

Climate condition/ sampling location	Room % RH ^A	SD ^B	Room Dewpoint ^C	SD ^B
Hi/Dry ^A (n=30)	42.0	6.5	48.1	4.0
Hi/Wet (n=30)	44.2	8.8	49.9	6.4
Low/Dry ^A (n=30)	51.3	5.9	52.8	4.2
Low/Wet (n=30)	51.4	7.4	54.5	5.3

^A Differences between Hi/-- and Low/-- categorical data were highly significant (p<0.001) for both Room % RH and Room Dewpoint (two-tailed two sample t-test).

^A Relative Humidity

^B Standard Deviation

^C Degrees Fahrenheit

A two-way ANOVA was performed on data for climatological conditions by sampling location within the building (table not presented). Results confirmed that climatological category (Hi/Low) was the major cause for the differences in environmental parameters (p values ranged from <0.001 - 0.007), as opposed to sampling location category (Wet/Dry) results. Tukey's pairwise confidence intervals confirmed that climatological category was the determining factor for the differences seen. On average there was a 19% (unit) elevation of mean relative humidity during climatological Low conditions compared with Hi conditions (two sample t-test, $\alpha=0.05$).

DISCUSSION

Results demonstrate that in office building occupancies interior bioaerosol concentrations and relative humidity vary in conjunction with exterior climatological events. It is rational to believe that the increased indoor bioaerosol levels seen, especially if due to one or two toxigenic or otherwise problematical microbial species, might also be elevated in certain facilities demonstrating Sick Building Syndrome. Persons investigating IAQ issues should

include an examination of exterior moisture events immediately prior to, or occurring during, such investigations with microbial implications that cannot otherwise be conclusively explained.

While the indoor bioaerosol blooms demonstrated might conceivably be attributed to the introduction of outside air into the facilities, there were no significant differences between outdoor air concentrations of either mesophilic fungi and bacteria, or selective fungi, by weather condition. Furthermore, because of the low replacement air used in the facilities (typically less than 15%) and the relatively energy efficient windows and other openings, mechanical introduction of outside contamination is not believed to contribute to the concentration increases seen. It is most likely that the bioaerosol increases are due to interior microbial growth precipitated by increases in total interior moisture resulting from exterior moisture intrusion.

Table 2 indicates that exterior rainfall events can result in moisture loading indoors. This finding is challenging to any notion that HVAC engineering setpoints are capable of maintaining interior relative humidity within tightly defined ranges, at least in this region of the country. With indoor temperatures in non-industrial or office buildings typically maintained about 21°C (70 °F), any significant elevation of the indoor dewpoint could bring temperature and dewpoint values close enough to result in localized condensation at surfaces. Those events, in turn, could potentially account for the microbial blooms found in this study.

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