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# Verifying Interpretive Criteria for Bioaerosol Data Using (Bootstrap) Monte Carlo Techniques

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*A number of interpretive descriptors have been proposed for bioaerosol data due to the lack of health-based numerical standards, but very few have been verified as to their ability to describe a suspect indoor environment. Culturable and nonculturable (spore trap) sampling using the bootstrap version of Monte Carlo simulation (BMC) at several sites during 2003–2006 served as a source of indoor and outdoor data to test various criteria with regard to their variability in characterizing an indoor or outdoor environment. The purpose was to gain some insight for the reliability of some of the interpretive criteria in use as well as to demonstrate the utility of BMC methods as a generalized technique for validation of various interpretive criteria for bioaerosols. The ratio of nonphyllplane (NP) fungi (total of Aspergillus and Penicillium) to phylloplane (P) fungi (total of Cladosporium, Alternaria, and Epicoccum), or NP/P, is a descriptor that has been used to identify “dominance” of nonphyllplane fungi (NP/P > 1.0), assumed to be indicative of a problematic indoor environment. However, BMC analysis of spore trap and culturable bioaerosol data using the NP/P ratio identified frequent dominance by nonphyllplane fungi in outdoor air. Similarly, the NP/P descriptor indicated dominance of nonphyllplane fungi in buildings with visible mold growth and/or known water intrusion with a frequency often in the range of 0.5. Fixed numerical criteria for spore trap data of 900 and 1300 spores/m<sup>3</sup> for total spores and 750 Aspergillus/Penicillium spores/m<sup>3</sup> exhibited similar variability, as did ratios of nonphyllplane to total fungi, phylloplane to total fungi, and indoor/outdoor ratios for total fungal spores. Analysis of bioaerosol data by BMC indicates that numerical levels or descriptors based on dominance of certain fungi are unreliable as criteria for characterizing a given environment. The utility of BMC analysis lies in its generalized application to test mathematically the validity of any given descriptor or criterion for bioaerosols, which can be an important tool in quantifying the uncertainty in interpreting bioaerosol data.*

**Keywords** bioaerosol, bootstrap, criteria, Monte Carlo

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## INTRODUCTION

The ability to associate health effects to building occupants with bioaerosol (fungi/mold) data generated through standardized sampling and analytical protocols is fundamental in the industrial hygiene and environmental/public health communities. However, until a standardized sampling and analytical protocol for bioaerosols is established and accepted, there can be no coherent epidemiologic data base on which to establish a health-based numerical airborne criterion for “acceptable” or “unacceptable” conditions for airborne fungi. In the interim, the basic guideline is that a given indoor environment should exhibit air quality that parallels or is not significantly different from the general outdoor environment. While there appears to be widespread agreement on the basic model, guidelines to identify differences between the indoor and outdoor air vary widely. A number of interpretive indices for bioaerosol data (to include various proportions, ratios, numerical values, and rank order analyses) have been proposed or have appeared in published studies. Other than use of (Spearman’s) rank correlation, none have been verified with regard to their ability to describe a suspect indoor environment or the variability associated with their use.<sup>(1–8)</sup>

Any given set of culturable or nonculturable/total spore count data (light microscopy) typically reports airborne concentrations for a variety of fungal species/genera, but because no one sampling methodology detects or is able to discriminate all fungal types that may be present at a given time, the types of fungi identified by a particular sampling and laboratory analysis define the criterion used to evaluate a given indoor environment. Some investigations may consider airborne concentrations of total fungi as the definitive criterion, while others may consider only certain fungal types of those detected, either as “markers” or as potentially important with regard to health effects.<sup>(1–3,5–7,9–21)</sup>

Consequently, a fundamental contributor to the wide variability in guidelines for bioaerosol data interpretation stems from differences in the nature of the data generated by a

particular sampling methodology, as well as how data may be truncated and/or processed to allow use of a particular descriptor.<sup>(5,16,22,23)</sup> As an example, an investigation comparing a suspect indoor environment with the outdoor air using differences in the ratio of phylloplane to nonphylloplane fungi (as discussed herein) must first define (limit) the types of fungi to be considered phylloplane and nonphylloplane fungi. Data for the designated species must then be combined into the respective grouping and each resulting group treated as a single contaminant. Not only does the species makeup of each group potentially alter the data (phylloplane and nonphylloplane species may vary among studies), but once established, comparison of nonphylloplane and phylloplane fungi across zones may in fact be comparing very different organisms identified within the same group.<sup>(3,5,6,22)</sup>

Even if the types of fungi determined to be relevant were universally agreed on, there is no consistent procedure in which data from a suspect indoor environment and an (outdoor air) control zone is compared. For example, each fungal type/species can be considered independently, with comparison of the frequency of each type (however defined) across indoor and outdoor zones irrespective of other microbial types that may be detected.<sup>(21,22)</sup> This is fundamentally different from the more common practice of comparing relative differences of fungal types detected within the control zone to relative differences of fungal types detected in a suspect indoor environment. The latter approach by rank order analysis is usually performed by simple inspection of the data but can also be formalized through utilization of Spearman's rank order correlation.<sup>(5,8,24,25)</sup> The concept of comparing rank ordered data is also reflected in the common laboratory practice of reporting airborne concentrations as well as relative percentage of each detected fungal type.<sup>(24)</sup> Another general approach is to compare actual indoor and outdoor numerical concentrations of the same fungal type or group. For example, mean total spore and/or the combined *Aspergillus/Penicillium* taxa (*Asp/Pen*) spores have been reported in an indoor/outdoor ratio. Alternatively, an agreement ratio calculated as the number of spore types found in both the outdoor air and test zone relative to the sum of all spore types found in both zones can also be reported.<sup>(5,6,25)</sup>

Fixed numerical airborne levels reflective of potentially problematic indoor environments have been suggested or implied for both spore trap/light microscopy and culturable spore data. In this procedure, spore trap data from buildings exhibiting characteristics such as visible mold growth, elevated moisture, and other key indicators have been used to derive suggested numerical levels for total spores, *Asp/Pen* spores, and basidiospores.<sup>(9,13)</sup>

Theoretically, a given criterion for bioaerosols, whether it be a numerical level, ratio, rank ordering, or similar relative descriptor can be validated against data collected under identical sampling and analytical protocols from the outdoor air.<sup>(8)</sup> A given descriptor or criterion ostensibly used to evaluate bioaerosol data in an indoor environment as "unacceptable" should not identify an outdoor air data set as unacceptable with

a frequency greater than, for example, 0.05–0.10, depending on the degree of uncertainty acceptable in the particular circumstance. That is, within the context of the "relative standard" model of comparing indoor and outdoor air, an "error rate" can be assigned to a given criterion based on how it performs against an outdoor air (or appropriate indoor control zone) data set.

Likewise (but less analytically), data sets from buildings assumed to be "contaminated" or "clean" based on water history or visible mold growth could also generate the frequency a particular criterion accurately reflects conditions. That is, a criterion should identify a problematic or contaminated environment with a sufficiently high frequency, such as 0.90. The frequency a given criterion does or does not adequately characterize a given zone is a random occurrence described as a probability value and is analogous to the Type I error rate (i.e., significance) traditionally used to describe deviation from the "accepted" or "true" condition.

Determination of an error rate for a given criterion is specific to each sampling and analytical technique due to the lack of standardization, and generating error rates from actual field data becomes impractical. However, the ability to estimate the probability of a given event through simulation of random variables, generally referred to as the Monte Carlo method, provides a solution around the problem. Any process influenced by random variables (such as the appearance of fungal species in the air or settled dust) can be modeled through the Monte Carlo method. Monte Carlo techniques are most often applied in those circumstances in which parametric statistics cannot accurately estimate probability and significance due to the distribution of the subject data.

Rather than assume a sampling distribution to enable an estimate of probability as required by standard statistical techniques, the Monte Carlo method relies on the actual unaltered data in the sample as the best estimate of the population. In the particular "bootstrap" version of the Monte Carlo method (BMC) discussed herein, the sampling distribution is generated empirically by repeated random sampling with replacement through the random number generating capability of the personal computer. Each "created" subset randomly drawn from the original (field generated) data pool varies slightly, and with enough simulated data, the frequency distribution from the repeated resampling reflects the overall variability in the population the original sample is intended to describe.<sup>(26,27)</sup>

Monte Carlo methods traditionally have been used to simulate the variations about a statistical parameter such as the mean. However, an extension of the ability of the Monte Carlo method to generate sampling distributions provides a mechanism to approximate the probability density of any random variable, such as a particular descriptor used for bioaerosol data.<sup>(28,29)</sup> In this case, the distribution of a given descriptor can be determined against base data that is either "acceptable" as general outdoor air (or an indoor environment with no visible mold or water history) or assumed to be "unacceptable" based on building conditions (i.e., visible

fungus growth). In this way, an estimate of the probability a given descriptor accurately describes an environment can be derived from the frequency distribution produced from the simulated data.

Culturable and nonculturable (spore trap) sampling at several sites served as a source of indoor and outdoor data to test various criteria using the BMC technique. The purpose was to gain some insight for the reliability of some of the interpretive criteria in use for bioaerosols and to demonstrate the utility of BMC simulation as a generalized analytical technique.

## METHODS

### Sampling and Analysis, General

Sample sets of outdoor and indoor air for culturable fungal spores and total fungal spores (spore trap/light microscopy) were collected as part of building evaluation at seven separate sites along the east coast of the United States from 2003–2006. The buildings were considered representative of what is encountered by practitioners evaluating fungal infestation as a result of suspect building envelope problems, water events (i.e., flooding), and/or inadequate cleanup following mold remediation (i.e., residual dust after removal of visible mold growth). At each site, 14–20 samples were collected intermittently in both the indoor and outdoor zones within the same 5- to 7-hour sampling period. All culturable and spore trap sample analysis was provided by EMLab P&K, Cherry Hill, NJ.

### Culturable Spore Sampling

Of the seven buildings involved in the study, culturable samples were collected at three sites, identified as Buildings PW, G, and E. All culturable samples were collected on malt extract agar (MEA) with an AeroTech/Anderson N-6 impactor (AeroTech Laboratories, Phoenix, AZ) beginning at approximately 8:30–9:00 a.m. and terminating between 2:00 and 4:30 p.m. Sampling period for all samples was 4 min at a calibrated flow rate of 283 L/min. Interim period between samples varied between 15–45 min. Samples were incubated at 25°C, and after development, were classified morphologically (genus/species) and counted under the light microscope.

Building PW was a newly constructed (1998–2000) six-story condominium in a central New Jersey suburb within the greater New York metropolitan area. Various roofing, mechanical, and foundation/grading characteristics resulted in several areas of localized water intrusion and visible mold growth. Sampling was conducted in the outdoor air (17 samples) and in the lower level (Zone T, 16 samples) of the building as part of a general building assessment in August 2003.

Building G was a modern, structural steel/curtain wall, multistory office building in a northeastern city. Significant flooding from the fifth floor to ground level occurred due to a domestic water valve failure in August 2005. Sampling in affected indoor areas was conducted in separate vertical zones of the building following dry down and removal of visibly moldy gypsum board as necessary. The Ground Floor (Zone

1) had minimal damage and was thoroughly dried and HEPA vacuumed prior to sampling (18 samples), while the two upper sections (Zones 4–5, and 2–3; 19 samples and 20 samples, respectively) exhibited residual dust after removal of affected gypsum board. Comparative outdoor air sample sets of 17, 20, and 22 samples (respectively) were collected for each of the indoor test areas.

Building E was a six-story condominium in an eastern seaboard resort, constructed in various renovation phases onto an older wood frame hotel. The building had a history of water intrusion and visible fungal growth within wall cavities under the three-component styrofoam, mesh, and thin coat stucco (exterior insulation and finish system, EIFS) applied onto the lower floors of the building. Sampling was conducted in October 2006, in two separate vertical zones (Zone G, and Zones 4–5; 15 and 14 samples, respectively) as well as the outdoor air (16 samples) to assess impact on the air quality of the building.

### Spore Trap Sampling

Spore trap samples were collected at four sites, designated Buildings L, SM, RM, and ENT. Sampling occurred during the 8:30 a.m. to 4:30 p.m. time period, using Air-O-Cell (Zefon International, Ocala, Fla.) cassettes at 15 L/min for 4 min. Interim periods between sampling varied from 15–45 min. Samples were analyzed by scanning the collecting surface of the sampler and providing a quantification of each presumptive spore type using direct microscopy.

Building L was a five-story hotel in suburban Washington, D.C., that had developed visible mold growth in several gypsum board walls as a result of condensation behind vinyl wall coverings. Uncovered in August 2003, mold remediation (removal of porous building materials with visible fungal growth, and a fine particulate cleaning of all surfaces) under standard isolation, containment, and cleanup protocols was to be undertaken.<sup>(30,31)</sup> However, removal of affected gypsum board and cleanup of dust from surfaces were insufficient on two attempts to complete the work. Sampling was conducted as part of the contractor release on two separate days in the remediation zone (16 samples each evaluation) and outdoor air (19 and 16 samples, respectively).

Building SM was a newly constructed (2004) church, community center, and two-story classroom complex located in a semirural setting in northern New Jersey. Indoor and outdoor sampling (15 samples each) was conducted as a final verification step following mold remediation in some of the lower level classrooms in August 2005. Final inspection indicated that all gypsum board with visible fungal growth had been removed, and all surfaces had been thoroughly cleaned to a dust-free condition.

Building RM was a multistory hotel in the midtown area of New York City in which visible mold growth on some gypsum board wall surfaces on two vacant floors was discovered as part of a general renovation in April 2006. Conditions observed indicated fungal growth was due to inherent conditions in the structure, and additional growth was suspected to be present in other areas of the building. Sampling was conducted in

the affected zones on two occasions after mold remediation (removal of gypsum board with visible mold growth and thorough surface cleaning). Each sampling consisted of 15 samples in both the indoor test zones and outdoor air.

Building ENT was a two-level office building in suburban New York state. A portion of the second floor had sustained a small amount of visible mold in some partitions, which was removed under standard mold remediation procedures. Sampling conducted in March 2006 in the remediation zone (Zone 2, 15 samples) and outdoor air (18 samples) was conducted as part of contractor release.

For purposes of data evaluation, outdoor air by definition was classified as “clean” or acceptable. Indoor air test zones considered “contaminated” were so classified based on the presence of greater than 1 ft<sup>2</sup> of visible mold and/or incomplete cleanup (or inadequate isolation) associated with mold remediation. This is consistent with other investigators who have evaluated bioaerosol data from buildings classified as problematic based on visual evidence of water intrusion and/or visible mold.<sup>(3,6,9,13)</sup> Three indoor zones (Building G, Zone 1; Building SM; and Building ENT, Zone 2) were considered acceptable (equivalent to outdoor air) in that they presented no visible mold growth or accumulated dust on surfaces via visual inspection following mold remediation. Table I summarizes the classification of acceptable (Clean) and problematic (Contaminated) indoor test zones from the buildings evaluated.

### Evaluating Criteria — Monte Carlo Simulation (Bootstrap)

The ratio of nonphyllplane (NP) fungi (airborne concentrations of all detected species of *Aspergillus* and *Penicillium*) to phylloplane (P) fungi (airborne concentrations of all detected species of *Cladosporium*, *Alternaria*, and *Epicoccum*) or NP/P, is one descriptor for bioaerosols that has some logical foundation. Given the strong association of *Aspergillus* and *Penicillium* with the decay processes in the general ecosystem, “dominance” of nonphyllplane fungi in an indoor environment (NP/P > 1.0) has been assumed

to be indicative of amplification of fungi and an unusual exposure/unacceptable condition.<sup>(3)</sup> Conversely, the outdoor air (or suitable indoor reference environment) should exhibit dominance by phylloplane fungi (NP/P < 1.0) if the NP/P descriptor is useful.

For analysis of the data by BMC simulation, each data point entered into the data pool was represented by a fungal type and an airborne concentration observed at some time. Data for a given fungal type that was nondetected were assigned a 0 value. The high frequency of nondetect values precludes the necessity of substitution of an arbitrary value based on a limit of detection, as any potential negative bias affects all data equally.<sup>(9,22)</sup>

The BMC analysis randomly selected a data point from the base field data and calculated the associated NP/P ratio. The process was repeated 10,000 times, and the frequency distribution of the various values of NP/P produced an estimate of probability the descriptor is useful. The number of randomly generated samples to calculate NP/P was also incrementally varied, in which case the ratio of the sum of the simulated samples was used in calculating the NP/P ratio. Two thousand simulations for each sample number from N = 2 to 15 were generated to determine the influence of increased sample size on the NP/P descriptor.

A fixed numerical level for mean total spore concentrations of spore trap samples greater than 900 spores per cubic centimeter (s/cm<sup>3</sup>), and greater than 750 s/cm<sup>3</sup> for the *Aspergillus/Penicillium* group, have been suggested as supplementary criteria (with visual inspection) to identify a problematic indoor environment.<sup>(9)</sup> The described BMC simulation was used to evaluate the total spore and *Aspergillus/Penicillium* criteria against simulated data from an acceptable zone.

## RESULTS

The distribution of 10,000 NP/P ratios for single samples selected by the BMC method from culturable spore data at each site is shown in Table II. The simulated ratios were generated from a total of 110 field samples (16–20 per site) from air assumed to be acceptable (i.e., outdoor air and Building G, Zone 1). The frequency that nonphyllplane fungi were dominant (NP/P > 1.0) in an acceptable environment was highly variable as shown by the frequency distributions in the highlighted columns of Table II.

The estimate of the total probability that the NP/P descriptor incorrectly identified nonphyllplane dominance (i.e., implying a problematic condition in an acceptable outdoor environment) was 0.377 or greater in five out of the six sample sets, as shown in the far right column of Table II. The same high variability was exhibited in the NP/P ratio if used for evaluation of multiple samples as shown in Table III. Two thousand BMC simulated sample sets from the same base outdoor data set, varying incrementally from N = 2 to 15, demonstrates that increasing sample size does not necessarily substantially improve the performance of NP/P. For example, two of three

**TABLE I. Classification of Indoor Sampling Zones**

Clean		Contaminated	
Culturable	Spore Trap	Culturable	Spore Trap
Building G Zone 1	Building SM	Building PW Zone T	Building L Zone 3 (first day)
	Building ENT Zone 2	Building G Zones 2–3	Building L Zone 3(second day)
		Building G Zones 4–5	Building ENT Zone 2 C
		Building E Zone G	Building RM Zones 9–11
		Building E Zones 4–5	Building RM Zones 6–8

**TABLE II. NP/P Distribution – Single-Sample Comparison (Culturable) “Clean” Air 10,000 Simulations**

Site	No. Field Samples	Freq	NP/P Ratio					Frequency NP Dominance (NP/P > 1.0)
			0–0.5	>0.5–1.0	>1.0–1.5	>1.5–2.0	>2.0	
PW (outdoor air)	17	Freq	0.800	0.139	0.005	0.021	0.040	0.066
G (outdoor air) <sup>A</sup>	17		0.481	0.139	0.028	0.026	0.323	0.377
G (outdoor air) <sup>A</sup>	20		0.164	0.061	0.025	0.012	0.738	0.775
G(outdoor air) <sup>A</sup>	22		0.228	0.077	0.058	0.045	0.594	0.697
E(outdoor air)	16		0.384	0.151	0.072	0.090	0.304	0.466
G (indoor zone 1) <sup>B</sup>	18		0.158	0.204	0.017	0.045	0.576	0.638

<sup>A</sup>Outdoor air sampling at Building G collected on 3 separate days.

<sup>B</sup>Indoor air from Building G, Zone 1, assumed to be “clean” based on visual inspection.

sets of outdoor air at Building G still exhibited nonphyllloplane dominance at a frequency of 0.365 or greater for sample sizes up to 15, while outdoor air at Building E exhibited dominance of nonphyllloplane fungi at a frequency of .109 or greater at all sample sizes less than 12. The NP/P ratio from the indoor air considered acceptable (Building G, Zone 1) actually exhibited increasing frequency of nonphyllloplane dominance (0.806 to 0.997) with increased sample size.

For the spore trap data, the variability of the NP/P ratio from outdoor air and indoor environments assumed to be acceptable was similar to that demonstrated in the culturable data. In this case, a total of 128 field samples (ranging from 15–19 per site)

were used to generate 10,000 single sample simulations, and 2000 simulations each for sample sets varying incrementally from N = 2 to 15. As shown in Table IV, the frequency of NP/P > 1.0 was 0.46 or greater in five out of the eight sample sets, with the highest frequency (0.6879) approximating the frequency exhibited in the culturable data. Similarly, increasing the sample size did not necessarily improve NP/P, with three of the outdoor data sets (Buildings RM and ENT) demonstrating marked increased frequency of nonphyllloplane dominance with greater sample sizes (Table V).

Assuming the logic that air from contaminated, indoor environments should exhibit dominance by nonphyllloplane fungi, NP/P should exceed 1.0 if NP/P is a useful descriptor. Eighty-four culturable spore samples (ranging from 16 to 20 at each site) from suspect indoor environments were used to generate 10,000 simulated single samples, and 2000 simulations each for sample sets varying incrementally from N = 2 to 15 as described previously.

As shown in Table VI, only one of five sets of data was identified as unacceptable (NP/P>1.0) at a frequency of at least 0.90. Two of the sites (Building G, Zones 4–5; and Building PW, Zone T) were evaluated as unacceptable at a frequency of approximately 0.50, while the two zones of Building E exhibited nonphyllloplane dominance less than a rate of 0.80. The performance of NP/P with increasing sample size is shown in Table VII. NP/P>1.0 for the data from Building G (both zones) and Building E, Zone G demonstrated a frequency of approximately 0.90 with minimal samples. However, the NP/P ratios from Building PW, Zone T and Building E, Zones 4–5 were essentially unaffected by increasing the sample size, and remained within the range of approximately 0.33 to 0.70.

BMC simulations for the spore trap data in outdoor air and (assumed) clean indoor air at the sites indicated in Tables IV and V were generated, using concentrations of greater than 900 s/cm<sup>3</sup> total spores and greater than 750 s/cm<sup>3</sup> of *Aspergillus/Penicillium* as the criteria for unacceptable air quality. Therefore, levels less than the fixed numerical criteria would be expected to occur in outdoor air at a relatively high frequency, if the criteria are useful. Ten thousand single sample

**TABLE III. NP/P Distribution – Multiple-Sample Comparison (Culturable) “Clean” Air 2000 Simulations**

Simulated Sample Size	Frequency NP/P > 1.0					
	Site					
	PW	G <sup>A</sup>	G <sup>A</sup>	G <sup>A</sup>	E	G <sup>B</sup>
2	.023	.257	.722	.639	.385	.806
3	.008	.168	.642	.551	.284	.875
4	.002	.142	.629	.483	.245	.902
5	.00	.099	.649	.464	.220	.936
6	.01	.071	.654	.438	.189	.955
7	.00	.061	.656	.422	.175	.973
8	.00	.042	.658	.392	.135	.977
9	.00	.033	.666	.391	.124	.984
10	.00	.024	.655	.402	.115	.989
11	.00	.022	.670	.391	.109	.991
12	.00	.021	.659	.365	.088	.996
13	.00	.014	.676	.370	.073	.997
14	.00	.008	.695	.369	.080	.997
15	.00	.012	.689	.370	.060	.997

<sup>A</sup>Outdoor air sampling at Building G collected on 3 separate days.

<sup>B</sup>Indoor air from Building G, Zone 1, assumed to be “clean” based on visual inspection.

**TABLE IV. NP/P Distribution – Single-Sample Comparison (Light Microscopy) “Clean” Air 10,000 Simulations**

Site	No. Field Samples		NP/P Ratio					Frequency NP Dominance (NP/P > 1.0)
			0–0.5	>0.5–1.0	>1.0–1.5	>1.5–2.0	>2.0	
L (outdoor air) <sup>A</sup>	19	Freq	5694	2044	.0915	.0454	.0893	.2262
L(outdoor air) <sup>A</sup>	16		4901	3693	.1026	.0315	.0065	.1406
SM (outdoor air)	15		8606	1095	.0258	.0038	.0003	.0299
RM (outdoor air) <sup>A</sup>	15		1549	3849	0	.0270	.4332	.4602
RM (outdoor air) <sup>A</sup>	15		2106	1677	.0491	.1219	.4507	.6217
ENT(outdoor air)	18		1694	2642	.0078	.0208	.5378	.5664
SM(indoor air)	15		1160	1961	.0489	.0546	.5844	.6879
ENT (indoor zone 2) <sup>B</sup>	15		.0963	2278	.0039	0	.6720	.6759

<sup>A</sup>Each outdoor air sampling at Buildings L and RM were conducted on separate days.

<sup>B</sup>Indoor air from Buildings SM and ENT, Zone 2, assumed to be “clean” based on visual inspection.

**TABLE V. NP/P Distribution – Multiple-Sample Comparison (Light Microscopy) “Clean” Air 2000 Simulations**

Simulated Sample Size	Frequency NP/P > 1.0							
	Site							
	L <sup>A</sup>	L <sup>A</sup>	SM	RM <sup>A</sup>	RM <sup>A</sup>	ENT	SM <sup>B</sup> (indoor)	ENT <sup>B</sup> (indoor)
2	.189	.146	.003	.606	.634	.688	.819	.791
3	.144	.057	0	.672	.619	.757	.888	.855
4	.112	.033	0	.736	.592	.790	.929	.857
5	.094	.007	0	.761	.620	.813	.965	.871
6	.074	.005	0	.792	.626	.814	.969	.887
7	.058	.006	0	.816	.640	.854	.982	.918
8	.032	0	0	.844	.663	.858	.988	.916
9	.038	.001	0	.864	.657	.855	.993	.931
10	.024	.001	0	.868	.631	.887	.996	.943
11	.021	0	0	.875	.671	.894	.996	.943
12	.018	0	0	.899	.671	.904	.998	.952
13	.019	0	0	.908	.662	.900	.999	.960
14	.013	0	0	.923	.701	.909	.999	.973
15	.010	0	0	.921	.699	.922	.999	.971

<sup>A</sup>Outdoor air sampling at Buildings L and RM collected on separate days.

<sup>B</sup>Indoor air from Buildings SM and ENT assumed to be “clean” based on visual inspection.

**TABLE VI. NP/P Distribution – Single-Sample Comparison (Culturable) “Contaminated” Air 10,000 Simulations**

Site	No. Field Samples		NP/P Ratio					Frequency NP Dominance (NP/P > 1.0)
			0–0.5	>0.5–1.0	>1.0–1.5	>1.5–2.0	>2.0	
Building PW, Zone T	16	Freq	.1859	.2698	.1487	.1318	.2638	.5443
Building G, Zones 2–3	20		.0359	.0485	.0288	.0370	.8498	.9150
Building G, Zones 4–5	19		.0563	.2924	.001	.0372	.6121	.6503
Building E, Zone G	15		.0350	.2036	.1437	.1521	.4656	.7614
Building E, Zones 4–5	14		.2509	.2714	.1337	.0971	.2469	.4770

**TABLE VII. NP/P Distribution – Multiple-Sample Comparison (Culturable) “Contaminated” Air 2000 Simulations**

Simulated Sample Size	Frequency NP/P > 1.0 Site				
	PW Zone T	G Zones 2–3	G Zones 4–5	E Zone G	E Zones 4–5
2	.581	.982	.928	.819	.464
3	.577	.997	.975	.877	.429
4	.615	.998	.994	.912	.443
5	.587	1.0	.998	.947	.416
6	.615	1.0	1.0	.954	.412
7	.621	1.0	1.0	.968	.409
8	.618	1.0	1.0	.974	.393
9	.629	1.0	1.0	.973	.397
10	.644	1.0	1.0	.988	.355
11	.648	1.0	1.0	.989	.355
12	.647	1.0	1.0	.993	.358
13	.645	1.0	1.0	.993	.364
14	.672	1.0	1.0	.994	.329
15	.650	1.0	1.0	.997	.345

simulations and 2000 simulations each for sample sets varying incrementally from N = 2 to 15 were conducted as previously described. In three of the eight data sets, all samples regardless of size exceeded the proposed criterion of 900 s/cm<sup>3</sup> total spores, while in three data sets, all samples regardless of size exhibited levels less than 900 s/cm<sup>3</sup> total spores. One of the sets of outdoor air data (Building RM) revealed a low frequency

(0.062) of samples greater than the criterion for single sample simulations, while all samples from N = 2 to 15 were less than 900 s/cm<sup>3</sup> total spores. Data from one site revealed a frequency exceeding the 900 s/cm<sup>3</sup> total spores criterion at a frequency of at least 0.223 regardless of sample size (Table VIII). Sample sets that exhibited a low frequency (<0.1) exceeding the 900 s/cm<sup>3</sup> total spores criterion also exhibited a low frequency (0.0)

**TABLE VIII. Total Spores Distribution (Light Microscopy) “Clean” Air 2000 Simulations**

Site	Field Samples	Frequency > 900 s/m <sup>3</sup> (Single-Sample Simulation)	Stability at Increased Samples N = 2 ... 15
L (outdoor air) <sup>A</sup>	19	1.0	Not applicable; all sample sizes mean total spores > 900/m <sup>3</sup>
L(outdoor air) <sup>A</sup>	16	1.0	Not applicable; all sample sizes mean total spores > 900/m <sup>3</sup>
SM (outdoor air)	15	1.0	Not applicable; all sample sizes mean total spores > 900/m <sup>3</sup>
RM (outdoor air) <sup>A</sup>	15	0	Not applicable; all sample sizes mean total spores < 900/m <sup>3</sup>
RM (outdoor air) <sup>A</sup>	15	0.062	Not applicable; sample sizes N= 2 ... 15 mean total spores < 900/m <sup>3</sup>
ENT(outdoor air)	18	0	Not applicable; all sample sizes mean total spores < 900/m <sup>3</sup>
SM(indoor air)	15	0.223	Frequency >900 m <sup>3</sup> varied from .284 (2 samples) to .494 (15 samples)
ENT (indoor zone 2) <sup>B</sup>	15	0	Not applicable; all sample sizes mean total spores < 900 m <sup>3</sup>

<sup>A</sup>Each outdoor air sampling at Buildings L and RM were collected on separate days.

<sup>B</sup>Indoor air from Building ENT, Zone 2, assumed to be “clean” based on visual inspection.

**TABLE IX. *Asp/Pen* Multiple-Sample Distribution (Light Microscopy) “Clean” Air 2000 Simulations**

Site	Field Samples	Frequency > 750 s/m <sup>3</sup> (Single-Sample Simulation)	Stability at Increased Samples N = 2 ... 15
L (outdoor air) <sup>A</sup>	19	.5190	Frequency >750 s/m <sup>3</sup> varied from .540 (2 samples) to .807 (15 samples)
L(outdoor air) <sup>A</sup>	16	.630	Frequency >750 s/m <sup>3</sup> varied from .769 (2 samples) to .946 (7 samples)
SM (outdoor air)	15	.2019	Frequency >750 s/m <sup>3</sup> varied from .159 (2 samples) to .0404 (5 samples)
RM (outdoor air) <sup>A</sup>	15	0	Not applicable; all sample sizes mean <i>Asp/Pen</i> spores < 750/m <sup>3</sup>
RM (outdoor air) <sup>A</sup>	15	0	Not applicable; all sample sizes mean <i>Asp/Pen</i> spores < 750/m <sup>3</sup>
ENT(outdoor air)	18	0	Not applicable; all sample sizes mean <i>Asp/Pen</i> spores < 750/m <sup>3</sup>
SM(indoor air)	15	.1940	Frequency >750 s/m <sup>3</sup> varied from .239 (2 samples) to .243 (15 samples)
ENT (indoor zone 2) <sup>B</sup>	15	0	Not applicable; all sample sizes mean <i>Asp/Pen</i> spores < 750/m <sup>3</sup>

<sup>A</sup>Each outdoor air sampling at Buildings L and RM were collected on separate days.

<sup>B</sup>Indoor air from Building ENT, Zone 2, assumed to be “clean” based on visual inspection.

exceeding the 750 s/cm<sup>3</sup> *Asp/Pen* criterion. Similarly, the sites that exhibited high frequency (1.0) of total spore levels greater than 900 s/cm<sup>3</sup> also exceeded the 750 s/cm<sup>3</sup> *Asp/Pen* criterion at a frequency of 0.20 or greater. As with the data for the NP/P ratio, increasing sample size did not necessarily improve and in some instances decreased the performance of the 750 s/cm<sup>3</sup> *Asp/Pen* criterion (Table IX).

Other descriptors/criteria evaluated by BMC, including the ratio of phylloplane fungi to total fungi (ratio greater than 0.5 for acceptability), nonphylloplane fungi to total fungi (ratio less than 0.5 for acceptability), total spore counts via spore trap/light microscopy (criterion greater than 1300 s/cm<sup>3</sup> defining problematic conditions), and the indoor/outdoor ratio of total spore counts via spore trap/light microscopy (ratio less than 1.0 for acceptability) revealed similar rates of error and unreliability regardless of sample size when tested by BMC.

## CONCLUSION AND DISCUSSION

All the selected descriptors/criteria for interpretation of bioaerosol data evaluated in this study exhibited very high variability when evaluated with BMC regardless of sample size. The single-sample BMC simulations that model the use of the respective descriptors for identification of “localized” contamination (for example, within a room) with a single sample further underscores unreliability with small sample sizes and/or single-sample comparisons.<sup>(7,8,22,23,32)</sup> More fundamentally, the fact that, in general, descriptors tested do not consistently improve with increasing sample size indicates

inherent unreliability with the particular descriptor/criterion rather than limitations due to small samples.

The numerous interpretive descriptors for bioaerosol data interpretation that have been utilized are based either on intuitive appeal, ease of calculation, and/or application in other fields. However, interpreting data using subjective and untested guidelines has significant potential ramifications given the public health, legal, and economic issues associated with indoor fungal contamination. There cannot be an objective basis for the assertion of an “unusual” population of airborne fungi in an indoor environment (thus implying problematic conditions) without a quantification of the uncertainty involved in the data generated and interpretative criteria utilized in a particular study.

Given the variety of sampling and analytical protocols for bioaerosols and the “relative standard” necessary for data evaluation, “objective” analysis is defined as much by the method in which differences between subject environments are determined from the laboratory data generated, as from the actual sampling and analytical methodology utilized. From this perspective, there is no given sampling protocol and laboratory analysis/technique any more or less inherently “objective” than another. Each dictates the mathematical nature (probability distribution) of the resulting data differently, which remains an outstanding, unresolved issue in applying interpretive guidelines or criteria for discriminating problematic indoor environments.<sup>(22–23,32–33)</sup> The utility of BMC analysis lies in its generalized application to establish the variability of any given descriptor or criterion for bioaerosols regardless of the sampling and analysis utilized and the distribution of the data. From this, investigators can estimate the probability a given

interpretive descriptor is useful in characterizing a suspect environment.

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