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A Regional Comparison of Mold Spore Concentrations Outdoors and Inside "Clean" and "Mold Contaminated" Southern California Buildings

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A total of 625 buildings and outdoor locations in the San Diego, California, area were monitored using the Allergenco Sampl-Air MK-3 impaction sampler or the Zefon Air-O-Cell slit bioaerosol cassette. Locations were classified by rigid criteria as clean commercial, commercial with mold growth, clean residential, residential with water staining, and residential with mold growth. In addition, coastal and inland outdoor locations were measured. Seven categories (total spores, Ascospores/Basidiospores, Cladosporium, *Smut/Myxomycetes-like,* Aspergillus/Penicillium *(AS/PE),* Alternaria, *and Unidentified/Other) were detected frequently enough that maximum likelihood estimate techniques could be used to determine distribution parameters and, thus, treat these as continuous variables. For total counts (no nondetectables) an analysis of variance was used to examine differences in location means. For the other categories Land's confidence limits were generated and visually compared for differences among locations. For 12 other categories (*Curvularia, Dreschlera, Epicoccum, Fusarium, *Mildew-like,* Pithomyces, *Rusts,* Stachybotrys, Stemphyllium, Torula, Ulocladium, *and Zygomycetes-like), detection generally occurred in less than 10% of samples. These genera were treated as dichotomous (detect/nondetect) data, and Chi-square analyses differentiated between locations. For total counts, values were significantly different on the order of clean $<$ outdoor $<$ moldy. There was a large difference between the moldy and other location classes. For AS/PE, moldy location means were clearly higher than those for clean buildings and outdoors, although the clean and outdoor means could not be differentiated. For all other genera the results tend to indicate little or no ability to discriminate location. For example, there were no differences in the probabilities of detecting* Stachybotrys *among the various locations. In our study only total counts, usually driven by AS/PE concentrations, had value in determining whether a building is mold contaminated employing our set of rigorous location classification criteria.*

Keywords mold, comparison, airborne

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Although aerobiologists studying the outdoor variability of aeroallergens have used slit impaction samplers for more than 20 years, it is only within the past 10 years that the use of this procedure to evaluate bioaerosols in indoor environments, including fungal (mold) spores, has become common practice. The most commonly used devices are the Zefon Air-O-Cell (Zefon International, Ocala, Fla.), Allergenco sampler (San Antonio, Texas), and Burkard personal sampler (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, England). Slit impaction sampling is currently the most commonly used method for the collection of both nonviable and culturable airborne mold spores. Because of size selection criteria associated with this type of collection device, particles larger than 2.7 μm in aerodynamic diameter are collected with at least 50% collection efficiency. This procedure is capable of determining the concentration of a spore fungal grouping (morphologically similar genus and/or species) present in the air regardless of its viability. Although identification down to genus and species is limited in some fungal groupings by this method, the advantages over viable sampling are significant. The ability to provide same day analysis and the identification of nonviable spores (i.e., those that will not culture) are two of many advantages.

The most common application of this procedure in air quality investigations is to help decide whether fungal concentrations in the indoor environment are "normal" or "atypical" and to decide when the remediation of mold-contaminated environments has been satisfactorily completed. In spite of this procedure's widespread use, very few comparative studies have been published regarding the expected range of mold spores in "clean" buildings, buildings with evidence of fungal growth, and regional outdoor concentrations as determined by this sampling procedure. Sampling approaches, as well as the statistical approaches used to declare a building clean or

contaminated, vary significantly among investigators. In the absence of standardized analysis methods, laboratory analysis and data reporting procedures are also highly variable. Investigators often rely on general comparisons of limited indoor sampling with limited samples from the local outdoor air without considering the regional variability (geographic, microclimatic, diurnal, and seasonal variability). Consequently, considerable variability both in the analytical results and their interpretation is common.

The majority of current regional outdoor data has been obtained using the Burkard 7-day sampler and is reported through the National Allergy Bureau Aeroallergen Network of the American Academy of Allergy, Asthma, and Immunology.[1] The majority of this data is collected from the rooftops of multistory buildings and over long time intervals (i.e., 5–7 days). In contrast, the majority of the data collected for the evaluation of buildings (indoors and outdoors) is based on short-term impaction samplers like those noted earlier. These devices are frequently used to collect 5- to 10-min samples at entry and exit points or fresh air intakes and supply locations of heating and air-conditioning systems.

Currently the American Industrial Hygiene Association[2] and the American Conference of Governmental Industrial Hygienists[3] (ACGIH®) recommend that indoor sampling data should be compared with the local outdoor environment and/or a control environment. Both of these organizations also acknowledge the current absence of existing standards or acceptable limits for the concentration of fungi due, in large part, to the absence of data sufficient to establish them.

The purpose of this study was to analyze short-term mold spore data collected from well-characterized residential and commercial buildings that have been classified based on predetermined visual inspection criteria. These results are compared with outdoor sampling results that have also been collected on a short-term basis at entry and exit points to buildings, including primarily ground level outdoor sampling that is more typically performed during routine indoor air quality investigations.

METHODS

Classification of Buildings—Inspection Procedures

The data presented in this study were collected in Southern California and analyzed by Environmental Analysis Associates (EAA) between 1994 and 2001. Although EAA has evaluated several thousand buildings and residences over this time period, this study included only buildings where a comprehensive building history and complete visual inspection information could be obtained.

All buildings in the study were systematically evaluated. We performed both an exterior and interior visual inspection for evidence of moisture intrusion or mold growth. The exterior inspection documented landscaping and topography, exterior building conditions, and other physical conditions potentially impacting moisture intrusion into the building. During our interior inspection we evaluated moisture and mold conditions of ceilings, walls, windows, baseboards, flooring, and foundation areas. We also evaluated plumbing utilities, including sinks, showers, toilets, and furnace and air-conditioning systems for any evidence of moisture intrusion, water leaks, or visible mold growth. We measured temperature, relative humidity, and surface moisture. Surface moisture measurements were collected using a Delmhorst penetrating pin probe meter (Towaco, N.J.) and/or the Tramex Moisture Encounter nonpenetrating moisture meter (Dublin, Ireland). Only those buildings for which all inspection information was available were included. This narrowed the usable number of buildings from more than 1000 to 190.

Classification of Buildings—Classification Procedures

Since water or moisture content is the primary determining factor in indoor mold growth, we divided buildings in this study into five classifications based on both visual inspection and the presence or absence of water staining or visible mold growth. Building classifications were:

1. "Clean" commercial office and nonindustrial workplace environments.
2. Commercial office and nonindustrial workplace environments with evidence of mold growth.
3. "Clean" residential single-family dwellings and apartments.
4. Residential single-family dwellings and apartments with evidence of water staining only. (There were insufficient data collected from commercial buildings with water staining to differentiate them from non-water-stained buildings.)
5. Residential single-family dwellings and apartments with evidence of mold growth.

The numbers of buildings and air samples in each classification are shown in Table I.

Criteria for Assigning a "Clean" Building Classification

To classify a building as "clean," it had to satisfy all of the following conditions:

1. No evidence or history of flooding was observed or found based on written records or by verbal communication with the tenant or building owner.
2. No evidence of moisture intrusion determined by our thorough systematic visual inspection.

TABLE I. Number of Buildings by Classification

Classification	No. of Buildings	No. of Samples
Residential buildings (clean)	19	55
Residential buildings (water stained)	30	108
Residential buildings (mold growth)	77	230
Commercial buildings (clean)	37	107
Commercial buildings (mold growth)	27	76

3. No history of sewer backups.
4. No visible mold growth.
5. No interior surface moisture measurements exceeding 15% (equivalent wood scales) using a Delmhorst penetrating moisture meter or Tramex Moisture Encounter nonpenetrating moisture meter.
6. Causes other than water intrusion or fungal growth were suspected or identified as the origin of the original air quality complaint.
7. Water staining was observed in no more than two windows. If staining was present, it could only be present on the operational side of the windowsill or in a jamb corner, and no visible mold growth was permitted. This exception was allowed because it is rare to find any building in Southern California with operational windows (commercial or residential) that does not have minor water staining present in at least one or two locations.

Criteria for Assigning a "Water Stained" Building Classification (Residential Only)

Buildings were classified as "water stained" if they met the following interior conditions:

1. Water staining was observed in single to multiple locations beyond the allowances for the clean building category.
2. No visual evidence of mold growth was observed.

Criteria for Assigning a "Mold Growth" Classification

For the building to be assigned a "mold growth" classification, interior visible mold growth on areas totaling greater than one square foot (0.09 m^2) was required. The majority of buildings in this category contained mold growth in areas exceeding 10 square ft (0.9 m^2).

Outdoor Sampling Data

In addition to the indoor sampling data, an outdoor regional database consisting of 589 samples collected from over 423 Southern California locations was also included in the study.

The outdoor sample database consists of samples collected during the course of routine indoor air quality investigations, as well as investigations specific to mold contamination. The outdoor sample database includes samples collected outside buildings in the study and buildings not included in the study in the same region. All outdoor samples were collected randomly throughout the year from Southern California in San Diego and Imperial counties, and west of the mountain ranges bordering the desert communities. Over 90% of samples were collected within San Diego County.

More than 90% of outdoor samples were collected at ground floor level outside the front or rear entrances to buildings that were being inspected for indoor air quality problems. The remaining outdoor samples were collected from the rooftops of multistory buildings (20 samples) or at a time-integrated sampling station located 20 ft (1.8 m) above ground level at EAA (60 samples). Samples collected within 5 miles of the coastline were classified as coastal samples. All other sample locations were classified as inland.

Sample Collection Procedures

Two slit impaction sampling devices were used in this study: the Allergenco Sampl-Air MK-3 impaction sampler and Zefon Air-O-Cell slit bioaerosol cassette. Both devices have similar slit design characteristics and recommended sampling flow rates. Preliminary side-by-side comparison measurements (conducted by EAA—unpublished data) of outdoor air using two Air-O-Cell samplers and two Allergenco samplers demonstrated similar reproducibility for the two devices. The mean values for three consecutive sets of quadruplicate side-by-side measurements collected over a half-hour period ranged from a low of 1813 spores/m^3 to a high of 3133 spores/m^3. One Air-O-Cell sampler was collected facing up, while the other sampler was facing down. Both Allergenco samplers were collected facing up.

The means of the three consecutive sampling events were 3275, 2943, and 2144 spores/m^3, respectively. The relative standard deviations for the three consecutive sampling events were 9%, 5%, and 11%, respectively. For purposes of this study, the devices are assumed to be equivalent. Based on a study by the University of Cincinnati Environmental Health Department,[4] the Air-O-Cell sampler has a measured 50% aerodynamic diameter cut point (i.e., particle diameter having a 50% collection efficiency) of 2.7 um at a flow rate of 15 L/min.

Sampling media used in both sampling devices consisted of a glass microscope slide or cover slip coated with the sticky transparent "acrylic" substrate as provided in the Air-O-Cell. This substrate is known to have greater adhesive properties during sample collection and retention of particles during staining than other commonly available alternatives, such as silicone grease.

Samples were collected at a calibrated flow rate of 15 L/min for both devices. Sample collection times ranged from 5 to 10 min. All indoor samples were collected 4–5 ft (1.5 m) above the ground and no closer than 3 ft (1 m) to any vertical obstructions, such as walls or room dividers. Samples from residential dwellings were collected with the windows closed at least 30 min prior to sampling. If the home had a furnace or air-conditioning system, and the occupants routinely used the system, the system was turned on 10 min prior to sampling and remained on during sampling. All samples were collected under normal building activity conditions, and no intentional disturbances to surfaces were performed prior to or during the collection of samples. Sample blanks were obtained for each lot of Air-O-Cell cassettes or Allergenco slides and hand carried to the microbiology laboratory.

Microscopic Analysis Procedures

Samples were prepared with lacto-phenol cotton blue stain and analyzed using bright field microscopy according to EAA's standard operating procedure.[5] Two different microscopes

TABLE II. Microscope Characteristics

Manufacturer/Model	Ocular	Objective	Magnification	Other Equipment
Nikon Labophot-2	20×	40× (planacromat)	800×	Polarizer, analyzer, full wave plate
American Optical	15×	40× (planacromat)	675×	Bright field only

were used throughout the project (Table II). The Nikon Labophot-2 microscope was used for approximately 80% of all analyses. Samples were counted according to the following procedures:

1. The area of deposition of an Air-O-Cell sample measures approximately 14.5 mm by 1.1 mm and visually resembles a pencil line. Because the device is an impaction sampler and not similar to filter sampling devices, the particle deposition density varies significantly along the width of the area from the center to each edge. Accurate counting requires mold spores to be counted in a series of slices, or traverses, perpendicular to the long axis of the rectangular particle deposition area. The typical field area or field-of-view counting method used in the analysis of asbestos filter samples (e.g., NIOSH Method 7400)[6] cannot be used. Instead, the diameter of the microscope field of view is measured and used to establish the width of the pathway covered by performing each traverse. The percentage of the sample analyzed is then calculated by multiplying the width of each traverse times the number of traverses, divided by the actual length of the deposition trace. When using the Nikon Labophot-2 microscope at a magnification of 800×, and counting 10 traverses, this corresponds to analyzing 19% of the entire trace.
2. A minimum of two traverse widths separated each analyzed traverse.
3. Where spore concentrations were relatively high, analysis stopping rules similar to NIOSH Method 7400[6] were employed. Once the spore concentration of any individual spore group exceeded 100, the sample analysis was stopped after completing that traverse.
4. Only spores found within the microscopic field of view or crossing the border of the microscope field of view by more than 50% of the spore area were counted.

Mold Spore Identification Procedures

Mold spores were identified based on comparison with known slide reference standards and reference atlases.[7–10] It is expected that in any sample some spores cannot be identified. When positive identification was not possible, the unknown spore was placed in the Unidentified category. It is not unusual for unidentified counts, especially in outdoor samples, to be 5–15% of the entire count.

Colorless (hyaline) spores including unidentifiable spores, possibly some Zygomycetes, *Paecilomyces*, and *Acremonium*, etc., were placed in the Other (hyaline or colorless) spore category. For statistical analysis the Other and Unidentified categories were combined.

Calculation of Mold Spore Concentrations

The calculation of spore concentrations was performed according to Equation 1.

$$C_p = \frac{L}{DN} \times \frac{P}{QT/1000} \quad \textbf{(1)}$$

where

C_p = concentration of particles per cubic meter of air (cts/m^3)
P = number of particles counted
L = length of entire deposition trace (mm)
D = microscope field of view and traverse width (mm)
N = number of sample traverses counted
Q = sample flow rate in liters per minute (L/min)
T = time (min)

Data Analysis Methods

<LOD Measurements and Concentration Mean Values within Buildings

One to 10 individual measurements were made per building. In almost all cases, sample sizes were <3, and in the majority of buildings only one measurement was made. In cases where a single measurement was made for the entire building, it was always collected from the room or office closest to the suspected origin of the air quality complaint or observed damage condition. When more than one measurement was made in a building, the arithmetic average for each spore category was used to represent that building. Values below the limit of detection (<LOD) were assigned a zero and included in the average.

Ideally, a more accurate method such as maximum likelihood estimation (see next section) would be used to estimate these censored or nondetected values; however, the small sample sizes (usually <3) per building limit this approach. Alternatively, a value such as LOD/$(2)^{0.5}$ would be used as being more representative of the values that exist in the distribution below the LOD. However, the LOD can vary depending on the sample air volume and the density of particles on the slide. The typical mold spore detection limit ranged from 40 spores/m^3 to over 3000 spores/m^3 depending on air sample volume and actual total spore density. High detection limits occurred when the 100-spore count stopping point was reached in only a few traverses. Thus, LOD values for a single fungal group and building can vary by fivefold or more. We decided the best approach was to use the constant value of zero. While this

created a negative bias in the true mean spore concentration when <LOD values were found, the bias existed for all comparison groups (i.e., building types).

Our objective was to calculate mean spore concentrations within a building classification (e.g., clean commercial). In most cases, a single measurement was made for the entire building. Table III shows more than 50% of the spore groups were rarely detected in most buildings. These groups, which were undetected or censored at least 50% of the time, were treated separately (described later). However, the six groups that were detected more than 50% of the time could be treated using a technique known as maximum likelihood, rather than assigning values of zero. In this case, the larger sample sizes (number of buildings) allowed use of this technique. Thus, the calculation of mean concentrations across buildings was performed differently than for the calculations of means within buildings.

When some of the measurements of a distribution are less than the detection limit (<LOD), the distribution is said to be censored. Total count was the only uncensored distribution. In addition, the ascospore/basidiospore and *Cladosporium* counts averaged <10% censored measurements.

One way to treat censored data sets is a technique that relies on the uncensored information to estimate the censored information, usually under the assumption of normality. While there are many techniques,[11] the advent of computers and spreadsheets with subroutines for iterative solution of systems of equations has made the maximum likelihood estimation (MLE) technique most useful.[12] For most environmental work there is no one concentration censoring point; rather, the LOD depends on the sample volume and the percentage of the sample actually analyzed. Consequently, the LOD will vary from sample to sample when the stopping rule is reached. The MLE technique is applicable to this type of data. The MLE was used to estimate the distribution parameters for those distributions having less than 50% total censoring (last column in Table III). The underlying distribution was assumed to be lognormal and the distributions of the log-transformed data were assumed to be normal.

Distribution Analysis

There were 21 distributions of total spore, ascospore/basidiospore, and *Cladosporium* concentrations across the five building classifications. These fungal groups were not highly censored and thus could be tested for lognormality (i.e., the distributions of the log-transformed data were tested for normality). Only 3 of the 21 log distributions were not normal based on 7 different tests. (These tests are outputs from the Number Cruncher Statistical Systems software, e.g., W-test, Martinez-Iglewicz, and D'Agostino Skewness.) For those three log distributions, only 2–5 values (<10%) from the distribution created non-normal skewed distributions.

A t-test is not available for comparing means of lognormal distributions.[13] Transforming the data to logs, and then

TABLE III. Percentage of Locations for Which a Fungal Group Was Detected

	Coastal Outdoor	Inland Outdoor	Clean Commercial	Clean Residential	Water Stained Residential	Moldy Commercial	Moldy Residential	All Sample Types
Number of measurements	216	207	37	19	30	27	77	613
Total spores	100	100	100	100	100	100	100	100
Asco/Basidiospores	98	96	81	100	97	85	97	95
Cladosporium	94	96	84	95	100	85	95	93
Other	79	77	57	79	80	56	81	76
Aspergillus/ Penicillium	67	65	97	89	80	100	92	74
Unidentified	59	49	89	74	77	89	70	62
Smut/Myxomycetes	58	55	43	53	100	56	75	59
Alternaria	62	57	32	47	80	37	62	57
Dreschlera	20	15	8	21	20	22	30	19
Rusts	14	20	11	16	43	11	26	18
Epicoccum	20	12	14	0	3	19	19	15
Mildew-like	13	14	3	16	17	4	21	14
Stemphyllium	11	10	5	5	10	4	17	10
Pithomyces	6	5	5	32	47	11	16	10
Stachybotrys	5	9	3	5	27	11	16	9
Torula	11	9	3	0	10	4	12	9
Curvularia	5	6	5	5	27	30	10	8
Ulocladium	1	3	5	5	3	0	6	3
Fusarium	3	1	3	0	0	0	3	2

using t-tests or analysis of variance, can lead to errors since the true variance is underestimated.[14] Similarly, since some distributions were censored, an analysis of variance (ANOVA) using log-transformed data was not possible. Thus Land's confidence limits for lognormal distributions[15] were calculated, and graphical analysis was used to look for differences in means across locations. The question is whether the mean concentrations for the fungal groups depend on location. An ANOVA was performed on the total counts, since there was no censoring. The ANOVA results were compared with the confidence interval results for the total group only.

Another approach was used for the distributions that were highly censored (>50%). Here, a fungal group in a given sample was considered to be detected or not. In this case, the question is whether a fungal group is more likely to be detected in one location than in another. For these fungal groups, the data were treated as discrete—each sample result was assigned a 0 or 1. We constructed 2×2 contingency tables, and chi-square analysis was used to detect differences in the proportion of results above the detection limit. As some of the 2×2 cells contained fewer than five observations, Fisher's exact test for significance was used.

Box plots were constructed to evaluate the degree of separation of the fungal distributions between the locations. These plots were only constructed for the least censored fungal groups: total, ascospore/basidiospore, *Cladosporium*, and *Aspergillus/Penicillium*.

RESULTS

ANOVA on Total Spore Concentrations

The ANOVA for total spore concentrations clearly showed that the means and confidence intervals for the two outdoor sample types (coastal and inland) were essentially identical. Similarly, there was almost perfect overlap in the two clean classifications (commercial and residential) and the residential water stained classification. These three locations were clearly discriminated from outdoor locations as the outdoor means, and confidence intervals were higher. The overlap for the confidence intervals (CI) of the two moldy environments (residential and commercial) was almost complete, but the residential CI was much smaller due to the larger sample size (77 versus 27). There was slight overlap and therefore no significant difference between the moldy commercial (but not the residential) and the outdoors locations. This may have been attributable to the larger CI (small sample size) for the commercial building classification. The trend in total count means was on the order of clean < outdoor < moldy.

Confidence Intervals on the Means

The comparison of Land's confidence intervals for total counts is shown graphically in Figure 1a and 1b. The data for moldy buildings (Figure 1b) was separated from the other classifications (Figure 1a) to maintain the smaller numerical scale for the values of the other locations. As for the ANOVA, the confidence intervals show that the water stained and clean building means are essentially the same. The outdoor mean spore concentration is larger than the means for clean commercial or residential buildings, with the rank order of means clean < outdoor < moldy.

Figures 1b–1h show the confidence intervals on the means for the other fungal groups.

A comparison of coastal with inland data for these groups showed no significant differences. Consequently, data collected from both coastal and inland locations were combined and used as the outdoor data set. The *Aspergillus/Penicillium* results mirrored those for total spore counts, and the intervals for moldy buildings were separated from the other classifications (Figure 1b and 1c) for the same reasons cited for the total counts. For the other fungal groups in Figure 1 there are no clear trends. The ascospore/basidiospore and *Cladosporium* results indicate that moldy residential and outdoor mean levels are higher than means for clean locations. For these two groups and the smuts/myxomycetes, the moldy commercial building mean is significantly lower than that for moldy residential buildings. This may be due to factors such as ascospore/basidiospore growth on wood stud construction in residential buildings and the presence of significant dry rot (basidiospore) fungi found in several of the moldy residential buildings in our study. Another contributing factor is the higher prevalence of unfiltered outdoor air in residential buildings because of open windows and relative absence of mechanical ventilation.

Chi-Square Analysis of Highly Censored Groups

Only three of the chi-square comparisons for the highly censored fungal groups were significant ($p < 0.05$). None of the water stained versus clean comparisons was significant, further decreasing the likelihood that the water stained classification is distinct from the clean classification with respect to fungal growth. For the clean versus moldy building comparisons there were two fungal groups that were less likely to be detected in the clean. In addition, *Epicoccum* was more likely to occur inland rather that in coastal outdoor locations. However, by chance, 2.4 significant comparisons (5%) would be expected among these 48 (4×12) comparisons at the $p = 0.05$ level; these three significant results appear to have no biological meaning and therefore their significance is questionable.

Comparison of Fungal Group Ranges Between Building Classifications

Figures 2–5 show box plots for the four least censored fungal groups. The plots show that the rank order for means (clean < outdoor < moldy) noted earlier also holds true for the ranges of total, *Aspergillus/Penicillium*, and ascospore/basidiospore concentrations. Yet, there is significant overlap (clean vs. moldy) between the two commercial classifications and the two residential classifications. This is particularly true above the 75th percentile for the clean buildings and below the 25th percentile for the moldy buildings. For *Cladosporium*, the third most detected group, there was even greater overlap between building classifications.

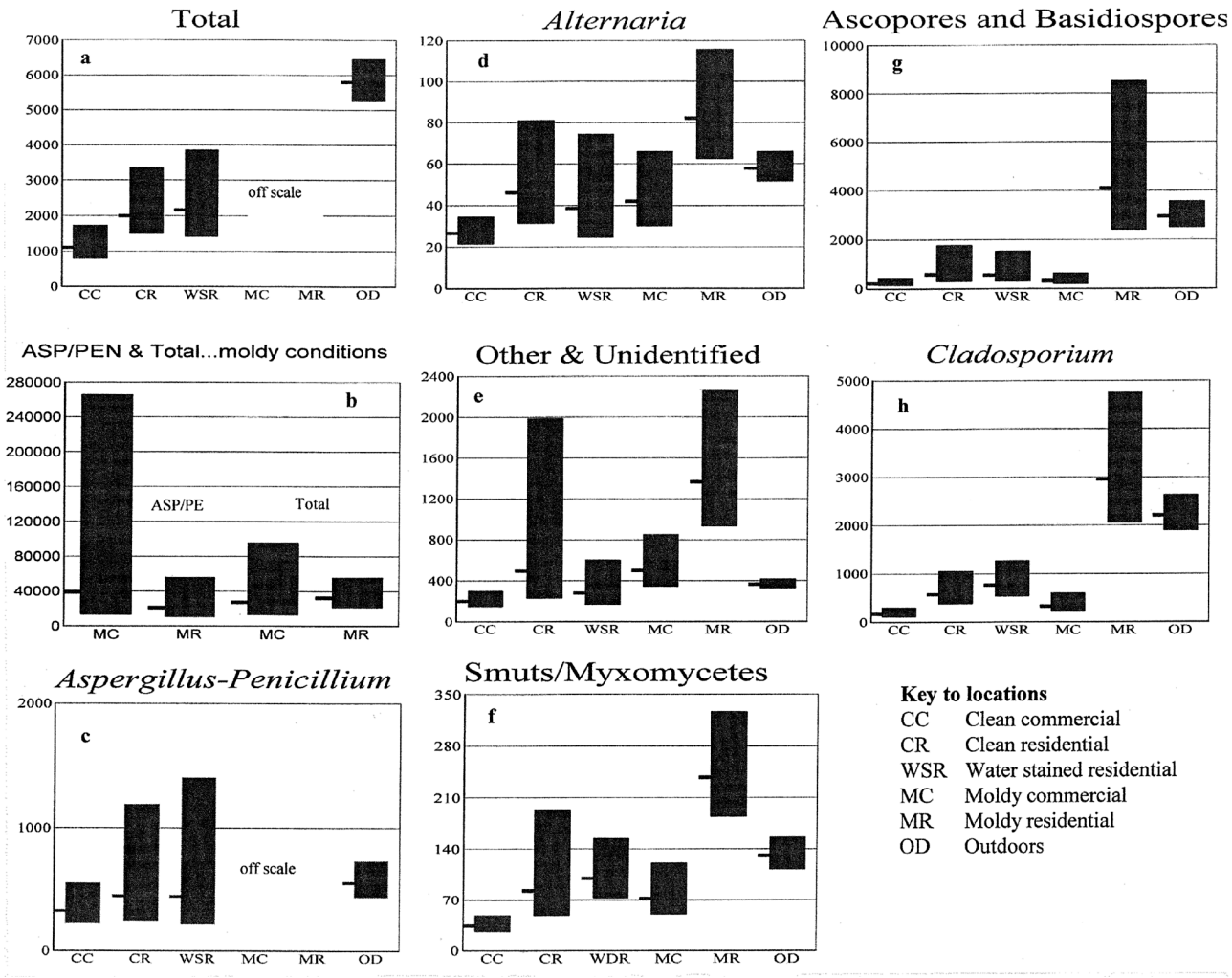

FIGURE 1. Maximum likelihood estimate of mean spore count/m^3 of air (small horizontal bar) and 90% confidence limits for the mean (large vertical bar) for seven fungal groups and six location types. Note that for *Aspergillus/Penicillium* and Total, the data for moldy buildings is separated in (b) to preserve scale in (a) and (c).

DISCUSSION

Potential Error Sources

All data collected in this study are potentially biased in two ways. First, the vast majority of indoor measurements were made in direct response to an alleged indoor air quality complaint. Inspections indicated that some of these complaint buildings were clean. This clean group became the control group for this study. Ideally, noncomplaint buildings chosen at random would have constituted a better population for the clean group. Second, building classifications have not been subdivided beyond identifying them as commercial or residential. For example, a one-room school building relying on a combination of window ventilation and window HVAC is not separated from a high-rise office building relying on HVAC systems for 100% of fresh air exchange. Thus, differences in commercial and residential buildings could be obscured by inadequate detail in classification.

Residential buildings that have pets have not been differentiated from homes without pets, nor were apartment units differentiated from single-family residences. These factors likely widen the distribution of airborne mold spore concentrations for any given building type and decrease the power to find differences between building types. Ideally, larger sample sizes would have allowed further subclassifications that might have yielded statistically meaningful differences.

Minor Fungal Groups

The elevation in airborne mold spore concentrations in most mold contaminated buildings is usually composed of a narrow range of fungal groups, most frequently containing *Aspergillus, Penicillium*, or molds of similar morphology. The detection of other spore genera, such as *Chaetomium* and *Stachybotrys*, are sufficiently infrequent that they require larger building sample sizes to provide any statistically meaningful conclusions

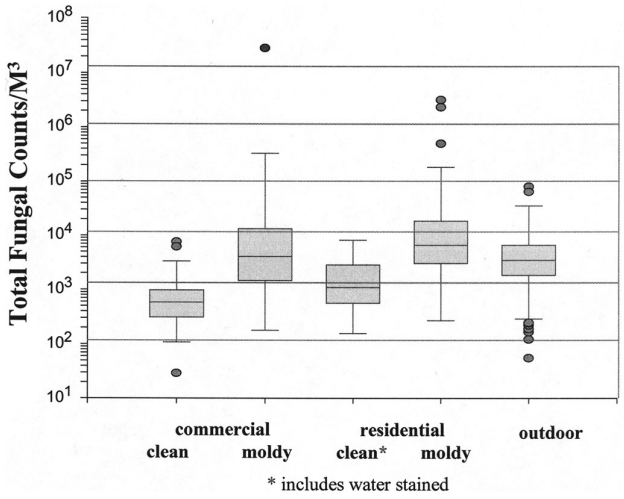

FIGURE 2. Box plot showing Total spore counts for five locations. Boxes are the approximate 25th and 75th percentiles with median in middle. Bars represent 1st and 99th percentiles. Circles are data outside the 1st and 99th percentiles.

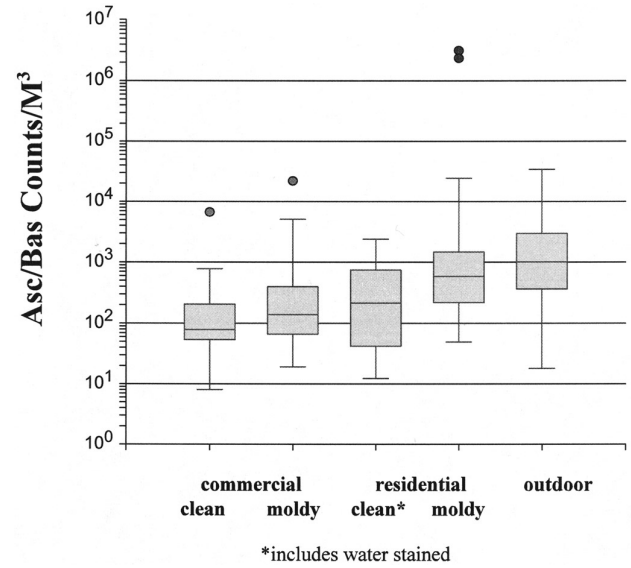

FIGURE 4. Box plot showing ascospores/basidiospores spore counts for five locations. Boxes are the approximate 25th and 75th percentiles with median in middle. Bars represent 1st and 99th percentiles. Circles are data outside the 1st and 99th percentiles.

about their significance. We were unable to detect a significant increase in these minor groups in moldy buildings in contrast with clean buildings or outdoors. Possibly, larger sample sizes or using a method to achieve lower detection limits would reveal such differences.

Stachybotrys is often found growing on indoor cellulosic materials (e.g., drywall paper, tar paper, ceiling tiles) where chronic water leaks or moisture intrusion are present. Because *Stachybotrys* spores do not become airborne as readily as those of *Aspergillus* or *Penicillium*, air sample analysis is usually not a good predictor of the presence of indoor growth of this fungus. In our experience, we rarely find *Stachybotrys* spores in airborne concentrations exceeding 1000 spores/m^3 except during the disturbance of contaminated materials.

Table III shows airborne *Stachybotrys* spores were detected in only 11% and 16% of measurements, respectively, in moldy commercial or residential buildings. When detected they were found in concentrations less than 1000 spores/m^3 in all cases (including less common detection occurrences in clean buildings). Although the detection occurrence in moldy commercial

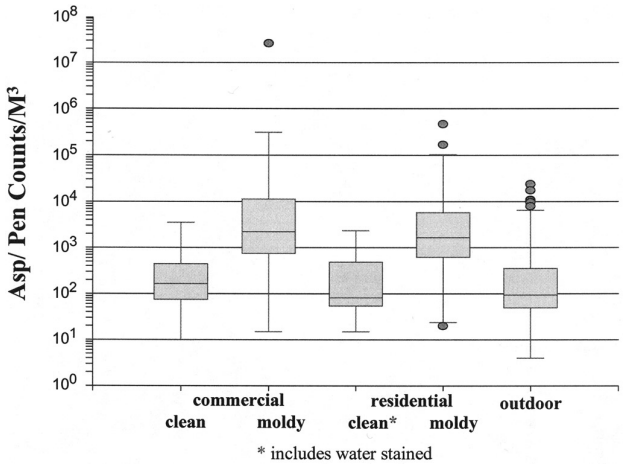

FIGURE 3. Box plot showing *Aspergillus/Penicillium* spore counts for five locations. Boxes are the approximate 25th and 75th percentiles with median in middle. Bars represent 1st and 99th percentiles. Circles are data outside the 1st and 99th percentiles.

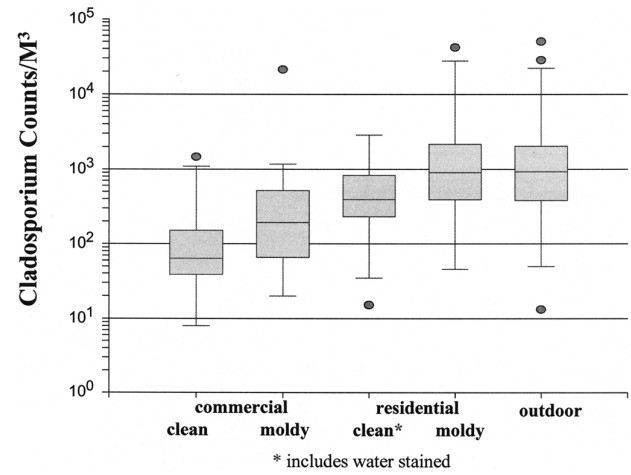

FIGURE 5. Box plot showing *Cladosporium* spore counts for five locations. Boxes are the approximate 25th and 75th percentiles with median in middle. Bars represent 1st and 99th percentiles. Circles are data outside the 1st and 99th percentiles.

and residential buildings was higher (11% and 16%) than in clean buildings (3% and 5%), these differences were not statistically significant. Collecting larger numbers of air samples within buildings may be able to differentiate airborne concentrations of *Stachybotrys* spores in contaminated buildings from background concentrations found in clean buildings. As with any mold, adequate sample sizes must be coupled with thorough visual inspection techniques to differentiate passive infiltration from indoor growth.

Outdoor Concentrations

The outdoor concentration and distribution of mold spores are known to vary greatly on a seasonal, daily, and even hourly basis depending on changing meteorological conditions. Based on information generated by the National Allergy Bureau of the American Academy of Allergy, Asthma, and Immunology,[1] as well as local outdoor data collected by EAA, the range of concentrations in any individual location can vary more than a hundredfold. For example, total mold spore concentrations at EAA's outdoor monitoring station in San Diego have ranged from 200 spores/m^3 during calm wind conditions to 80,000 spores/m^3 during high Santa Ana wind conditions (wind speeds greater than 20 miles per hour). The most predominant fungal groups found outdoors include ascospore/basidiospore and *Cladosporium* species.

Occasionally, elevated concentrations of other fungal groups, including *Aspergillus/Penicillium, Alternaria*, and smuts/myxomycetes, can also be found. Even with this variability, the large number of outdoor samples collected for this study allowed the mean outdoor total count to be differentiated from mean total building counts in the rank order of clean < outdoor < moldy.

Water Stained Buildings

The residential "water staining" data (Figure 1) show that evidence of moisture intrusion is not associated with elevated airborne mold spore mean concentrations when compared with clean residences. For the other more censored fungal groups, there also appeared to be no statistically significant differences between the water stained classification and the clean classification. Thus there appears to be no reason to exclude houses with some water staining, in the absence of other evidence for indoor fungal amplification, from the clean category.

Discriminating Building Classification

Our findings might argue against the need for air sampling in cases where buildings are easily classified after a thorough inspection and review of the buildings' environmental history. In this study, buildings were classified based on a simple and clearly stated set of criteria. Only those buildings that fit these criteria were included in the study. Our results demonstrate that mean total and *Aspergillus/Penicillium* spore concentrations can differentiate clean from moldy buildings. Based on our data, if a building was classified as "clean," one could predict that total indoor airborne mold spore concentrations are not likely elevated compared to outdoors. Conversely, if a building was classified as "moldy," one could predict that total indoor airborne mold spore concentrations would likely be elevated.

Although the mean total spore concentrations for the various building classifications are statistically differentiated, there is still overlap in the ranges or distributions. The box plots (Figures 2–4) show that neither the interquartile ranges (25th–75th percentiles) for the residential buildings or the commercial buildings overlap. However, the 1st and 99th percentiles clearly overlap. Consequently, results of airborne fungal analyses alone may not be sufficient to classify a building as clean or moldy without a thorough visual inspection.

Developing Guidelines for Acceptable Airborne Concentrations

ACGIH[3] recommends using two sources of comparison data as a guide for acceptable mold spore concentrations in buildings: (1) a clean building control group, and (2) regional outdoor mold spore concentrations. Any comparison with outdoor data should consider the climatic variation of the region. All comparisons of data should utilize the calculated confidence limits of the means and not just the mean values.

It would seem unreasonable to set an acceptability limit for the concentration in a building equal to a clean building mean or its upper confidence limit (calculated from many samples as in this study), as too many "clean" buildings would erroneously be categorized as "moldy." Such a criterion is too stringent. Conversely, setting a guideline at the 90th or 95th percentile, as recommended by ACGIH, might include too many contaminated buildings in the "acceptable" category. For this study the ranges for three of the least censored fungal groups are shown in Figures 2–4. Using an ACGIH criterion for acceptability of less than the 90th percentile of clean buildings, it is clear that approximately 20–40% of the moldy buildings in our study would have been miscategorized as "acceptable" based on the air sampling data alone.

As can be seen in Figures 2–4, using a smaller percentile from the clean building distribution (say, the 75th) as an acceptability criterion will reduce false negatives (i.e., a moldy building being labeled clean). The 25th (moldy) and 75th (clean) percentiles demarcate the least overlap for these two building classifications. However, overlap still exists. In our study, at least three possible explanations should be considered when the total spore concentration exceeds 1300 spores/m^3 (the 25th percentile for moldy buildings) in the absence of significant mold growth or finding excessive moisture during the inspection. The first possibility is the existence of a yet unidentified source of fungal amplification indoors. A second is an unusual outdoor condition contributing to the elevated mold spore concentrations indoors. A final possibility is the accumulation of a reservoir of mold spores in settled dust as a result of inadequate housekeeping.

Sampling outdoor concentrations for comparison with indoor concentrations is appropriate, but sample size must be carefully considered. Because of the great variability in fungal spore concentrations (see Table IV for geometric standard deviations, GSDs, derived from this data), small sample sizes

TABLE IV. Variability in Total Spore Counts for Eight Sample Types

Measurement	n	GSD
Clean commercial	37	3.0
Moldy commercial	19	10.1
Clean residential	30	2.5
Clean water-stained	27	3.2
Moldy residential	77	5.0
All outdoor	423	2.8
Coastal outdoor	216	2.6
Inland outdoor	207	3.0

will yield large confidence limits. Even a sample size of 10 from a population with a GSD of 3 will yield an upper confidence limit (UCL) about 2–3 times higher than the estimated mean.[14] Thus, a mean of 3000 estimated with a sample of 10 will have a UCL of 6500. Note that 6500 is the UCL of the outdoor total mean fungal spore concentration from this study (Figure 1). In conclusion, comparison of an indoor mean with a contemporaneous outdoor sample mean (as opposed to a larger historical sample) will likely lead to the conclusion that the two are not different, unless the sample sizes are quite large.

The addition of measurements to this Southern California database and the development of carefully collected databases from other geographical regions will increase the power to detect real differences between indoor and outdoor concentrations when they exist. For the moment, limiting geographical representation is necessary to limit the variability that could increase if outdoor data from one climatic zone were combined with those from a different climatic zone.

The suggested criteria in Table V provide values that could be used to confirm a conclusion to classify a building as clean based on visual inspection. A common use of such data is to measure airborne fungal spore concentrations to classify a building as clean following abatement. If air sampling yields values less than those in the Clean columns of Table V, then satisfactory abatement would be further supported. If not, this result sends a precautionary warning that further inspection is needed and outdoor spore concentrations should be measured for comparison. Similarly, if a complaint building is inspected and suspect mold or moisture conditions are found, air sampling values exceeding moldy building limits as shown in Table V would suggest that cleaning and/or abatement may be necessary.

CONCLUSIONS

Currently, there is a lack of carefully conducted studies that systematically evaluate buildings and statistically analyze visual and laboratory data that identify and quantify fungal growth. As a result, investigators often use diverse investigation approaches and take wide liberties in their interpretation of the environmental significance of airborne fungal spore concentrations. Careful statistical evaluation of our data supports the following conclusions and recommendations:

1. A systematic visual inspection can be an accurate predictor of the level of airborne fungal spore concentrations.
   a. In general, the absence of visible mold growth in readily accessible areas of buildings is an accurate predictor of the absence of elevated fungal spore concentrations. Exceptions are most likely to occur in nonresidential buildings.
   b. The presence of water staining alone in a residential building is more likely to yield sample results similar to clean rather than moldy buildings.
   c. The presence of significant visible fungal growth of more than one square foot total is a good predictor of elevated airborne fungal spore concentrations in that room.
2. Because airborne fungal spore distributions are lognormal, the application of statistical tests based on normally distributed data is inappropriate.[17]
3. Our data from Southern California demonstrate typical clean building total mold spore concentrations are less than 4000 spores/m^3 in approximately 90% of buildings sampled (Figure 2). While buildings classified as "clean" will occasionally exceed this concentration, total spore concentrations for buildings classified as "moldy" range from approximately 200 to greater than 2,000,000 spores/m^3.

TABLE V. Suggested Airborne Acceptance or Rejection Criteria (Southern California) Based on 75th and 25th Percentiles

	Acceptance or Rejection Criteria (counts/m^3)[A]					
Building Type	Total Spores		*Aspergillus/Penicillium*		Asco/Basidiospores	
	Clean[B]	Moldy[C]	Clean	Moldy	Clean	Moldy
Residential	<1200	>1300	<750	>900	<1200	>1300
Commercial	<900	>1000	<750	>900	<1000	>1100

[A] Air sampling data as the sole indication of "amplification" or indoor growth is not advised. The results of a thorough visual inspection may be confirmed with air sampling data.
[B] 75% of all clean buildings (as defined in this study) have measured mold spore concentrations below these values.
[C] 25% of all mold-contaminated buildings (as defined in this study) have measured mold spore concentrations below these values.

4. When airborne spore concentrations are elevated as a result of indoor fungal amplification, the airborne spores present are usually from a few fungal groups, primarily *Aspergillus* and *Penicillium* genera.
5. For total, *Aspergillus/Penicillium*, and ascospore/basidiospore concentrations, the least overlap or greatest differentiation between clean and moldy buildings is found at the 25th percentile of moldy buildings and the 75th percentile of clean buildings. These values are approximately equal. There is not a single cut-off value that would completely differentiate clean from moldy buildings without yielding false positive or false negative results. Using the 25th percentile of fungal spore concentrations for moldy buildings in this study provides a reasonable target for confirmation that a building has *potential* mold problems. If no visible mold growth is present, and the levels exceed the 25th percentile for moldy buildings, further investigation is warranted to evaluate the possibilities of hidden indoor mold growth, outdoor infiltration, and inadequate housekeeping.
6. In general, fungal spores other than *Aspergillus/Penicillium*, ascospore/basidiospore, and *Cladosporium* are found less frequently and in lower concentrations in indoor air, even in the presence of excessive moisture and mold growth. This limits our ability to derive any reliable useful information regarding the analysis of the distribution or ratio of these other fungal groups.
7. Although the data in our study are limited to the identification of fungal spores at genus level or morphological classification, we statistically demonstrate that discriminating between clean and moldy buildings and the outdoor environment is possible without having specific species identification. The relatively small sample size of our study did not permit differentiation of airborne fungal spore concentrations in clean versus moldy buildings for less commonly occurring genera (e.g., *Chaetomium, Epicoccum, Stachybotrys*). Of note, the sample sizes in our study were larger than are typically used by field investigators to interpret the significance of their own data.

RECOMMENDATIONS

At this time, indoor fungal spore concentrations should be compared with regional data from inside "clean" buildings to establish an upper range suggesting the absence of fungal amplification.

Limited outdoor sampling data is most appropriately used to roughly assess the potential for infiltration from outdoor sources. It should be used cautiously in direct comparisons with contemporaneous indoor data for the determination of indoor amplification.

If outdoor data are used for comparison, sufficient numbers of measurements representing the local microclimate environment are required to establish statistical significance. A confidence limit test (on the mean of outdoor values) should be applied to determine if the indoor environment is contaminated by outdoor sources.

Additionally, these cutoff values could be used to help determine when remediation of fungal growth in a building has been successfully performed. However, other factors, such as the results of a thorough post remediation visual inspection and sampling design, must be considered.

Finally, airborne fungal spore data obtained without adequate sample sizes can be used to suggest classification of "clean" or "moldy" buildings. However, in such cases, conclusions based exclusively on sampling results are subject to a greater likelihood of error. Combining airborne data with a careful systematic visual inspection can minimize erroneous conclusions.

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