COMPARISON OF INDOOR AND OUTDOOR AIRBORNE FUNGAL SPORE CONCENTRATIONS IN RESIDENTIAL PROPERTIES

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ABSTRACT

A total of 235 outdoor and 422 indoor samples for total airborne fungal spores were collected from residential properties in 23 cities in nine states: Arizona, California, Florida, Georgia, Illinois, Louisiana, Maryland, Nevada, and Texas. The samples were collected during site visits to properties in which a mold investigation had been requested, and represented 108 sets of data in which corresponding outdoor and indoor spore counts were collected.

The projects were characterized by small sample sizes, total spores were collected on Air-O-Cell cassettes using 5-minute grab samples, and all the samples for a project were collected on the same day and over a relatively short period of time. Forty-six percent (%) of the 108 properties were characterized by three or fewer samples, and 86 % by five or fewer samples.

These data were used to assess the association between outdoor and indoor concentrations of *Cladosporium* and *Aspergillus/Penicillium* (*Asp/Pen*) under the conditions tested; and a possible association between indoor *Asp/Pen* concentrations and the character of the indoor environment. The assessments were performed for both the average and maximum concentrations for each project.

Cladosporium accounted for 35 % of the outdoor spores and 17 % of the indoor spores, and was the most common spore detected outdoors. The GM concentration for the average outdoor *Cladosporium* concentrations was 481 spores/m^3, while the indoor GM concentration was 99 spores/m^3; a ratio of outdoor to indoor concentrations of 4.9.

There was essentially no correlation between outdoor and indoor concentrations of airborne *Cladosporium* spores. A one-way ANOVA for the log-transformed average concentrations resulted in an F-value of 42.9 with a critical value of 3.9 ($P = 4 \times 10^{-10}$). The coefficients of determination (r^2) for the correlations were 0.07 for average concentrations and 0.09 for maximum concentrations.

Asp/Pen type spores represented 46 % of indoor spores but only 15 % of outdoor spores, and were the most common spore type detected indoors. The GM concentration for the average outdoor *Asp/Pen* concentrations was 99 spores/m^3, while the indoor GM concentration was 463 spores/m^3, with a ratio of outdoor to indoor concentrations of 0.21.

There was essentially no correlation between outdoor and indoor concentrations of airborne *Asp/Pen* type spores. A one-way ANOVA for the log-transformed average concentrations resulted in an F-value of 25.8 with a critical value of 3.9 ($P = 8 \times 10^{-7}$), with a similar result for maximum concentrations. The r^2-values were 0.13 for average concentrations and 0.07 for maximum concentrations.

Therefore, under the conditions tested in this study, which were typical of residential mold investigations, the method of data interpretation based on comparing indoor to outdoor concentrations of airborne spores had limited utility.

A simple rank order analysis of indoor *Asp/Pen* concentrations suggested a transition in the character of indoor environments, possibly from uncontaminated to contaminated, occurred at approximately 1,200 spores/m^3 for average concentrations and 1,400 spores/m^3 for maximum concentrations. The value for average concentrations was similar to the 1,000 spores/m^3 reported in a previous study (Baxter et al, 2005).

INTRODUCTION

Background

The comparison of indoor concentrations of airborne fungi to outdoor concentrations has been recommended as a method for assessing the presence of fungal contaminants in the indoor environment. The American Conference of Governmental Industrial Hygienists (ACGIH) and the American Industrial Hygiene Association (AIHA) have both recommended this method.[1, 2]

The recommendations by both organizations are similar. The ACGIH recommended a sampling plan in Table 5.6 of the referenced document that included a minimum of two outdoor sampling locations and a minimum of four indoor sample series. In addition, these samples were to be repeated at least twice on two or more days. An implicit assumption in these recommendations, as indicated by the reference to air delivery systems and zones as sampling locations, was that the sampling site was a commercial building.

Table 5.9 of the ACGIH document recommended that duplicate samples should be collected at a minimum of three locations to estimate worst-case exposures, and on at least three consecutive days to estimate average exposures. Again, the implication was that the project lasted for an extended period of time, and was probably a reference to commercial projects.

Section 14.2.3.2 of the ACGIH document indicates that comparing indoor to outdoor concentrations is a common method for assessing fungal contamination indoors. However, several limitations on the use of this method are also discussed. First, the use of this method requires that the fungi be identified to species. This limits this recommendation to the collection of culturable fungi. Second, only multiple pairs of indoor/outdoor samples are to be interpreted, which requires replicate samples to be collected both indoors and outdoors. Third, the ratio of indoor-to-outdoor concentrations should be based on data with low variability, which implies the need for large sample sizes and/or samples collected over an extended period of time.

Sheldon et al examined an extensive database of over 12,000 samples of culturable airborne fungal concentrations collected both indoors and outdoors. The samples had been collected throughout the United States and across seasons.[3] They reported that the median concentrations of total spores were lower indoors (82 CFU/m^3) than outdoors (540 CFU/m^3), with a median ratio of 6.6. The results of the study by Sheldon et al supported the indoor-to-outdoor comparison method. Both the mean and median

concentrations of fungi indoors were correlated with the corresponding outdoor concentrations.

Baxter et al evaluated a large database of total airborne fungal spore data that had been collected in southern California.[4] The study included 393 samples collected in 126 residential properties, and the conditions of the properties were well documented. The residential properties were stratified into groupings of clean, water stained, and moldy buildings. The comparisons were based on the concentration distributions of outdoor and indoor spores, but the results for matched indoor and outdoor data were not reported. Baxter et al concluded that the comparison of indoor concentrations of airborne spores to outdoor concentrations was an acceptable method for interpreting the data; although this conclusion was not consistent with the ACGIH recommendation that indoor/outdoor comparisons should be limited to the comparison of fungal species.

However, these authors concluded that the utility of this method was limited by the large sample sizes that were required. For example, almost all of the projects included in the study by Baxter et al were characterized by a sample size of three or less, with the majority of buildings were characterized by only one sample. Therefore, none of the 126 residential building investigations included in their study met the criterion of adequate sample size. This is not presented as a criticism of the study, but rather to emphasize the difficulty of collecting large sample sizes over multiple days during residential investigations, even in a large, well-documented study.

Section 14.2.3.2 of the ACGIH document states that all contaminant fungi detected indoors were ultimately derived from the outdoors.[1] This statement provided the basic premise for the indoor to outdoor comparison. However, McGrath et al reported that even though fungal profiles in the outdoor air changed continually, the fungal profiles inside contaminated buildings tended to remain unchanged.[5] These results suggested that airborne spores present in the indoor micro-environment may not always be closely associated with those spores present in the outdoor macro-environment.

In addition, Baxter et al concluded that indoor concentrations of airborne *Aspergillus/Penicillium* (*Asp/Pen*) type spores, as compared to other spore types, had the most utility in determining whether a building was contaminated with mold..[4] Similarly, McGrath et al reported that complaint buildings frequently had high airborne concentrations of *Penicillium* species, while *Cladosporium* was usually dominant in buildings with few complaints.[5] These studies suggested that airborne concentrations of *Asp/Pen* type spores may be useful in differentiating clean from contaminated indoor environments.

Purpose

Many of the limitations placed on the indoor-to-outdoor comparison method by the ACGIH and AIHA documents may not be met in many mold investigations, especially in residential mold investigations. One purpose of the current study was to evaluate the utility of the indoor-to-outdoor comparison method under conditions typical of residential

mold investigations. For example, only total spores are often collected rather than culturable fungi, few samples are collected, short-term grab samples are collected without replication, and all the samples are generally collected on the same day and within a short period of time. The association between the average indoor and outdoor concentrations of *Asp/Pen* (dominant indoors) and *Cladosporium* (dominant outdoors) type spores was compared for 108 projects using a one-way ANOVA.

A second purpose of the study was to compare the concentration distributions of *Asp/Pen* type spores detected indoors with the data reported by Baxter et al.[4] The concentration distributions for the average indoor and outdoor concentrations of *Asp/Pen* and *Cladosporium* type spores were evaluated; as well as the rank order of indoor concentrations.

Finally, the variability of sample results within residential projects was also briefly examined, since this affects the minimum sample size required to perform a useful statistical analysis. This issue was examined by evaluating the room-to-room variability of the sample results for a small but representative selection of the projects included in the study.

METHODS

The concentrations of total airborne fungal spores were available for microbial investigations performed in 23 cities located in nine states. Those databases were searched for residential mold investigations conducted during 2003 and 2004, and a total of 108 residential properties were selected. The 108 properties represented 422 indoor samples and 235 outdoor samples, which had all been collected during the same site visit.

Comparisons of indoor and outdoor spores were limited to the two dominant spore types, *Asp/Pen* type spores for indoor samples and *Cladosporium* spores for outdoor samples. The data were further simplified by limiting comparisons to (1) the average spore concentration for each project, and (2) the maximum spore concentration reported for each project.

The airborne samples were collected for 5 minutes using Air-O-Cell cassettes at an airflow rate of 15 lpm. The airflow rate was calibrated using a rotameter. The cassettes were positioned at a downward angle of about 45°. Windows had typically been closed prior to and during sampling. Two outdoor samples were generally collected during each investigation. All samples were analyzed at a laboratory qualifying in the Environmental Microbiology Laboratory Accreditation Program (EMLAP) administered by AIHA.

The ANOVA and coefficients of correlation (r) were calculated using standard templates in Quattro Pro 11 (Corel, Inc., Ottawa, Ontario, Canada). Additional statistical parameters were calculated using readily available equations.[6, 7]

The *Asp/Pen* and *Cladosporium* concentration distributions were treated as lognormal distributions, although the actual distributions were not verified.[4, 8-11] Censored data

were assigned a value of LOD/2 for statistical calculations. Since the log-plots were not constructed using a log-probability scale, concentrations were plotted versus the normal deviates (ND) rather than cumulative percentiles (CP) in order to linearize the plots. The ND is equivalent to the standard deviation, except it is centered on the GM (ND = 0) rather than the arithmetic mean. For example, ND = -1 at the 16^{th} percentile and

RESULTS

Indoor-Outdoor Associations

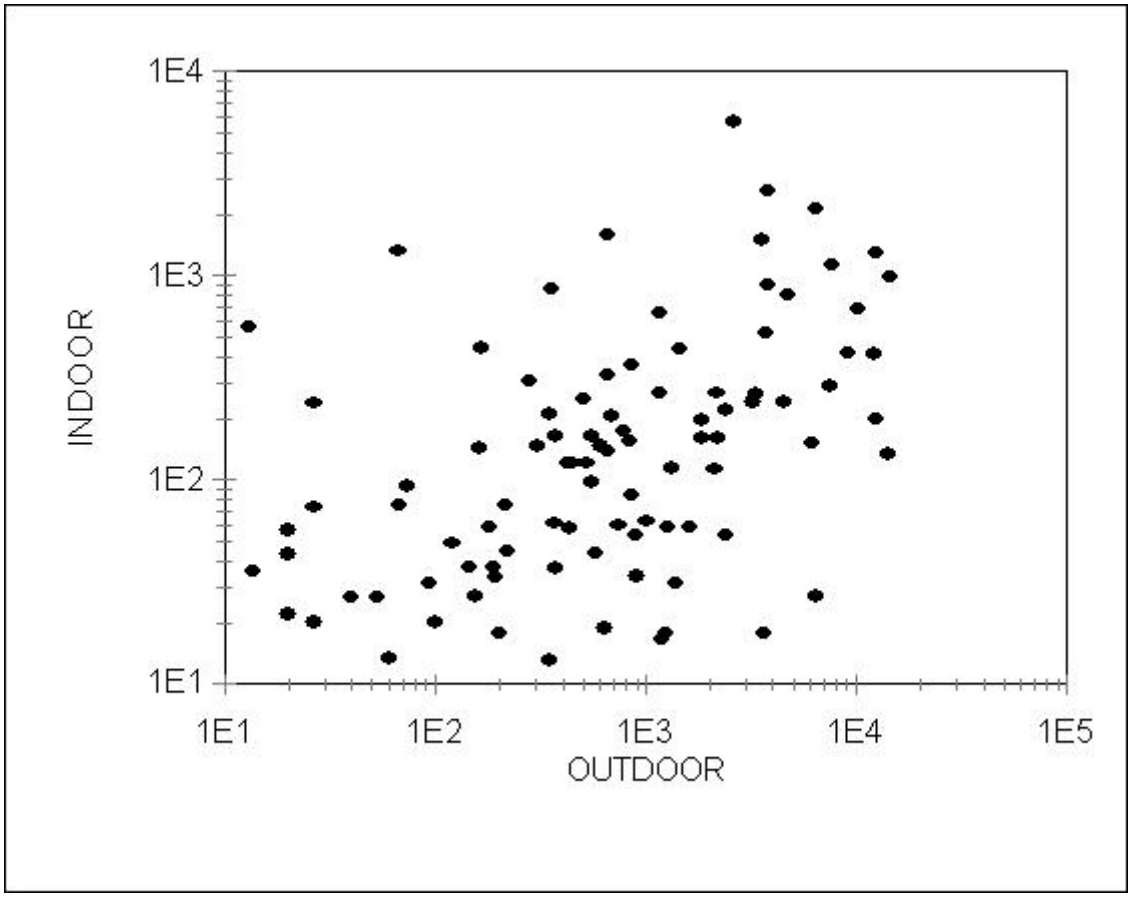
Cladosporium Data

The statistical parameters describing the indoor and outdoor distributions of *Cladosporium* concentration are contained in Table I. The parameters include the concentration range, the geometric mean (GM), the 95 % confidence interval (CI) on the GM, the geometric standard deviation (GSD), arithmetic mean, and the minimum variance unbiased estimator of the mean (MVUE). The GM concentration for average outdoor *Cladosporium* was 480 spores/m^3 and the GM concentration for average indoor *Cladosporium* was 99 spores/m^3, a ratio of 4.8.

PARAMETER	Outdoor Avg	Indoor Avg	Outdoor Max	Indoor Max
Minimum	0	0	0	0
Maximum	14,500	5,700	20,000	20,000
95 % LCL, GM	330	73	450	134
GM	480	99	650	183
95 % UCL, GM	700	133	940	250
GSD	7.1	4.8	6.8	5.1
MEAN	1,947	328	2,520	775
MVUE	2,800	320	3,500	650

The GSD's for the average outdoor and indoor concentration distributions for *Cladosporium* in Table I were similar, with an average value of 6. The GSD's for the maximum outdoor and indoor concentration distributions were also similar, with an average value of 6.

A total of 108 sets of average indoor *Cladosporium* concentrations were plotted versus the corresponding average outdoor concentrations in Figure 1. There was essentially no association detected between the concentrations of outdoor and indoor airborne *Cladosporium* spores. The coefficients of determination (r^2) for the correlation were 0.07 for average concentrations and 0.09 for maximum concentrations. A one-way ANOVA for the log-transformed average concentrations resulted in an F-value of 42.9 with a critical value of 3.9 ($P = 4 \times 10^{-10}$). Maximum concentrations resulted in an F-value of 27.2 with a critical value of 3.9 ($P = 4 \times 10^{-7}$).



Aspergillus/Penicillium Data

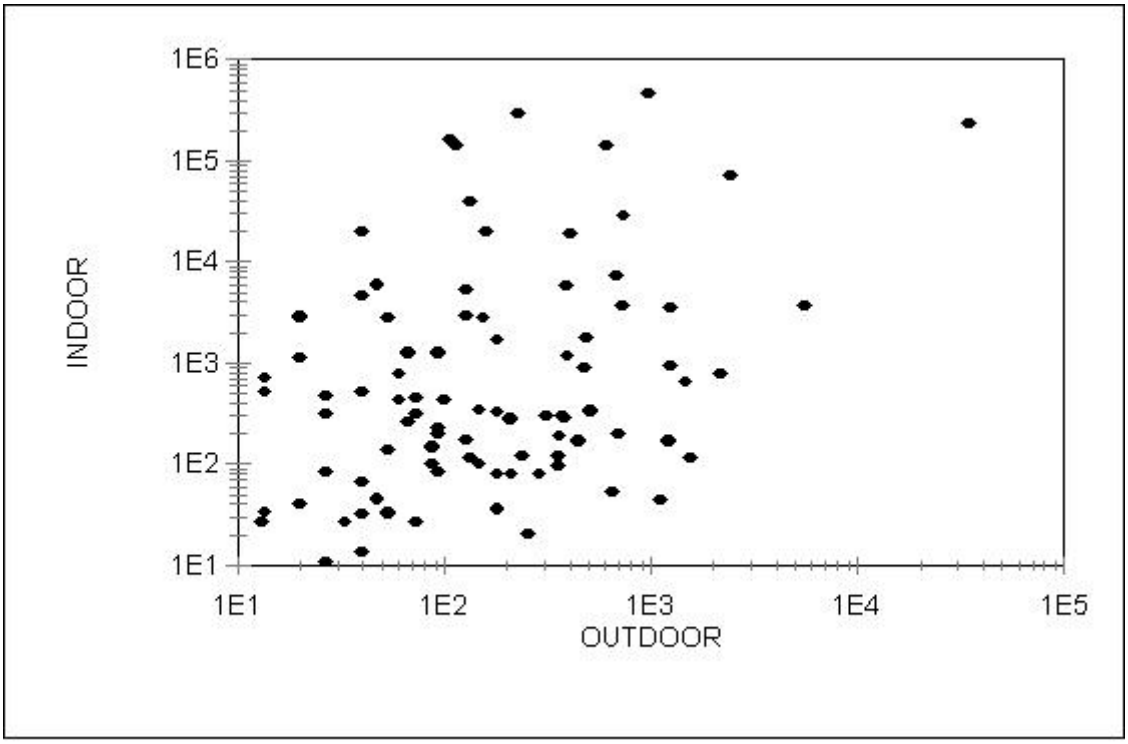
The statistical parameters describing the indoor and outdoor distributions of *Asp/Pen* concentration are contained in Table II. The GM concentration for average indoor concentrations of *Asp/Pen* was 463 spores/m^3, and 99 spores/m^3 for indoor concentrations, a ratio of 4.6. The GM concentration for maximum indoor concentrations of *Asp/Pen* was 942 spores/m^3, and 143 spores/m^3 for outdoor concentrations, a ratio of 6.6.

PARAMETER	Outdoor Avg	Indoor Avg	Outdoor Max	Indoor Max
Minimum	0	0	0	0
Maximum	34,800	458,000	60,200	667,000
95 % LCL, GM	70	280	100	570
GM	99	460	140	940
95 % UCL, GM	140	760	200	1,560
GSD	5.9	13.4	6.4	14.0
MEAN	642	16,115	1,139	31.353
MVUE	440	7,300	710	15,700

The GSD values for outdoor *Asp/Pen* concentrations were similar to those for outdoor *Cladosporium*, which averaged about 7 for *Cladosporium* and 6 for *Asp/Pen*. However,

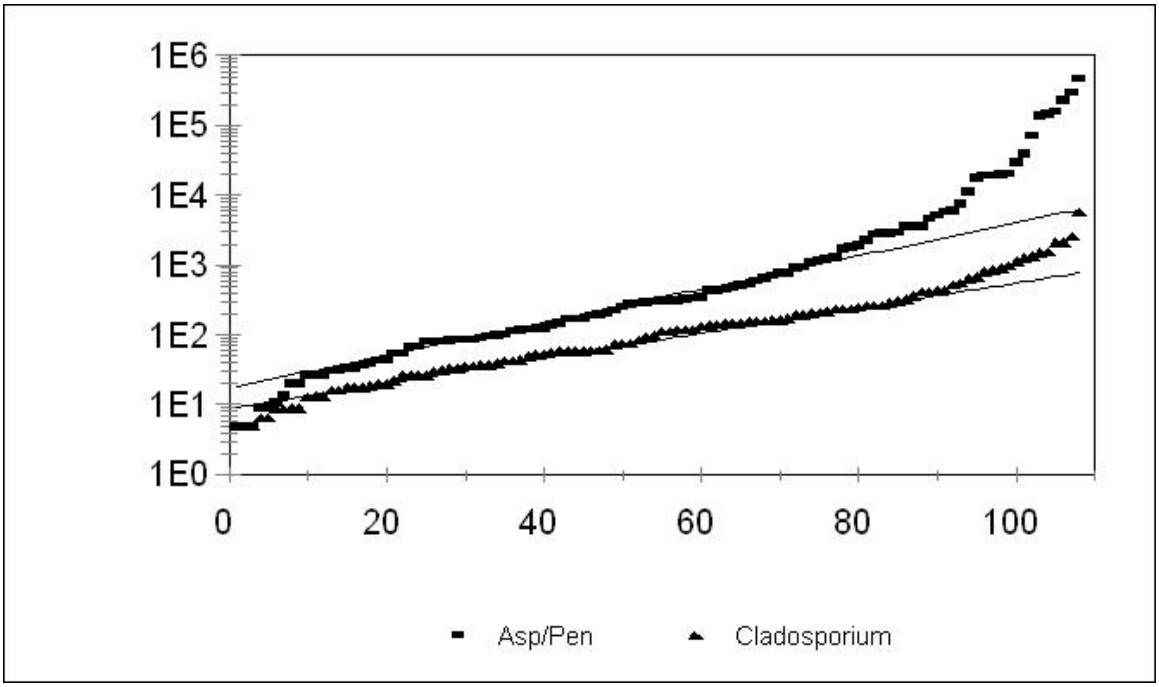
the GSD's for the outdoor and indoor concentration distributions of Asp/Pen type spores were not similar. For average concentrations, the GSD for indoor *Asp/Pen* was 13.4 versus 5.9 for outdoor spores. For maximum concentrations, the GSD for indoor *Asp/Pen* was 14 versus 6.4 for outdoor spores.

A total of 108 sets of average indoor *Asp/Pen* concentrations were plotted versus the corresponding average outdoor concentrations in Figure 2. There was essentially no association detected between the concentrations of outdoor and indoor airborne *Asp/Pen* spores. The r^2 values for the correlation were 0.13 for average concentrations and 0.07 for maximum concentrations. A one-way ANOVA for the log-transformed average concentrations resulted in an F-value of 25.8 with a critical value of 3.9 ($P = 8 \times 10^{-7}$). Maximum concentrations resulted in an F-value of 36.9 with a critical value of 3.9 ($P = 5 \times 10^{-9}$).



Indoor Environments

Figure 3 illustrates the rank order of average indoor *Asp/Pen* and *Cladosporium* concentrations for the 108 projects. The data in Figure 3 became nonlinear at an approximate *Asp/Pen* concentration of 1,200 spores/m^3, and 450 spores/m^3 for *Cladosporium*. For maximum concentrations, the nonlinearity occurred at approximately 1,400 spores/m^3 for *Asp/Pen* and 550 spores/m^3 for *Cladosporium*.



Rank Order

The rank order of airborne spores detected both outdoors and indoors is illustrated in Table III. Only those spore types averaging more than 10 % of the sample were included in the table. Spore types other than *Asp/Pen* and *Cladosporium* did not yield a usable sample size for analysis. For example, *Chaetomium* had a GM concentration of 77 spores/m^3 and a maximum concentration of 21,300 spores/m^3. However, this spore was only detected in 0.7 % of the 422 indoor samples.

SPORE TYPE	OUTDOORS	INDOORS
Cladosporium	34 %	17 %
basidiospores	24 %	13 %
Aspergillus/Penicillium	15 %	46 %
ascospores	14 %	6 %

The rank orders for *Cladosporium*, basidiospores and ascospores were the same indoors and outdoors. *Cladosporium* represented 34 % of the spores in the outdoor samples but only 17 % of the spores in the indoor samples. *Asp/Pen* type spores represented 46 % of the spores in the indoor samples, but only 15 % of the outdoor spores. *Asp/Pen* type spores were three-times as prevalent in the indoor samples as compared to outdoor samples. Based on the total number of spores detected in the 108 projects, *Asp/Pen* type spores represented 87 % of the total indoor spore count.

For the 422 samples collected indoors, 12 % of the *Cladosporium* samples and 17 % of the *Asp/Pen* samples were censored; and 21 % of the 235 outdoor *Asp/Pen* samples were censored.

Sample Size

The distribution of sample sizes for the 108 residential projects included in the study is described in Table IV. The number of indoor samples collected for each project ranged from two to 15. The largest fraction of the projects (46 %) were characterized by a sample size of three or less, a total of 74 % by four or fewer samples, and 86 % of the projects by five or fewer samples. Except for one project in which four outdoor samples were collected, two outdoor samples were collected for each project.

NUMBER OF PROJECTS	SAMPLE SIZE	PERCENT
50	2 – 3	46 %
30	4	28 %
13	5	12 %
7	6	6 %
5	7	5 %
3	9 – 15	3 %

Variability by State

The GM concentrations of *Asp/Pen* type spores detected indoors were compared between states. The number of samples collected in each state, the concentration range, and the GM and 95 % CI on the GM are reported in Table V. The sample sizes for Corpus Christi (CC) and Houston (H), Texas were sufficiently large to allow the data for each city to be analyzed separately.

STATE	N	MIN	MAX	LCL	GM	UCL	GSD
LA	23	27	9,200	90	200	450	7
AZ	26	13	50,700	80	210	520	10
GA	34	27	9,300	180	290	480	4
NV	23	27	19,600	150	365	870	8
IL	66	13	480,000	270	465	798	9
CA	15	13	30,000	170	700	2,850	14
FL	56	13	400,000	370	770	1,600	16
TX-CC	41	27	667,000	330	840	2,100	19
MD/DC	18	27	68,900	450	1,300	4,000	10
TX-H	48	13	587,000	600	1,400	3,300	19

The large GSD values in Table V reflect the variation between the individual projects in each state, and simply indicate that the samples in individual projects were not drawn from the same distribution of concentrations. However, even with this limitation, the 95 % CI on the GM concentrations were similar for each of the grouped samples, except MD/DC and Houston, Texas.

Similar Exposure Areas

The indoor *Asp/Pen* concentrations for individual samples collected from five projects with representative GSD values are presented in Table VI. The data are the concentrations of *Asp/Pen* type spores reported in each indoor sample, with each sample collected in a different room within the house. The examples were limited to projects in which five indoor samples had been collected in order to achieve a reasonable sample size for comparison.

PROJECT	Room 1	Room 2	Room 3	Room 4	Room 5	GSD
1	190	210	330	400	600	3
2	40	200	800	1,700	6,700	7
3	70	90	500	800	10,000	7
4	0	0	0	0	14, 500	33
5	70	8,000	106,000	667,000	667,000	48

The indoor *Asp/Pen* concentrations for individual samples varied widely within the same project, ranging over several orders of magnitude. In general, each room within a residential property had the potential of being a distinct similar exposure area (SEA).

DISCUSSION

It is common practice to compare concentrations of airborne spores detected indoors to outdoor concentrations. The rationale for this comparison includes the assumption that the airborne spores detected indoors are associated with those detected outdoors.[1, 2, 4] However, if the perimeter penetrations (windows and doors) have been closed for a reasonable period of time prior to sampling (8-12 hours), and one assumes modern housing (low to moderate infiltration rates), then there may be little association between the indoor and outdoor concentrations of airborne spores.

Therefore, it was initially assumed that the interior micro-environment rather than the exterior macro-environment would be the dominant influence on indoor spore concentrations. To illustrate this concept, assume two pieces of wet drywall are visibly contaminated with equal areas of the same fungus, such as *Penicillium chrysogenum*. The two materials are each in rooms of the same size, the water activities of the drywall are the same, room temperatures are the same, and the contaminated areas are subject to equivalent amounts of disturbance. The only difference is that one room is located in the sunny southwest while the other is located in the snow-covered northeast. One might expect the airborne spore concentrations in the two rooms to be similar if the indoor micro-environment was the dominant factor affecting indoor spore concentrations.

Second, mold is typically not the problem; the amplification of mold in the indoor environment is generally the problem. The concept of fungal amplification suggests that a single viable spore of *P. chrysogenum* entering the indoor environment, over time, could generate several thousand spores/m^3 in the indoor environment. Therefore, especially in complaint properties, contaminant spores detected indoors would not

necessarily be expected to originate from the outdoors; nor would a correspondence between indoor and outdoors concentrations of *P. chrysogenum* necessarily be expect based on this example.

If the original assumption that indoor spore concentrations were determined by the indoor micro-environment was plausible, (1) there would be little association between indoor and outdoor concentrations of spores in complaint properties, and (2) the distributions of indoor spore concentrations in complaint properties should be similar across a variety of geographical areas and seasonal changes. The data reported in this study supported both conditions.

Indoor to Outdoor Comparisons

There was little association between outdoor and indoor concentrations of *Cladosporium* spores, as illustrated in Table I and Figure 1. The r^2 value for average concentrations was 0.09, and a one-way ANOVA performed on these data indicated that the association between average indoor and outdoor concentrations of *Cladosporium* spores was not statistically significant. A similar correlation for maximum concentrations resulted in $r^2 = 0.07$, and an ANOVA also indicated that the association for maximum concentrations was not statistically significant.

The GSD for average *Cladosporium* concentrations was 7.1 for outdoor samples and 4.8 for indoor samples. Based on maximum *Cladosporium* concentrations, the respective GSD values were 6.8 and 5.1. The GSD values for the distribution of *Cladosporium* concentrations collected indoors were similar to the values for outdoor samples.

It was also concluded that the association between the indoor and outdoor concentrations of *Asp/Pen* type spores was not statistically significant, as illustrated in Table II and Figure 2. The r^2 value for the average concentrations was 0.13, and $r^2 = 0.07$ for maximum concentrations of *Asp/Pen* type spores. An ANOVA also indicated that the association for maximum concentrations was not statistically significant.

The GSD for average *Asp/Pen* concentrations was 5.9 for outdoor samples and 13.4 for indoor samples. Based on maximum *Asp/Pen* concentrations, the respective GSD values were 6.4 and 14. The GSD values for the outdoor *Asp/Pen* distributions were similar to the GSD values (7.1 and 4.8) for the outdoor distributions of *Cladosporium* concentrations. However, the GSD values for the distributions of indoor concentrations

All of the 108 properties were the subject of a microbial investigation. Therefore, the properties included in the study were typical of those expected to be encountered in a microbial field investigation. The projects providing the basis for these results were characterized by small sample sizes, the collection of short-term grab samples on the same day and over a short period of time, and the collection of total spores rather than speciated fungi.

In a similar study based on total spore counts, Baxter et al concluded that sampling outdoor spore concentrations for comparison with indoor concentrations was appropriate.[4] However, that study did not directly compare indoor concentrations to outdoor concentrations. The results of the current study, which directly compared indoor to outdoor concentrations for *Asp/Pen* (dominant indoors) and *Cladosporium* (dominant outdoors), did not support the conclusion that comparing outdoor concentrations with indoor concentrations was a useful method for interpreting the sample results under the conditions tested.

However, the inconsistency in the two conclusions was of little practical significance. Both studies concluded that comparing indoor to outdoor concentrations was of limited practical value, either because of a lack of association between indoor and outdoor concentrations or the limitations imposed by the sampling conditions recommended by ACGIH and AIHA.[1, 2]

Indoor Environments

The rank order of average indoor *Asp/Pen* and *Cladosporium* concentrations for the 108 projects is illustrated in Figure 3. A simple rank order analysis of concentrations plotted on a log scale indicated that the average indoor concentrations of *Asp/Pen* type spores deviated from linearity at about 1,200 spores/m^3. A similar nonlinearity occurred at a value of 1,400 spores/m^3 for maximum indoor *Asp/Pen* concentrations. The average indoor concentrations of *Cladosporium* spores deviated from linearity at about 450 spores/m^3, with a similar nonlinearity at 550 spores/m^3 for maximum indoor C*ladosporium* concentrations.

These data suggested a possible difference between indoor environments in which the average *Asp/Pen* concentration was above or below approximately 1,200 spores/m^3; or an average *Cladosporium* concentration of about 450 spores/m^3.

Baxter et al concluded that for residential properties, a mean *Asp/Pen* concentration of 750 spores/m^3 or less indoors was indicative of an uncontaminated building, while mean concentrations of 950 spores/m^3 or more indoors were indicative of a contaminated building.[4] The upper limit of 950 spores/m^3 was similar to the value of 1,200 spores/m^3 obtained in the current study.

The similarity of the upper limits for the mean indoor *Asp/Pen* concentration was interpreted as indicating a good agreement between the two studies. The data in Figure 3 supported the conclusion of Baxter et al that indoor *Asp/Pen* concentrations of 1,000 spores/m^3 or higher were possibly indicative of a contaminated indoor environment.

Dominant Spore Types

Spore types other than *Asp/Pen* and *Cladosporium* were detected indoors too infrequently to yield a usable sample size for analysis. For example, *Chaetomium* had a GM concentration of 77 spores/m^3 and a maximum concentration of 21,300 spores/m^3.

However, it was only detected in 0.7 % of the 422 indoor samples. Therefore, the comparison of indoor and outdoor spores was limited to *Cladosporium* and *Asp/Pen* type spores.

Cladosporium was the dominant spore type detected outdoors, as indicated in Table III. This spore represented 34 % of the outdoor spores but only 17 % of the indoor spores. Based on average concentrations, indoor concentrations of *Cladosporium* were less than outdoor concentrations in 83 % of the projects. The GM for average concentrations of *Cladosporium* indoors (99 s/m^3) was 21 % of the average outdoor concentration (481 s/m^3).

Based on maximum concentrations, indoor concentrations of *Cladosporium* were less than outdoor concentrations in 80 % of the 108 projects. The GM for maximum concentrations of *Cladosporium* indoors (183 s/m^3) was 28 % of the outdoor concentration (650 s/m^3).

Cladosporium was typically not the amplified spore type in the sampled indoor environments. Except for *Asp/Pen* type spores, the rank order for outdoor and indoor spores was the same for the spore types in Table III. In addition, indoor concentrations averaged about half the outdoor concentrations, a ratio that is often considered typical for indoor spores with an outdoor source. However, the data in Figure 1 still indicated a lack of association between indoor and outdoor concentrations of *Cladosporium* under the conditions tested.

The average percentage of *Asp/Pen* type spores in the indoor air (46 %) exceeded the percentage in the outdoor air (15 %) by a factor of three. *Asp/Pen* type spores were the dominant spore type detected indoors, and concentrations were significantly higher indoors as compared to the outdoor concentrations. Based on the total number of spores detected in the 108 projects, *Asp/Pen* type spores represented 87 % of all the spores detected indoors.

Over 65 % of the data sets had an indoor to outdoor ratio greater than 1.0 for average concentrations of *Asp/Pen* type spores, and 76 % of the ratios were greater than 1.0 for maximum concentrations. The ratio of indoor to outdoor GM concentrations of *Asp/Pen* type spores was 4.6 for average concentrations, and 6.6 for maximum concentrations. Since the average indoor GM concentration (463) was typically 4.6 times the average outdoor GM concentration (99). Therefore, it was unlikely that the sources of the indoor *Asp/Pen* type spores were outdoor reservoirs. This result supported the concept that indoor concentrations of *Asp/Pen* type spores (contaminant spores) were associated with conditions in the indoor micro-environment rather then the outdoor macro-environment.

The dominance of *Asp/Pen* type spores in contaminated indoor environments was consistent with the results reported in at least three other studies. Baxter et al reported that the concentrations of airborne *Asp/Pen* type spores were statistically higher in moldy versus clean houses, and that *Asp/Pen* was the dominant spore type in moldy houses. [(4)] McGrath et al reported that *Penicillium* species were the dominant organism in a

documented complaint building, representing 90 % or more of the airborne fungi indoors.[5] In addition, Engelhart and Exner reported that *Aspergillus* and *Penicillium* represented more than 20 % of the airborne fungi in non-industrial indoor environments, and were the most prevalent indoor fungal genera during the sampling period.[12]

Sample Size

The distribution of sample sizes for indoor samples collected in the 108 residential projects is contained in Table IV. About half (46 %) of the data sets contained three or fewer samples; and the sample size was five or less for 86 % of the projects. The samples for each project were collected during the same day and during a relatively short period of time; conditions which may be typical of residential mold investigations.

The sample sizes reported by Baxter et al were typically 3 or less; and the conditions in a majority of the buildings were assessed based on a single sample.[4] The results reported by Baxter et al, and those reported in the current study, indicated that small sample sizes were typical for residential investigations, limiting the utility of indoor-to-outdoor comparisons on that basis alone.

Both AIHA and ACGIH recognize that small sample sizes are often a limitation, and recommend that professional judgment be used as the default method of assessment when a statistical analysis can not be performed.[1, 2] As indicated by the small sample sizes typical in these two studies, professional judgment may often be the default method of assessment for residential properties.

Assessing the significance of airborne spore concentrations based on professional judgment essentially involves comparing the current data to previously interpreted data. This implies that experienced investigators have access to either a literal or internally derived database of sample results. It also implies that the investigator has formulated decision criteria that are applied to that database in order to assess the significance of the current data. These decision criteria are rarely discussed, but they must exist. Otherwise, there would not be a rational basis for collecting samples, since even experienced investigators would not have a basis for interpreting the results.

Geographical Area

The geographical distribution of projects is described in Table V. The large GSD values in Table V, generally ranging from 7 to 19, reflect the fact that multiple concentration distributions had been combined for each state, as was evident from the experimental design. This reduced the overall utility of any comparisons based on these data.

However, even with this limitation, the 95 % CI on the GM concentrations of indoor *Asp/Pen* type spores in Table V were within the same range for nine of the ten locations. Only the data for Houston, Texas was higher than the range for the other nine groups of data. This similarity in the range of indoor *Asp/Pen* concentrations across states

suggested that the distributions of indoor concentrations of *Asp/Pen* type spores may have been similar for samples collected over a wide geographical area and in different seasons.

Similar Exposure Areas

Large commercial buildings are often stratified (subdivided) into "similar exposure areas" (SEA) or "sampling zones" by the investigator prior to sampling.[1, 6, 7] The stratification is based on the information obtained during the visual inspection, the incident history, and the occupant interviews. The magnitude to which this concept may influence the variability of sample results obtained in residential properties is illustrated in the following three anecdotal examples (Spurgeon, unpublished data):

(1). When six Air-O-Cell (AOC) cassettes were used to collect side-by-side samples at a concentration of about 10,000 spores/m^3 in a settling chamber, the CV was 9 % for 10-minute samples. This was a relatively low CV, indicating a homogeneous environment within the test section of the chamber.

(2). When six AOC were used to collect side-by-side samples at a concentration of about 5,500 spores/m^3 in a living room, the CV was 28 % for 10-minute samples. This value suggested a more heterogeneous but still similar environment, representative of samples collected in a single SEA.

(3). During the investigation of an apartment complex, an AOC was collected in each of the three bedrooms in about 20 apartments, with three 5-minute AOC samples collected per apartment. The CV for the three samples collected in each apartment ranged from 80 % to 150 %, with an average CV of about 100 %. These data suggested that each of those bedrooms was probably a different SEA, and was subject to different concentration distributions of airborne contaminants.

The indoor *Asp/Pen* concentrations for individual samples collected from several representative projects are presented in Table VI. The between-sample variability of *Asp/Pen* concentrations within the 108 projects was often large, as indicated by the GSD values in Table VI. For example, the average outdoor concentration of *Asp/Pen* type spores for Project # 4 in Table VI was 127 spores/m^3. *Asp/Pen* spores were not detected in four of the five indoor samples, with 14,500 spores/m^3 detected in one sample.

It was not unusual to detect elevated concentrations in only one or two of the sample locations, resulting in large GSD values. This suggests that assessing the conditions of interior spaces in residential properties may be more appropriately based on a comparison with control areas, whenever possible, rather than on a comparison with outdoor concentrations.[5]

The between-sample variability illustrated in Table VI supports the consideration of similar exposure areas (SEA) in residential mold investigations.[6, 7] The variability in the concentrations of airborne *Asp/Pen* spores within a property, as illustrated by the

examples in Table VI, suggests a need to stratify the interior spaces into SEA based on professional judgment prior to sampling.

The default method of assessment, especially in residential investigations, is often professional judgment, which avoids the need for statistical analysis of the data. However, the data in Table VI suggest that an intent to rely on statistical analysis would require the collection of multiple short-term samples in each SEA, rather than treating all the interior spaces as a single SEA.

Limitations of the Study

There were at least four recognized limitations affecting the conclusions of the current study. First, the number of indoor samples for each project was limited, with three or fewer samples collected for 46 % of the projects, and six or fewer samples collected for 93 % of the projects. However, these conditions are representative of residential mold investigations, and are therefore relevant to the conclusions of the study.

Second, although an effort was made to include projects from a wide geographical area, the projects were limited to only nine states. The majority of those states were in the southern region of the US; and none of the projects were in the northeast or northwest regions.

Third, the extent of water intrusion and/or mold associated with the 108 residential properties was not included in the database. This factor limited the detail to which the dominance of the indoor micro-environment versus the outdoor macro-environment could be evaluated.

Fourth, the comparisons were based on the sample results for short-term airborne “grab” samples. The results were therefore expected to be more variable than for those collected with long-term samples, and less representative of average concentrations.

CONCLUSIONS

Asp/Pen type spores were the dominant contaminant spore type detected indoors, representing an average of 46 % of the spores detected indoors, but 15 % of the outdoor spores. *Cladosporium* represented an average of 34 % of the outdoor spores and 17 % of the indoor spores.

The comparison of indoor to outdoor concentrations of either *Cladosporium* or *Asp/Pen* type spores had limited utility for assessing indoor environments. The coefficients of correlation, one-way ANOVA, and 95 % CI on the GM concentrations all indicated there was no association between the indoor and outdoor concentrations for the two dominant spore types, *Cladosporium* and *Asp/Pen*.type spores, in the 108 residential properties included in the study.

About 46 % of the 108 projects included in the study were characterized by a sample size of three or less, and 86 % of the projects were characterized by a sample size of five or less. When combined with the results reported by Baxter et al, the two studies suggest that spore concentrations in residential investigations are typically assessed based on small sample sizes, and short-term grab samples that are collected on the same day and over a short period of time.

The spore concentration data described in this study, which were drawn from a wide geographical area, suggested that *Asp/Pen* concentrations in potentially complaint indoor environments may be similar over a relatively wide geographical area and across seasons. To the extent this observation was generally applicable; the indoor micro-environment may be a greater determinant of indoor concentrations of airborne spores than the outdoor macro-climate.

The assessment of the indoor environment, by either statistical methods or professional judgment, should include the concept of stratification, or similar exposure areas.

The study published by Baxter et al, when combined with this study, suggest at least two significant results. First, Baxter et al concluded that their data demonstrated the utility of numerical guidelines for the assessment of indoor environments. The data described in this study, drawn from a wider geographical area, not only supported the use of numerical guidelines as decision criteria, but the guidelines for identifying contaminated environments were similar in both studies.

Second, the comparison of indoor to outdoor concentrations as a method for interpreting sample results was described as having limited utility both in the study by Baxter et al and in the current study. However, the methodology that was applicable in both studies was the comparison of current data to a database containing the concentrations of indoor spores that had been collected in previous investigations.

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