

MONITORING VIABLE FUNGAL AND BACTERIAL BIOAEROSOL CONCENTRATIONS TO IDENTIFY ACCEPTABLE LEVELS FOR COMMON INDOOR ENVIRONMENTS

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Abstract

Bioaerosol concentrations between 200 - 450 CFU/m³ have remained largely uncharacterized with respect to potential indoor air quality concerns. This research provides for further description of indoor bioaerosol concentrations and concludes that ≤ 300 CFU/m³ of non-toxicogenic or non-pathogenic organisms should be typical for normal, non-immunocompromised environments. With the exception of *Cladosporium*, no organism should individually contribute more than 150 CFU/m³. Furthermore, it is concluded that > 300 CFU's/m³ and/or not meeting the above criteria should incite some additional level of investigation with respect to; the bias of prominent outdoor bioaerosol(s), adequate filtration, indoor humidity and microhumidity environments, and/or potential indigenous contamination source(s). The author cautions that the >300 CFU/m³ threshold is not intended to represent any threshold having medical or health significance and/or necessarily representative of an unacceptable indoor environment. The >300 CFU/m³ is intended to be a "reactionary threshold" to incite further investigation as to the cause(s) of what is perceived to be an above average viable concentration for indoor bioaerosols. The author further concludes that outdoor sampling should only be utilized in the relative comparisons to individual indoor components and that indoor/outdoor ratios involving total CFU/m³ concentrations should not be utilized as a specific mechanism to evaluate acceptable indoor bioaerosol concentrations.

Introduction

Human exposure to bioaerosols has been documented in a variety of adverse health effects, including allergy, hypersensitivity, respiratory problems, toxicological, and infectious disease(1-13). Various fungal and bacterial bioaerosols have been identified and enumerated using several sampling methodologies, yet the specific effects of many airborne microorganisms on human health is not clearly understood. The lack of understanding is in part due to the extreme complexities that

exists with respect to sampling methodologies, and bioaerosol data interpretation, as well as, the complexities involving individual human sensitivity. Several methodologies are available to evaluate bioaerosols (14-18); however, there is a current absence of information with regard to the comparability of results. Currently, viable sampling methodologies have been utilized to document fungal and bacterial bioaerosols with respect to indoor environments; however, additional research is necessary in order to develop concentration standards involving exposure, in general.

The purpose of this research was to evaluate viable bioaerosol concentrations to aid in the development of guidelines for the interpretation of indoor fungal and bacterial bioaerosols. The specific objectives of this study were to determine the total concentrations of indoor bioaerosols in a variety of indoor environments in the United States and to specifically characterize individual bioaerosol components with respect to population and concentration.

Materials and Methods

This research evaluated a total of 1116 bioaerosol samples collected over a nine (9) month period from eighteen (18) states. Nine hundred and thirty four (934) of the samples were collected from indoor commercial and residential environments equipped with forced heating, ventilating, and air conditioning systems. The remaining 182 samples were collected from the outdoor environment in those same geographical areas. Bioaerosol samples were collected with an Andersen N-6 single stage microbial sampler and the Andersen 10-850 two stage microbial sampler. Data obtained from the first stage of the Andersen 10-850 was excluded from the sample population. All samplers were calibrated at a flow rate of 28.3 liters per minute. Indoor sampling times ranged from 2 to 5 minutes which provided detection limits ranging between 18 and 7 colony forming units (CFU) per cubic meter (m³), respectively. Outdoor sampling ranged from 1 to 3 minutes which provided detection limits ranging between 35 and 12

CFU's/m³, respectively. Collection was made on sterile 100 mm x 15 mm polystyrene petri plates containing 30 milliliters of 2% malt extract agar (19,20). All samples were sent to Mycotech Biological, Inc., by 1-3 day courier services for further processing. Immediately upon arrival, collections were incubated at 25°C with 12 hour light-dark cycles. Isolates were examined 7 days after incubation.

Morphological structures of filamentous fungi were characterized utilizing standard light microscopic technique (22-25). Mature sporulating fungal colonies were identified to genus and enumerated as CFU/m³. Yeast, bacteria, Actinomycetes and sterile hyphae were reported as yeast, bacteria, Actinomycetes and sterile hyphae CFU's/m³, respectively. Data obtained from these collections was not adjusted relative to the positive hole conversion.

Results

The evaluation of 934 indoor samples indicated fungal and bacterial bioaerosols that ranged from 0 CFU/m³ to 6077 CFU/m³. This sample set demonstrated an average of 157 CFU/m³. Eighty seven (87) percent of the sample set indicated indoor concentrations below 300 CFU/m³. Seven (7) percent of the samples ranged between 300-500 CFU/m³ and six (6) percent were over 500 CFU/m³. The number of individual bioaerosols per sample range from 0 to 10 with an overall average of 4 bioaerosols observed per sample.

Thirty seven (37) genera of fungi were identified from indoor bioaerosol samples. Unspecified groups of yeast, sterile hyphae, bacteria, Actinomycetes, and unidentified Hyphomycetes were also observed. Seven (7) of the forty two (42) total bioaerosol components were distinguished with respect to the frequency of occurrence. These included *Cladosporium*, yeast, sterile hyphae, *Penicillium*, *Aspergillus*, *Alternaria*, and *Curvularia*. *Cladosporium* was recovered from 77% of the indoor sample set and averaged 92 CFU/m³. Yeast and sterile hyphae were both observed in 56% of the sample set; however, yeast averaged 52 CFU/m³ while sterile hyphae averaged 29 CFU/m³. *Penicillium* was observed in 50% of the indoor samples and averaged 48 CFU/m³. *Aspergillus* was documented to occur in 33% of the indoor samples and demonstrated an average airborne concentration of 20 CFU/m³. *Alternaria* occurred in 17% of the samples and averaged 30 CFU/m³. *Curvularia* was observed in 7% of the samples and demonstrated an average of 20 CFU/m³. The remaining 34 components were

observed in 3% or less of the total sampling population. Despite relatively low frequency of occurrence, several of these incidents, when present, indicated a moderate contribution to bioaerosol concentration. These incidents included; *Geotrichum*, *Fusarium*, *Drechslera*, *Nigrospora*, *Monocillium*, *Cunninghamella*, *Trichoderma*, *Humicola*, and *Pithomyces*.

The evaluation of 182 outdoor samples indicated fungal and bacterial bioaerosols that ranged from 0 CFU/m³ to 12668 CFU/m³. This sample set demonstrated an average of 860 CFU/m³. Thirty three (33) percent of the sample set indicated outdoor concentrations below 300 CFU/m³. Sixteen (16) percent of the samples ranged between 300-500 CFU/m³ and fifty one (51) percent were over 500 CFU/m³.

Thirty three (33) genera of fungi were identified from outdoor bioaerosol samples. Unspecified groups of yeast, sterile hyphae, bacteria and Actinomycetes were also observed. Fourteen (14) of the thirty seven (37) total bioaerosol components observed were distinguished with respect to frequency of occurrence. These included *Cladosporium*, sterile hyphae yeast, *Penicillium*, bacteria, *Alternaria*, *Aspergillus*, *Geotrichum*, *Curvularia*, *Fusarium*, *Epicoccum*, *Drechslera*, *Acremonium*, and *Phoma*. The remaining twenty three (23) components were observed in 4% or less of the total sampling population. Despite relatively low frequency of occurrence, most of these incidents, when present, indicated a moderate to substantial contribution to bioaerosol concentration.

Conclusions

The data obtained from this study indicates that indoor average of 158 CFU/m³ is 5.5 times less, or eighteen percent (18%) of the average outdoor concentration of 860 CFU/m³. This data is consistent with other studies involving the comparison of indoor and outdoor concentrations (26). The indoor average of 158 CFU/m³ is similar to indoor concentrations observed in other research (27,28). The eighteen percent (18%) value obtained in this study falls below the thirty three percent (33%) indoor/outdoor ratio recommended for indoor environments established by the American Conference of Governmental Industrial Hygienists (20). However, the data observed in this research indicates that outdoor bioaerosol fluctuation introduces an inherent problem with respect to utilizing indoor/outdoor ratios as a mechanism to establish recommendations for

indoor exposure. Considerable variation has been documented to occur in outdoor bioaerosol concentrations (29,30). Ten percent (10%) of the total outdoor samples evaluated by this research indicated outdoor concentrations less than 100 CFU/m³, with two (2) samples indicating 0 CFU/m³. The application of the 33% indoor/outdoor ratio to a 100 CFU/m³ outdoor concentration would establish 33 CFU/m³ as a recommended indoor concentration; however, only 27% of the indoor sample collected in this investigation indicated concentrations less than 33 CFU/m³. This data indicates that the application of the 33% indoor/outdoor ratio, when applied to below average or low outdoor concentrations, results in a recommended indoor concentrations which does not reflect the typical indoor data observed in in this study; and furthermore, may be to extreme with respect to providing acceptable concentration limits for indoor environments. Additionally, the application of the 33% indoor/outdoor ratio to 12668 CFU/m³, which was the highest outdoor bioaerosol concentration observed by this study, would establish a concentration of 4180 CFU/m³ as a recommended indoor concentration level. However, only 0.1% of the indoor environments observed indicated indoor concentrations in excess of 4180 CFU/m³. This data indicates that the application of the 33% indoor/outdoor ratio, when applied to above average or high outdoor concentrations, results in a recommended indoor concentration which does not reflect the typical indoor data observed in in this study, and furthermore, may be to liberal with respect to providing acceptable concentration limits for indoor environments. The author suggests that such numerical extremes would potentially exist with respect to any value selected as an indoor/outdoor ratio. Therefore, total indoor/outdoor CFU/m³ comparisons should, in general, be avoided as a basis for evaluating and recommending exposure levels for indoor bioaerosols.

The specific comparison of individual indoor and outdoor components may be of some benefit in evaluating the potential risk for exposure. Literature is of record that indicates that indoor bioaerosols should not be significantly different that outdoor bioaerosols (30). Of the total 45 bioaerosol incidents recovered in this study, twenty percent (20%) were found exclusively indoors. Although present, these bioaerosols occurred in three percent (3%) or less of the indoor sampling population and may be of little concern with respect to establishing general guidelines.

Further investigation is necessary to determine if the recovery of these organisms has some specific relationship with indoor environments in general. Seven percent (7%) of the incidents were found exclusively outdoors. Thirty three (33) of the total forty five (45) bioaerosol components observed were common to both indoor and outdoor samples. Of this sample set, ninety four percent (94%) of the average CFU/m³ were in greater concentration outdoor when compared to indoor. Individual outdoor components ranged from 3 to 71 percent higher in average CFU/m³ with respect to indoor components, respectively. This data is consistent with referenced literature indicating indoor and outdoor populations should be similar, with indoor concentrations being lower than outdoor concentrations for individual organisms. This study indicated *Pithomyces* and *Humicola* were found in a greater indoor concentration when compared with outdoors. Further investigation is necessary to determine if the recovery of these organisms has some specific relationship with indoor environments in general.

The data obtained from this study indicates that concentrations ≤ 200 CFU/m³ may be considered typical for indoor bioaerosols and supports other conclusions involving research on indoor bioaerosols (27,28). Miller, et. al., has suggested that indoor airborne samples should contain less than 300 CFU/m³ of common fungi (e.g. *Cladosporium*) and less than 150 CFU/m³ of all other mixed species other than pathogenic or toxigenic species (32). This statement would suggest that 450 CFU's/m³ would be a maximum recommended value for indoor environments. This value represents 2.9 times the average of 158 observed in this study and 2.25 times the value of 200 CFU/m³ suggested by Yang, et.al. as acceptable indoor concentrations. Holt and Reponen, in separate investigations have sited 500 CFU/m³, or 3.2 times the average indoor CFU/m³ value observed in this study as representative of abnormal indoor fungal sources (30,33).

Evidence is of record that generally identifies bioaerosol concentrations that are considered typical and/or abnormal with respect to indoor environments. Values of less than 200 CFU/m³ have been identified as acceptable for normal environments while values of 450-500 CFU/m³ have been utilized to support the presence of various unacceptable indoor air quality situations. The data gained from this research, in conjunction with other bioaerosol research, provides for the basis of further

characterization of indoor bioaerosol concentrations between 200 and 450 CFU's/m³. The author supports the following recommendations for non-adjusted indoor bioaerosol concentrations:

1. ≤ 300 CFU/m³ of non-toxicogenic or non-pathogenic organisms should be typical for normal, non-immunocompromised environments because 87% of the sample population indicated less than ≤ 300 CFU/m³. With the exception of *Cladosporium*, no organism should individually contribute more than 150 CFU/m³.
2. >300 CFU's/m³ and/or not meeting the above criteria should incite some additional level of investigation with respect to; the bias of prominent outdoor bioaerosol(s), adequate filtration, indoor humidity and microhumidity environments, and/or potential indigenous contamination source(s).

The author cautions on the indiscriminate use of any bioaerosol data and/or recommendations. As with any sampling methodology, false negatives and false positive may occur, as well as diurnal, seasonal or transient variation. Given these factors, the above recommendations are suggested only as general guidelines with respect to interpreting general bioaerosol data. The >300 CFU/m³ threshold is not intended to represent any threshold having medical or health significance and/or necessarily representative of an unacceptable indoor environment. The >300 CFU/m³ is intended to be a "reactionary threshold" to incite further investigation as to the cause(s) of what is perceived to be an above average viable concentration for indoor bioaerosols. The author further suggests that outdoor sampling should only be utilized in the relative comparisons to individual indoor components and that indoor/outdoor ratios involving total CFU/m³ concentrations should not be utilized as a specific mechanism to evaluate acceptable indoor bioaerosol concentrations.

More research is needed to further evaluate and characterize acceptable indoor bioaerosol concentrations and populations. These areas include detailed geographical and seasonal influences, toxigenic and pathogenic organisms, specific yeast and bacteria bioaerosols, as well as investigations into non-typical, immuno-compromised and/or medical environments. New sampling methodologies are being developed to evaluate bioaerosol

concentrations. These include ergosterol, beta Beta 1-3 Glucan, DNA fingerprinting, and others. While these methodologies may offer alternative means to evaluate bioaerosols in the future, the practical use of these technologies is not currently in place. As with all sampling methodologies, certainly these new methodologies will have benefits as well as shortcomings. However, at the present, viable bioaerosol recovery does provide an investigator with a readily available means to evaluate bioaerosols. Despite all the associated variables associated with this methodology, viable bioaerosol sampling offers the clearest avenue of data comparability to current bioaerosol references. This investigation has further contributed to this growing volume of bioaerosol reference and has provided the additional interpretive information with respect to current field investigations.

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