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Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes

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Abstract

In this study, the culturability of indoor and outdoor airborne fungi was determined through long-term sampling (24-h) using a Button Personal Inhalable Aerosol Sampler. The air samples were collected during three seasons in six Cincinnati area homes that were free from moisture damage or visible mold. Cultivation and total microscopic enumeration methods were employed for the sample analysis. The geometric means of indoor and outdoor culturable fungal concentrations were 88 and 102 colony-forming units (CFU) m^{-3}, respectively, with a geometric mean of the *I/O* ratio equal to 0.66. Overall, 26 genera of culturable fungi were recovered from the indoor and outdoor samples. For total fungal spores, the indoor and outdoor geometric means were 211 and 605 spores m^{-3}, respectively, with a geometric mean of *I/O* ratio equal to 0.32. The identification revealed 37 fungal genera from indoor and outdoor samples based on the total spore analysis. Indoor and outdoor concentrations of culturable and total fungal spores showed significant correlations ($r = 0.655$, $p<0.0001$ and $r = 0.633$, $p<0.0001$, respectively). The indoor and outdoor median viabilities of fungi were 55% and 25%, respectively, which indicates that indoor environment provides more favorable survival conditions for the aerosolized fungi. Among the seasons, the highest indoor and outdoor culturability of fungi was observed in the fall. *Cladosporium* had a highest median value of culturability (38% and 33% for indoor and outdoor, respectively) followed by *Aspergillus*/*Penicillium* (9% and 2%) among predominant genera of fungi. Increased culturability of fungi inside the homes may have important implications because of the potential increase in the release of allergens from viable spores and pathogenicity of viable fungi on immunocompromised individuals.

Keywords

Indoor air; Outdoor air; Total fungal spore; Culturable fungi; Culturability

1. Introduction

Fungi are common in indoor and outdoor environment, and nearly 10% of people worldwide have fungal allergy (Burge, 2001). Numerous studies have shown that exposure to fungi may be associated with acute toxic effects, allergies, and asthma (American Conference of Governmental Industrial Hygienes (ACGIH), 1999; Burge, 2001; Bush and Portnoy, 2001).

Modern technology provides the investigators with a variety of ways for analyzing fungi while each method has certain advantages and drawbacks (Pasanen, 2001). Many studies utilized culture-based analysis as well as total spore analysis using optical microscopy. Although the

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culture-based methods have several limitations including lack of reproducibility, selection effect, limitation of sampling time and underestimation of total number of fungal propagules, they allow accounting for culturable fungi (Douwes and Pearce, 2003; Douwes et al., 2003; Meyer et al., 2003). Culture-based analysis is commonly used when species level identification is needed and is a surrogate for viable fungi. Viable fungi can induce infectious disease in immunocompromised individuals (Burge, 2001;Eduard, 2003). Furthermore, recent studies have shown that spores release allergens during their germination (Mitakakis et al., 2001; Green et al., 2003). Thus, viable fungal spores may release more allergens than dead ones after deposition in the respiratory system (Sercombe et al., 2004). On the other hand, the total spore enumeration can reveal human exposure to both non-culturable and culturable fungal spores when counting error is minimized (Douwes et al., 2003; Eduard, 2003). The total spore count has shown better exposure to response relationship compared to the culture-based method (Eduard, 2003). However, in order to elucidate personal exposure to airborne fungi that may act as aeroallergen or pathogen in the residential environment, both the levels of culturable fungi and total fungal spores as well as culturability (the ratio of the culturable to total fungi) should be evaluated properly. The culturability of airborne fungi may be associated with adverse health effects on humans as well as plant and animal diseases. It also can provide a relationship between the dynamics of airborne fungi and environmental variables (Adhikari et al., 2004). Little, however, is known about the culturability of fungi in indoor and outdoor residential environments.

In many published studies, the investigators utilized different sampling techniques for measuring culturable and total fungal spores (Adhikari et al., 2004; Chakraborty et al., 2000; Garrett et al., 1997). However, it is well known that different samplers may differ considerably by their sampling efficiency characteristics, such as the cut-off size (d_{50}), or by the spore extraction efficiency from the collection media (e.g., a filter or a slide). If the samples enumerated for the colony-forming units (CFU) and the total spore number were collected with samplers, which had different d_{50}-values and/or different properties of the collection surface, the comparison of the two databases may not be appropriate. Thus, we believe that the airborne fungi should be collected for the CFU and total enumeration using the same sampler and the same collection media. E.g., Toivola et al. (2002) utilized filter collection of airborne microorganisms with subsequent extraction for culturable and total fungi.

The objective of this study was to investigate the culturability of airborne fungi collected in indoor and outdoor environments in six Cincinnati area single-family homes using long-term (24-h) air sampling with an inhalable sampler, which aimed at generating an exposure database and examine the relationship between the indoor and outdoor culturability of fungi. In the present study, airborne fungal particles were collected with a Button Personal Inhalable Aerosol Sampler, which was designed to measure an inhalation exposure as its inlet efficiency reasonably well fits the inhalable convention of ACGIH/CEN/ISO. The sampler has several advantages including low sensitivity of its performance characteristics to the wind direction, high filter collection uniformity, and the ability to screen out very large particles (Grinshpun et al., 1998). Adhikari et al. (2003) reported that the sampler is efficient for collecting outdoor pollen and fungal spores. In addition, the Button Sampler has also been used for personal and micro-environmental measurement of fungal spores and bacteria (Toivola et al., 2004).

2. Materials and methods

2.1. Selection of sampling sites

The field sampling of indoor and outdoor bioaerosols was performed from March 2004-February 2005 in six typical single-family homes selected from a group of household recruited in the Cincinnati metropolitan area (South-West Ohio and Northern Kentucky). Based on the walk-through database, the homes were selected to meet the following criteria: no visible mold,

no smell of mold, and no previous and present moisture damage. The selected homes were different by the age, window type and house material, and the efficiency of indoor air filtration. Five houses located in residential neighborhoods of the Greater Cincinnati and Northern Kentucky area and were occupied during the study. One house, located in downtown Cincinnati, was mostly unoccupied. The age of houses ranged from 24 to 115 years. Additionally, the largest total finished area was 581 m^2, and the smallest was 71 m^2. Four other houses had approximately the same total finished areas (ranging from 150 to 190 m^2).

2.2. Measurement of indoor and outdoor airborne fungi

Button Personal Inhalable Aerosol Samplers (SKC Inc., Eighty Four, PA, USA) loaded with 25 mm polycarbonate filters (1 μm pore size, GE Osmonics Inc., Minnetonka, MN, USA) were utilized to collect airborne fungi inside the selected homes and outdoors in the vicinity of the homes. The Button Sampler was washed before each 24-h measurement with 5% bleach and 70% ethanol solution, and then sterilized in hot air oven at 180 °C for 1 h. After that, a sterile polycarbonate filter was loaded to the sampler inside of a Class II Biosafety cabinet (Baker, Stanford, ME, USA), covered with a clean cap, and carried in a dust-free box to the sampling sites. Each sampling device operated at a sampling flow rate of 4 L min^{-1}, which was maintained by a small pump (Model 224-PCXR4, SKC Inc., Eighty Four, PA, USA) and verified with DryCal® DC-Lite Calibrator (Bios International Corporation, Butler, NJ, USA) before and after each 24-h measurement. The outdoor samplers were fixed on a tripod with a rain shield at a height of 1.5m and at a distance of 1-2m from the house outside wall. The pumps of indoor samplers were placed inside noise-insulated enclosures to reduce the residents' noise exposure in homes. Each indoor air sampling set-up was placed in a child's primary activity room in order to collect a representative sample of the airborne fungi inhaled by the child. Both indoor and outdoor samplers were oriented vertically relative to the ground. The residents stayed at home and performed their normal activities during the measurements. The sampling sessions were conducted for five subsequent 24-h periods—from Monday mornings to Saturday mornings—in each site. One sampling campaign was carried out in the spring (March-May), the second one was conducted in the fall (September and October), and the third one was performed in winter (January and February). According to Adhikari et al. (2005), the fungal spore concentration levels during late spring and summer seasons in Cincinnati area are rather similar, which may be attributed to moderate differences in outdoor temperature and relative humidity. For this reason, no measurements were conducted during the period from June-August. The meteorological information, including air temperature and relative humidity indoors and outdoors, as well as the outdoor precipitation level and wind speed was recorded during sampling sessions using a meteorological station (Vantage Pro, Davis Instruments, Hayward, CA, USA) operating on each site.

2.3. Sample analysis

After air sampling, the filters were unloaded from the sampler to 50 mL test tubes (Fisher Scientific, Pittsburgh, PA, USA) containing 10 mL extraction liquid. Extraction fluid was prepared from 1 L of deionized filtered sterilized water, 1 g of Bacto™ Peptone (Becton, Dickinson and Company, Sparks, MD, USA), and 0.05% of Tween 80 (Fisher Chemicals, Fair Lawn, NJ, USA). The collected particles were extracted from the filters using a touch mixer (Model 231, Fisher Scientific, Pittsburgh, PA, USA) that operated for 2 min followed by a 10-min ultrasonic bath (FS20, Fisher Scientific, Pittsburgh, PA, USA) agitation. Our earlier study revealed that a method combining vortexing with ultrasonic agitation exhibited an extraction efficiency of 96-98% when the microorganisms were extracted from polycarbonate filters; at the same time, it did not considerably reduce the culturability of microorganisms, including sensitive bacteria such as *Pseudomonas fluorescens* (Wang et al., 2001). All the filter preparation and handling procedures were performed in a Class II Biosafety cabinet.

The concentration of culturable fungi was obtained after 0.1 mL of extracted fluid was inoculated onto triplicate malt extract agar (MEA, Difco Laboratories, Detroit, MI, USA) supplemented with streptomycin sulphate (40 mg L^{-1}, Fisher Scientific, Fair Lawn, NJ, USA). The colony forming units of culturable fungi were counted after 7-14 days of incubation at a temperature of 25±2 °C.

The microscopic analysis was applied to obtain the concentration of total fungal spores. Following the above-described extraction process; the extracted solution was filtered on a mixed cellulose ester (mixture of cellulose acetate and cellulose nitrate, Pore size 1.2 μm, diameter 13 mm, Millipore corporation, Bedford, MA, USA) membrane filter and cleared by a modified acetone vaporizing unit (Model: Quixfix, Environmental monitoring system, Charleston, SC, USA) with a continuous flow of acetone vapor. The filter was stained with glycerin jelly (gelatin 20 g, phenol crystals 2.4 g, glycerol 60 ml, water 70 ml) mixed with calberla's stain for light microscopic analysis. The fungal spores were identified and counted under the 100 × or 400 × magnification of a high-resolution light microscope (labophot 2, Nikon Corp.; Japan) in at least 40 randomly selected microscopic fields.

The identification of fungi was conducted up to the genus, family, or class level based on their morphological characteristics. Reference slides (Aerobiology Instruction and Research, Brookline, MA, USA) and illustrated identification manual by Smith (1990) were used for fungal spore identification.

The culturability of airborne fungi, defined as the ratio of culturable fungi concentration to the total fungal spore concentration, was calculated as

$$\text{Culturability airborne fungi} \quad (\%) = \frac{\text{Concentration of culturable fungi} \quad \left(\text{CFUm}^{-3}\right)}{\text{Concentration of total fungal spores} \quad (\text{sporesm}^{-3})} \times 100.$$

2.4. Data analysis

Descriptive statistics included range, median, geometric mean (GM) and geometric standard deviation (GSD) were employed for the data analysis. Before analysis, a value of one (CFU m^{-3} or spores m^{-3}) was added to all concentration results. This allowed us to account for the underestimation associated with the levels below limit of detection (LOD) and to obtain GM and GSD. In the end of this procedure, value one was deducted from the calculated GM (Eudey et al., 1995).

In accordance with the normality test (PROC Univariate, SAS/Stat 9.1, SAS Institute Inc., Cary, NC, USA) of indoor and outdoor concentrations of culturable fungi and total fugal spores, only indoor total fungal spore concentration showed a lognormal distribution as determined by the Shapiro-Wilk W statistics. Therefore, non-parametric statistics was used in the data analysis. Wilcoxon Signed Rank test (PROC Npar1way wilcoxon, SAS/Stat 9.1) was applied to compare the difference of indoor and outdoor concentration for culturable fungi and total fungal spores and their *I/O* ratios. To study the correlation between indoor and outdoor concentrations for both airborne fungi concentrations, Spearman Correlation coefficients (PROC Corr Spearmam, SAS/Stat 9.1) were determined. Spearman correlation was also used to study the relationship between fungal concentrations and simultaneously measured meteorological factors.

3. Results and discussion

3.1. Indoor and outdoor concentration of culturable fungi

The indoor concentrations of culturable fungi were between 0 and 1362 CFU m^{-3} with a median of 89 CFU m^{-3}. The outdoor concentration of culturable fungi ranged from 0 to 3882 CFU m^{-3} with a median of 168 CFU m^{-3}.

The indoor and outdoor GM and as well as the indoor to outdoor (*I/O*) ratios—all for culturable fungi—integrated over the six tested homes for each of the three seasons are presented in Table 1. According to Wilcoxon signed rank test, the indoor and outdoor CFU levels integrated over the entire test period (three seasons) were not significantly different ($p = 0.080$). As to the CFU concentrations averaged over a specific season, only the fall database showed statistically significant difference between the indoor and outdoor levels ($p = 0.005$). Shelton et al. (2002) have shown that fungal concentration in American Midwest generally reaches the lowest level in the winter season. Increasing through the spring, it peaks during summer and fall seasons. The indoor and outdoor culturable fungal spore concentration levels obtained in this investigation were mostly lower than those reported in previous studies. E.g., the median levels of indoor and outdoor culturable fungi in 80 homes in Latrobe Valley, Australia were 812 and 1042 CFU m^{-3}, respectively (Garrett et al., 1997). The median indoor and outdoor concentrations of 1717 buildings located across the United States were 82 and 540 CFU m^{-3}, respectively (Shelton et al., 2002). In addition, a study conducted in Greater New Haven, Connecticut revealed higher mean levels of culturable fungi (Ren et al., 1999) than found in the present study. However, the data collected in the Atlanta area and reported by Horner et al. (2004) are close to our data with respect to the indoor median concentration of culturable fungi. This might be because (a) the climate conditions and vegetation in Atlanta are similar to those we observed in Cincinnati and (b) Horner et al. pre-screened the sampling sites before measurement to focus on the houses free from water damage for minimizing the indoor sources of airborne fungi (similarly to our approach). Twenty six genera for indoor and outdoor culturable fungi were detected in malt extract agar. The most prevalent culturable fungal type indoors was non-sporulating fungi (frequency of occurrence 81.1%). It was followed by *Penicillium* (76.7%), *Cladosporium* (63.6%), *Aspergillus* (41.1%), Yeast (17.8%), and *Chrysosporium* (11.1%). Other genera occurred in frequencies below 6% and most of them were found in low concentrations. In outdoor environment, the non-sporulating fungi (81.1%) was the predominant type followed by *Cladosporium* (70.0%), *Penicillium* (60.0%), *Aspergillus* (43.3%), *Alternaria* (24.4%) and Yeast (13.3%). The predominant culturable fungi reported in this study are consistent with previous studies (Ren et al., 1999;Shelton et al., 2002;Adhikari et al., 2005). Indoor and outdoor environments revealed exactly the same frequency of 81.1% of non-sporulating culturable fungi, which may be originated from Ascomycetes and Basidiomycetes. The concentration of non-sporulating fungi significantly correlated with that of Ascospores and Basidiospores (indoor: $r = 0.423$, $p = 0.0004$, and outdoor: $r = 0.516$, $p<0.0001$). It is explainable given that species, which belong to Ascomycetes or Basidiomycetes, may grow but are unlikely to sporulate on malt extract agar (Horner et al., 1994;Levetin et al., 1995). Certain types of genera such as *Paecilomyces* and *Sporothrix* were recovered only from indoor samples while *Aureobasidium*, *Curvularia*, Diatrypaceae, *Epicoccum*, *Humicola*, *Paracoccodioides*, *Pithomyces*, and *Ulocladium* were recovered only from outdoor samples.

3.2. Indoor and outdoor concentration of total fungal spores

The indoor level of total fungal spores ranged from 13 to 6302 spores m^{-3} with a median value of the entire database equal to 246 spores m^{-3}. The outdoor levels of total fungal spores were between 0 and 7704 spores m^{-3} with a median of 778 spores m^{-3}.

The indoor concentrations of total fungal spores integrated over the three-season period were significantly different from those detected outdoors ($p<0.0001$). The indoor concentration integrated per season also showed statistically significant differences according to Wilcoxon singed rank test (spring: $p<0.001$, fall: $p<0.0001$, winter: $p = 0.048$).

Spores from 37 different types were detected in homes and outdoors. With respect to the total spore count, the prevailing indoor total fungal spore genera/class/groups were *Aspergillus*/*Penicillium* (98.9%), Ascospores (82.2%), *Cladosporium* (70.0%), Basidiospores (46.7%), Smut spores (30.0%), *Ganoderma* (12.2%), and *Epicoccum* (11.1%). The frequency of unidentified spores occurred in 73.3% of the samples. For outdoors, *Aspergillus*/*Penicillium* (98.9%) was the most prevalent genus followed by Ascospores (86.7%), *Cladosporium* (81.1%), Basidiospores (65.5%), Smut spores (53.3%), *Ganoderma* (33.3%), *Epicoccum* (32.2%), and *Alternaria* (31.1%). The frequency of unidentified spores in outdoor samples was 87.8%. *Sporidesmium* and *Tetracoccosporium* were detected only in homes while many genera including *Botrydiplodia*, *Cercospora*, *Chaetomium*, *Coprinus*, *Drechslera*, *Fusarium*, *Panaeolus*, *Pestalotiosis*, *Phaeotrichochonis*, *Podospora*, *Polythrincium*, Rust spores, *Sordaria*, *Spegazzinia*, and *Sporormiella* were detected solely in the ambient air. Li and Kendrick (1995) reported indoor and outdoor total fungal spores in 15 Canadian residences. The list of predominant fungi identified by these investigators is almost the same as in our study but our concentration levels are lower. It should be noted that the referred investigation was performed in the geographic regions different from Southwest Ohio in terms of climate characteristics and vegetation. The differences may also be attributed—although to a smaller extent—to the fact that our measurements were conducted with an inhalable sampler, which has the collection efficiency of <100% for larger fungal spores.

3.3. Comparison of the culturable and total counts

While the concentrations of culturable airborne fungi were lower than those of total fungal spores ($p = 0.0002$ for indoor concentration and $p<0.0001$ for outdoor one), the *I/O* ratio of culturable fungi exceeded that of total fungal spores ($p = 0.0002$). The *I/O* ratios of culturable fungi ranged from 0.04 (measured in fall) to 53.34 (measured in spring) with a median of 0.58. These are greater than the *I/O* ratios reported by Burge et al. (2000) for office buildings. It may be mainly due to the usage of central HVAC system with supply air filtration in the commercial buildings. The median of *I/O* ratio for *Penicillium* was the highest among the dominant genera (1.09) of culturable fungi followed by non-sporulating fungi (0.49), *Aspergillus* (0.24), and *Cladosporium* (0.17). The *I/O* ratio of total fungal spores ranged from 0.01 to 61.78 with a median of 0.35. The median of *I/O* ratio for Smut spores (0.48) was the highest among the most common fungal types as determined from microscopic count of total fungal spores followed by *Aspergillus*/*Penicillium* (0.42), Ascospores (0.34), Basidiospores (0.22), *Cladosporium* (0.20), and *Ganoderma* (0.13). The seasonal analysis revealed that both fall and winter *I/O* ratios for culturable fungi were significantly greater than those of total fungal spores ($p = 0.0004$ and $p = 0.005$, respectively).

Table 2 shows Spearman Correlation Coefficients between the concentrations of airborne culturable fungi, total fungal spores, and simultaneously measured meteorological factors. The indoor culturable fungi had a significant correlation with the outdoor culturable fungi, indoor temperature, and outdoor temperature. Meanwhile, we found that the correlation coefficient between the indoor culturable fungi and both indoor and outdoor total fungal spores was low. The outdoor culturable fungi had a strong positive correlation with outdoor temperature, indoor temperature, and outdoor total fungal spores. The indoor total fungal spores showed a significant correlation with the outdoor total fungal spores and indoor and outdoor temperature but had a negative correlation with outdoor relative humidity. The outdoor total fungal spores had a positive correlation with indoor and outdoor temperature. It was noted that the

temperature correlated better with the concentration of airborne fungi than relative humidity. This finding is consistent with our previous study of Adhikari et al. (2005) conducted in the Greater Cincinnati area, in which the outdoor airborne concentration of fungi was shown to be significantly affected by temperature while the correlation between concentration of airborne fungi and relative humidity were neither statistically significant nor strong. It may be because certain types of fungal spores including *Cladosporium*, *Alternaria*, and *Epicoccum* belong to dry-air spora that generally exhibits the highest concentration in the atmosphere with low humidity condition, while the others such as Ascospores and Basidiospores belong to wet-air spora that requires moisture for spore release (Levetin, 1995;Troutt and Levetin, 2001). Thus, different fungal species react differently to the air humidity changes, while the air temperature affects the dispersal of different species in a similar way.

3.4. Culturability

The culturability data are presented in Fig. 1. The indoor levels ranged from 1% to >100% with a median of 55% and outdoor levels were between 1% and >100% with a median of 25%. The measured culturability values above 100% (some were much greater than 100%) may be attributed to the growth of fungal mycelium fragments on the nutrient medium. Fungal mycelium cannot be counted as fungal spores under the microscope. However, both fungal spores and mycelium can form colonies on the agar medium (Lee et al., 2005). Li and Kendrick (1995) reported that the average concentration of hyphal fragments was 146 pieces m^{-3} (6.3% of total counts) inside 15 homes in Canada. Interestingly, the concentration of indoor hyphal fragment was mostly higher in indoors than in outdoors.

Throughout the three seasons tested in this study, the indoor culturability levels of airborne fungi were significantly greater than those obtained from outdoor samples ($p = 0.0005$). With respect to specific seasons, the indoor fungal spore culturabilities determined in fall and winter were greater than those found outdoors ($p = 0.006$ and 0.02, respectively). The indoor median values of culturability of airborne fungi for spring, fall, and winter were 37%, 87%, and 51%, respectively, and the outdoor median values for these seasons were 15%, 32%, and 18%, respectively. The overall culturability was found to be higher in indoor environments than outdoors. It might be related to the environmental variables as discussed below.

Since each air sample resulted from a 24-h collection of airborne spores on the filter, we anticipated that desiccation would affect the culturability (although fungal spores are stress-resistant). However, the culturability obtained in our measurements was greater than the culturable bioaerosol levels reported by some other investigators. For instance, Karlsson and Malmberg (1989) found culturability of approximately 17% including bacteria, actinomycetes, and fungi together in an agricultural environment. Eduard et al. (1990) reported that the culturability of fungi in a sawmill and a pig confinement ranged from 3% to 98%. The culturability level obtained by Hanhela et al. (1995) was between 1% and 10%; however, they also included actinomycete spores in the total count. It should be noted that measurements reported in the above-cited papers were conducted in environments with extremely high levels of the fungal spore concentration: up to 10^{10} spores m^{-3} and 10^{10} CFU m^{-3}. Toivola et al. (2002) measured the fungal spore culturability in a personal exposure study performed in winter in Finland. The investigators found very low culturability levels (below 1% on the average) that may be attributed to low longterm air temperatures outdoors during their measurements.

The indoor and outdoor culturability that we found in spring and fall had a significant correlation ($r = 0.737$, $p<0.0001$ and $r = 0.785$, $p<0.0001$) but the winter data showed non-significant correlation ($r = 0.352$, $p = 0.09$). The median of the *I/O* ratio for culturability of airborne fungi in spring, fall, and winter were 1.4, 2.2, and 3.3 respectively. The *I/O* ratio of culturability for spring was significantly lower than for fall ($p = 0.004$) and winter ($p = 0.038$). However, fall and winter data did not show any difference with respect to the *I/O* ratio for

culturability of airborne fungi. The median of *I/O* culturability ratio was greater than one when integrated over the entire three-season period. The average and standard deviation of indoor and outdoor temperature during the measurements were 22±4 °C and 11±8 °C, respectively. Among prevalent genera of indoor and outdoor airborne fungi, *Cladosporium* had a highest median value of culturability (38% for indoor and 33% for outdoor environment) followed by *Aspergillus*/*Penicillium* (9% for indoor and 2% for outdoor).

In conclusion, the culturability of indoor airborne fungi was mostly greater than that of fungi measured outdoors, which indicates that indoor environment provides more favorable conditions for survival of airborne fungal spores. The indoor and outdoor airborne fungi concentrations had a significant positive correlation. At the same time, the *I/O* ratio was mostly below one, which suggests that the indoor inhalation exposure to airborne fungi is largely influenced by outdoor airborne fungal concentrations. The pilot data from this study will help in understanding human exposure to airborne fungi in relatively clean residential homes. Increased culturability of fungi in indoor homes is significant because it may lead to the increased allergen release from spores and some culturable fungi may cause infections in immunocompromised individuals. The health-effect potential of live versus dead airborne spores has been addressed in several recent studies. Hirvonen et al. (1997) reported that culturable actinomycete (not fungal) spores induced more reactive oxygen species than dead spores in macrophages; no difference, however, was found in the nitrite production. Mitakakis et al. (2001), who studied the allergen release from individual *Alternaria* spores utilizing the Halogen immunoassay method, reported that more *Alternaria* allergen released from germinated spores. In addition, Green et al. (2003) detected allergen from 11 fungal species before and after germination and found that germination of spores increased the allergen level significantly. It suggests that live spores may have a greater potential to evoke inflammatory diseases than dead spores when they are deposited in the respiratory tract.

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References

Adhikari A, Martuzevicius D, Reponen T, Grinshpun SA, Cho S, Sivasubramani SK, Zhong W, Levin L, Kelly AL, St. Clair H, LeMasters G. Performance of the button personal inhalable sampler for the measurement of outdoor aeroallergens. Atmospheric Environment 2003;37:4723–4733.

Adhikari A, Sen MM, Gupta-Bhattacharya S, Chanda S. Airborne viable non-viable and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations. Science of the Total Environment 2004;326:123–141. [PubMed: 15142771]

Adhikari A, Reponen T, Grinshpun SA, Martuzevicius D, LeMasters G. Correlation of ambient inhalable bioaerosols with particulate matter and ozone: a two-year study. Environmental Pollution 2005;140:16–28. [PubMed: 16183184]

American Conference of Governmental Industrial Hygienes (ACGIH). Health effects of bioaerosols. Bioaerosols: Assessment and Control; Cincinnati, OH: 1999. Chapter 3

Burge HA. Fungi: toxic killers or unavoidable nuisance? Annals of Allergy, Asthma & immunology 2001;87(6 Suppl 3):52–56.

Burge HA, Pierson DL, Groves TO, Strawn KF, Mishra SK. Dynamics of airborne fungal populations in a large office building. Current Microbiology 2000;40:10–16. [PubMed: 10568797]

Bush RK, Portnoy JM. The role and abatement of fungal allergens in allergic disease. Journal of Allergy & Clinical Immunology 2001;107(3):S430–S440. [PubMed: 11242604]

Chakraborty S, Sen SK, Bhattacharya K. Indoor and outdoor aeromycological survey in Burdwan, West Bengal, India. Aerobiologia 2000;16:211–219.

Douwes J, Pearce N. Invited commentary: is indoor mold exposure a risk factor for asthma? American Journal of Epidemiology 2003;158:203–206. [PubMed: 12882941]

Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effect and exposure assessment progress and prospects. British Occupational Hygiene Society 2003;47(3):187–200.

Eduard W. The performance of culture-based methods and microscopy for quantification of noninfectious airborne microorganisms in epidemiological studies of highly contaminated work environments. American Industrial Hygiene Association Journal 2003;64:684–689. [PubMed: 14521428]

Eduard W, Lacey J, Karlsson K, Palmgren U, Strom G, Blomquist G. Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated occupational environments. American Industrial Hygiene Association Journal 1990;51(8):427–436. [PubMed: 2203242]

Eudey, L.; Su, HJ.; Burge, HA. Biostatistics and Bioaerosols. In: Burge, HA., editor. Bioaerosols. CRC Press Inc.; Boca Raton, FL: 1995. p. 269-307.

Garrett MH, Hooper BM, Cole FM, Hooper MA. Airborne fungal spores in 80 homes in the Latrobe Valley, Australia: levels, seasonality and indoor-outdoor relationship. Aerobiologia 1997;13:121–126.

Green BJ, Mitakakis TZ, Tovey ER. Allergen detection from 11 fungal species before and after germination. Journal of Allergy & Clinical Immunology 2003;111:285–289. [PubMed: 12589346]

Grinshpun SA, Aizenberg V, Willeke K, Smith JP, Baron PA. Personal aerosol sampler with curved porous inlet: wind tunnel evaluation using a full-size manikin. Journal of Aerosol Science 1998;29:1137–1138.

Hanhela R, Louhelainen K, Pasanen AL. Prevalence of microfungi in Finnish cow barns and some aspects of the occurrence of Wallemia Sebi and Fusaria. Scandinavian Journal of Work, Environment, and Health 1995;21:223–228.

Hirvonen MR, Ruotsalainen M, Savolainen K, Nevalainen A. Effect of culturability of actinomycete spores on their ability to stimulate production of nitric oxide and reactive oxygen species in RAW264.7 macrophages. Toxicology 1997;124:105–114. [PubMed: 9458000]

Horner WE, Lehrer SB, Salvaggio JE. Fungi. Immunology and Allergy Clinics of North America 1994;14 (3):551–566.

Horner WE, Worthan AG, Morey PR. Air- and dustborne mycoflora in houses free of water damage and fungal growth. Applied and Environmental Microbiology 2004;70(11):6394–6400. [PubMed: 15528497]

Karlsson K, Malmberg P. Characterization of exposure to molds and actinomycetes in agricultural dusts by scanning electron microscopy, fluorescence microscopy and the culture method. Scandinavian Journal of Work Environment & Health 1989;15:353–359.

Lee, S.; Adhikari, A.; Grinshpun, SA.; McKay, R.; Shukla, R.; Reponen, T. Journal of Occupational & Environmental Hygiene. 2005. Personal exposure to airborne dust and microorganisms in agricultural environments. in press

Levetin, E. Fungi. In: Burge, HA., editor. Bioaerosols. CRC Press Inc.; Boca Raton, FL: 1995. p. 87-120.

Levetin E, Shaughnessy R, Fisher E, Ligman B, Harrison J, Brennan T. Indoor Air quality in schools: exposure to fungal allergens. Aerobiologia 1995;11:27–34.

Li DW, Kendrick B. A year-round comparison of fungal spores in indoor and outdoor air. Mycologia 1995;87(2):190–195.

Meyer HW, Würtz H, Suadicani P, Valbjørn O, Sigsgaard T, Gyntelberg F. Molds in floor dust and building-related symptoms in adolescent school children. Indoor Air 2003;14:65–72. [PubMed: 14756847]

Mitakakis TZ, Barnes C, Tovey ER. Spore germination increases allergen release from Alternaria. Journal of Allergy & Clinical Immunology 2001;107:388–390. [PubMed: 11174210]

Pasanen P. A review: fungal exposure assessment in indoor environments. Indoor Air 2001;11:87–98. [PubMed: 11394015]

Ren P, Jankun TH, Leaderer BP. Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American county. Journal of Exposure Analysis and Environmental Epidemiology 1999;9:560–568. [PubMed: 10638841]

Sercombe JK, Green BJ, Tovey ER. Recovery and identification of fungal spores from the nasal cavity. Journal of Allergy and Clinical Immunology 2004;113(2):S231.

Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. Applied and Environmental Microbiology 2002;68(4):1743–1753. [PubMed: 11916692]

Smith, EG. Sampling and Identifying Allergenic Pollens and Molds. Blewstone Press; San Antonio, TX: 1990.

Toivola M, Alm S, Reponen T, Kolari S, Nevalainen A. Personal exposures and microenvironmental concentrations of particles and bioaerosols. Journal of Environmental Monitoring 2002;4:166–174. [PubMed: 11871701]

Toivola M, Nevalainen A, Alm S. Personal exposures to particles and microbes in relation to microenvironmental concentrations. Indoor Air 2004;14:351–359. [PubMed: 15330795]

Troutt C, Levetin E. Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. International Journal of Biometeorology 2001;45:64–74. [PubMed: 11513049]

Wang Z, Reponen T, Grinshpun SA, Gorny RL, Willeke L. Effect of sampling time and air humidity on the bioefficiency of filter samplers for bioaerosol collection. Journal of Aerosol Science 2001;32:661–674.

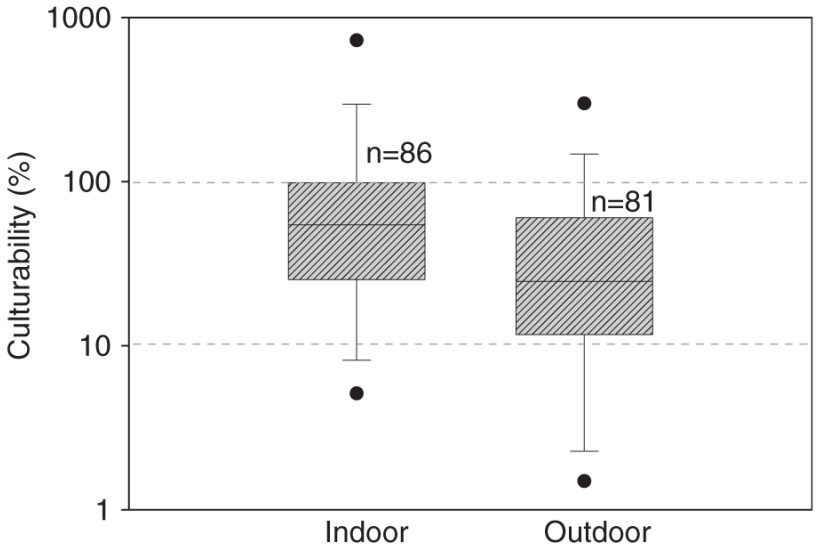

Fig. 1.

The box plot of indoor and outdoor culturability (%) for airborne fungi. The horizontal lines in the box plot from bottom to top indicate 10th, 25th, 50th (median), 75th, and 90th percentiles. The circles indicates the 5th (lower circle) and 95th (upper circle) percentiles. *N* is the number of samples.

Table 1

Indoor and outdoor concentrations of culturable fungi and total fungal spores integrated per each season and over the entire three-season database (overall)

Seasons	Number of samples	Culturable fungi (CFU m^{-3})			Total fungal spores (spores m^{-3})		
		Indoor	Outdoor	*I/O* ratio	Indoor	Outdoor	*I/O* ratio
Spring	30	90(4.69)	171(5.63)	0.54(3.37)	487(2.32)	1192(2.28)	0.41(2.57)
Fall	30	142(4.63)	355(4.10)	0.38(2.56)	155(3.76)	959(2.97)	0.16(2.39)
Winter	30	53(3.23)	17(7.78)	1.71(4.00)	124(3.66)	193(4.11)	0.51(3.24)
Overall	90	88(1.47)	102(2.16)	0.66(3.74)	211(1.50)	605(2.33)	0.32(3.05)

Each cell lists the geometric mean with the geometric standard deviation indicated in parentheses.

Table 2

Spearmean correlation coefficients between the concentrations of airborne culturable fungi, total fungal spores, and simultaneously measured meteorological factors

Variables	Outdoor culturable fungi	Indoor total fungal spores	Outdoor tota l fungal spores	Indoor temperature	Outdoor temperature	Indoor relative humidity	Outdoor relative humidity	Wind speed	Precipitation
Indoor culturable fungi	**0.655 (<0.0001, *n* = 90)**	**0.323 (0.002, *n* = 90)**	**0.364 (0.0004, *n* = 90)**	**0.509 (<0.0001, *n* = 88)**	**0.547 (<0.0001, *n* = 88)**	0.111 (0.303, *n* = 88)	-0.132 (0.220, *n* = 88)	—	—
Outdoor culturable fungi	—	0.157 (0.141, *n* = 90)	**0.550 (<0.0001, *n* = 90)**	**0.659 (<0.0001, *n* = 88)**	**0.743 (<0.0001, *n* = 88)**	0.189 (0.077, *n* = 88)	-0.013 (0.902, *n* = 88)	-0.038 (0.727, *n* = 88)	0.118 (0.275, *n* = 88)
Indoor total fungal spores	—	—	**0.633 (<0.0001, *n* = 90)**	**0.343 (0.001, *n* = 88)**	**0.428 (<0.0001, *n* = 88)**	0.085 (0.431, *n* = 88)	**-0.256 (0.016, *n* = 88)**	—	—
Outdoor total fungal spores	—	—	—	**0.647 (<0.0001, *n* = 88)**	**0.717 (<0.0001, *n* = 88)**	0.195 (0.068, *n* = 88)	-0.170 (0.113, *n* = 88)	0.149 (0.166, *n* = 88)	-0.087 (0.422, *n* = 88)

Significant correlations are marked bold.

p values and number of samples are mentioned in the parenthesis.