Published in final edited form as:
Indoor Air. 2006 February ; 16(1): 37–47.

Relationship between indoor and outdoor bioaerosols collected with a button inhalable aerosol sampler in urban homes

T. Lee[1], **S. A. Grinshpun**[1], **D. Martuzevicius**[1,2], **A. Adhikari**[1], **C. M. Crawford**[1], **J. Luo**[3], and **T. Reponen**[1]

1 *Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, Cincinnati, OH, USA*

2 *Department for Environmental Engineering, Kaunas University of Technology, Kaunas, Lithuania*

3 *Center for Biostatistical Services, Department of Environmental Health, University of Cincinnati, Cincinnati, OH, USA*

Abstract

This field study investigated the relationship between indoor and outdoor concentrations of airborne actinomycetes, fungal spores, and pollen. Air samples were collected for 24 h with a button inhalable aerosol sampler inside and outside of six single-family homes located in the Cincinnati area (overall, 15 pairs of samples were taken in each home). The measurements were conducted during three seasons – spring and fall 2004, and winter 2005. The concentration of culturable actinomycetes was mostly below the detection limit. The median indoor/outdoor ratio (I/O) for actinomycetes was the highest: 2.857. The indoor of fungal and pollen concentrations followed the outdoor concentrations while indoor levels were mostly lower than the outdoor ones. The I/O ratio of total fungal spores (median = 0.345) in six homes was greater than that of pollen grains (median = 0.025). The low I/O ratios obtained for pollen during the peak ambient pollination season (spring) suggest that only a small fraction penetrated from outdoor to indoor environment. This is attributed to the larger size of pollen grains. Higher indoor concentration levels and variability in the I/O ratio observed for airborne fungi may be associated with indoor sources and/or higher outdoor-to-indoor penetration of fungal spores compared to pollen grains.

Keywords

Bioaerosol; Exposure; Indoor; Outdoor; Bacteria; Fungal spore; Pollen

Introduction

Adequate assessment of human exposure to biological aerosols has been recognized as an important need as many studies have linked adverse health effects to bioaerosol hazards [Burge and Rogers, 2000; Douwes et al., 2003; Institute of Medicine (IOM), 2004; Verhoeff and Burge,

S. A. Grinshpun, 3223 Eden Avenue, PO Box 670056, Cincinnati, OH, 45267-0056, USA, Tel:+1 513 558 0504, Fax: +1 513 558 2263, e-mail: sergey.grinshpun@uc.edu.

Practical Implication

This study addresses the relationship between indoor and outdoor concentrations of three different types of bioaerosols, namely actinomycetes, fungal spores, and pollen grains. The results show that actinomycetes are rare in indoor and outdoor air in Midwest, USA. Exposure to pollen occurs mainly in the outdoor air even during peak pollen season. Unexpectedly high fungal spore concentrations were measured outdoors during winter. The presented pilot database on the inhalable levels of indoor and outdoor bioaerosols can help apportion and better characterize the inhalation exposure to these bioaerosols. Furthermore, the data can be incorporated into existing models to quantify the penetration of biological particles into indoor environments from outdoors.

1997]. Lierl and Hornung (2003) indicated that elevated outdoor pollen concentration levels observed in spring and summer had a strong association with exacerbations of asthma in children living in the Cincinnati metropolitan area. In addition, ambient fungal spore level has been correlated with hospital visits for asthmatic symptoms in Canada (Dales et al., 2000). Several types of fungal genera such as *Cladosporium* and *Epicoccum* in outdoor air were associated with deficit in peak expiratory flow rates in children (Neas et al., 1996). Dampness and mold in buildings have also been associated with increased incidence of upper respiratory tract (nasal and throat) symptoms, cough, wheeze, and asthma symptoms in sensitized asthmatic persons (Bornehag et al., 2001; IOM, 2004). A recent cohort study on 849 infants published by Gent et al. (2002) reported an association between increased concentration of airborne culturable fungi and persistent cough and wheeze. Stark et al. (2003) reported that exposure to high fungal concentrations among 499 children increased the risk of lower respiratory illnesses during infancy.

Actinomycetes are a group of soil bacteria that can grow both in a yeast form and produce mycelium and spores like filamentous fungi. These organisms have been implicated in hypersensitivity pneumonitis cases in agricultural environments (Lacey and Dutkiewicz, 1994). Actinomycetes have been listed as one of the indicator microorganisms for mold problems in buildings (Samson et al., 1994). These spores may be found in office or residential buildings that have excessive microbial growth because of moisture accumulation within the structure or inside the heating, ventilation and air conditioning (HVAC) system (Cole et al., 1994; Nevalainen et al., 1991).

While people, particularly infants and toddlers, spend approximately 90% of their time in indoor environments (Klepeis et al., 2001), bioaerosol sources are often located outdoors. Therefore, the influence of these sources on human exposure largely depends on the fraction of outdoor bioaerosol, which is subjected to indoor penetration.

Studies by Jones et al. (2000); Thatcher et al. (2003); Vette et al. (2001) and others have investigated the relationship between indoor and outdoor airborne aerosol concentrations and particle size distributions. Although the investigators reported that indoor air quality generally follows the ambient (outdoor) air quality, the indoor–outdoor relationship has not been systematically studied for various types of biological particles. Most of the information is based on the simultaneous measurement of culturable fungal spores performed indoors and outdoors, as this approach is generally accepted for investigating mold-problem buildings. For example, Shelton et al. (2002), who measured airborne fungi in 1717 buildings and outdoor environments in the USA, found that the median indoor-to-outdoor ratio (I/O ratio) for culturable airborne fungal concentration was 0.16 and 25th-to-75th percentile of I/O ratio laid between 0.049 and 0.45. A review paper (Gots et al., 2003) referred to various studies with regard to indoor and outdoor fungi concentrations but most of those studies were limited to culturable fungi. Furthermore, many investigators based their conclusions on short-term air sampling. These conclusions may not fully represent the long-term trends in exposure. It is well known that a shorter sampling time is likely to decrease the representativeness of samples and increase the variability between samples, especially for those of biological origin. In contrast, a long-term sampling has a greater potential to generate an adequate database (Pasanen, 2001). Stock and Morandi (1988) are among the few authors that have addressed multiple types of biological particles in their measurements. Resulting from their monitoring of fungal spores and pollen inside and outside of twelve Houston area houses, the investigators reported that the I/O ratios were 0.2 and 0.3, for fungal spores and pollen, respectively. Sterling and Lewis (1998) reported the indoor and outdoor concentration data on fungal spores and pollen obtained with Rotorod sampler in mobile homes around Huston and El Paso. They found that I/O ratios of fungal spores measured in winter, spring, and summer were 0.45, 0.08, and 0.70, respectively, while for pollen these seasonal levels were 0.43, 0.37, and 0.91, respectively. However, it should be

noted that – among several limitations of the Rotorod sampler – its sampling efficiency curve does not represent the inhalation curve. At the same time, to better understand the potential inhalation exposure to bioaerosol agents, which may cause respiratory effects (including aeroallergens), it is important that the indoor and outdoor concentrations of different types of bioaerosols (as well as their spatial and seasonal variability) be measured using a personal inhalable aerosol sampler well-characterized as to its sampling efficiency.

In this study, the indoor and outdoor concentrations of actinomycetes, total fungal spores and pollen were obtained using a button inhalable aerosol sampler, which has previously been validated for bioaerosols, such as bacteria, fungi, and pollen (Adhikari et al., 2003; Toivola et al., 2004). The measurements were performed inside and outside of six houses during five consecutive 24 h periods. The I/O ratios, determined for specific genus, family, or class level of fungi and pollen, were compared with aerodynamic size of respective biological particle type.

Materials and methods

Selection of sampling site

Field sampling of indoor and outdoor bioaerosols was performed from March 2004 through February 2005 in six typical single-family homes selected from the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) cohort. CCAAPS includes more of 800 households recruited in south-west Ohio and northern Kentucky (the Cincinnati metropolitan area) and aims at investigating the interaction between diesel engine exhaust particles and aeroallergens in the development of children's allergy and asthma. In accordance with the walkthrough database of the CCAAPS, six homes were selected to meet the following criteria: no visible mold, no smell of mold, no previous and present moisture damage, and no indoor plant population in the homes. However, the homes were different by the age, window type and house material, and the efficiency of indoor air filtration. The summary of home characteristics is presented in Table 1. Five houses (nos 1, 2, 3, 5, and 6), located in residential neighborhoods of the Greater Cincinnati and northern Kentucky area, were occupied during the study. One house (no. 4), located in downtown Cincinnati, was mostly unoccupied. The age of houses ranged from 24 (no. 3) to 115 years (no. 4). Additionally, the largest total finished area was in house no. 4 (581 m^2), and the smallest was in house no. 1 (71 m^2). Four other houses had approximately the same total finished areas (ranging from 150 to 190 m^2).

Measurement of indoor and outdoor bioaerosols

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 actinomycetes, fungal spores, and pollen in indoor and outdoor environments. The inlet efficiency of this device reasonably well fits the inhalable convention of ACGIH/CEN/ISO (for example, the sampling efficiency in particles of 7, 29, and 70 μm were approximately 70%, 40%, and 40%, respectively). The sampler design has several advantages providing low sensitivity of the performance characteristics to the wind direction, high filter collection uniformity, and the ability to screen out 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 microenvironmental measurement of fungal spores and bacteria (Toivola et al., 2004).

The button sampler was washed before each 24-h measurement with 5% bleach and 70% ethanol solution, and then autoclaved in an oven at 180°C for 1 h. After that, a 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, which was maintained by a small pump (Model 224-PCXR4; SKC Inc.) 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 1.5 m. 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 bioaerosol 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 (except for house no. 4 that was unoccupied most of the time). The residents were requested to record their indoor activities, i.e. opening and closing windows, using HVAC system and vacuum. 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 through May) when the ambient pollen concentration was at its peak level; the other one was conducted in the fall (September and October) when the ambient fungal spore concentration reached the highest annual level; and the third one was performed in winter (January and February) when the outdoor bioaerosol concentrations were expected to be at their lowest. The winter measurement cycle is of particular interest because most of the previous ambient bioaerosol monitoring studies did not cover the winter season. The meteorological information, including air temperature and relative humidity indoors and outdoors, as well as outdoor precipitation level and wind speed, was recorded in parallel to the bioaerosol measurements using a meteorological station (Vantage Pro; Davis Instruments, Hayward, CA, USA).

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 using 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 by using a touch mixer (Model 231; Fisher Scientific) for 2 min followed by a 10-min ultrasonic bath (FS20; Fisher Scientific) 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 does 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 actinomycetes was obtained using the culture-based method. A volume of 0.1 ml of extracted fluid was inoculated onto a triplicate International Streptomyces Project agar 2 (ISP2; Difco Laboratories, Detroit, MI, USA) with added cycloheximide (500 mg/l; Sigma-Aldrich, St Louis, MO, USA). The colony forming units (CFU) of actinomycetes were counted after 14 days of incubation at a room temperature of 25 ± 2°C. The limit of detection (LOD) of actinomycetes was 6 CFU/m^3.

The total count method was applied to obtain the concentration of total fungal spores and pollen grains. Following the extraction process described above, 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). 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

(Adhikari et al., 2003). The total number of pollen grains was counted under the 100× or 400× magnification high-resolution light microscope (Labophot 2, Nikon Corp., Japan). At least, 40 randomly selected microscopic fields were examined. For fungal spores, 400× or 1000× magnification were used.

The identification of fungal spores and pollen grains 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 microbial identification. The LOD of fungal spores and pollen grains were eight spores per cubic meter and one pollen per cubic meter, respectively.

Data analysis

To explain the relationship between the indoor and outdoor concentration of different types of bioaerosols, different statistical models were utilized. As the indoor and outdoor aerosol concentrations of fungal spores and pollen were measured in 5 consecutive days for each combination of season and house, the repeated measurement issue must be considered to fit the models. To account for underestimation associated with the levels below LOD for each fungal genus and to apply the log-transformation to the entire database, a value of one (spores per cubic meter) was added to all concentrations and consequently deducted from the mean values thus obtained following the protocol of Eudey et al. (1995). The exchangeable and the first autoregressive correlation structures were alternatively chosen, and the Akaike's Information Criterion (AIC) was applied to model selection. For fungal spores, general linear models with repeated measurements (PROC Mixed, SAS/Stat 9.1; SAS Institute Inc., Cary, NC, USA) were applied to natural log transformed data on the indoor and outdoor concentrations and their ratios. Season, house, relative humidity, wind speed, and precipitation were analyzed to determine their effects on fungal spore concentrations and the I/O ratios. For pollen, the data analysis involved the Poisson regression modeling with repeated measurements (PROC GENMOD, SAS/Stat 9.1). The pollen data analysis was rather complicated as so many points were near the limit of detection and a rather high variability of counts occurred across the entire dataset. For this reason, it was not appropriate to assume that the pollen counts were lognormally distributed. Thus, the Poisson regression with repeated measurements was chosen to fit the models dealing with the pollen counts. Similar to the models used for fungal spores, the effects of temperature and relative humidity were also assessed for the pollen database. To verify the correlation between indoor and outdoor concentrations for both fungal spores and pollen, Spearman correlation coefficients were determined. For the samples collected in spring, I/O ratios of fungal spores and pollen were compared to each other using the paired *t*-test.

Results

The indoor and outdoor concentrations and the I/O ratio for airborne actinomycetes

Many actinomycetes samples were below the LOD, which was as low as 6 CFU/m^3. In the spring campaign, only three indoor samples out of thirty and none of outdoor samples contained actinomycetes. For this reason, the concentration of actinomycetes showed the greatest season-dependency. During the fall and winter campaigns, actinomycetes were detected in higher numbers both indoors and outdoors. In the fall campaign, the indoor concentration ranged from 6 to 138 CFU/m^3 (>LOD in 9 days); the outdoor concentrations were between 6 and 90 CFU/m^3 (>LOD in 5 days). Overall four pairs of indoor and outdoor concentrations were available to calculate the I/O ratios of actinomycetes in the fall campaign. The ratios ranged from 0.973 to 5.636 with a median of 2.857. House no. 5 had higher indoor and outdoor levels of culturable actinomycetes compared with other houses. In the winter campaign, the indoor concentrations of actinomycetes ranged from 6 to 23 CFU/m^3 (>LOD in 8 days), but only one outdoor sample

contained a detectable concentration. In general, more CFUs of actinomycetes were observed in indoor than in outdoor samples.

The indoor and outdoor concentrations and the I/O ratio for airborne fungi

The total fungal spore concentrations measured in five consecutive days indoors and outdoors were averaged, and the geometric mean and geometric standard deviation were recorded separately for each house and each season. The data are presented in Figure 1. The indoor concentration of total fungal spores followed outdoor concentration, and the outdoor levels were usually greater than the indoor ones. As seen in Figure 1, the increase of outdoor concentrations resulted in a corresponding increase of indoor concentrations in most houses. The multiple regression with repeated measurement analysis revealed that the indoor concentration was influenced by factors such as outdoor concentration ($P = 0.003$), season ($P = 0.021$) indoor relative humidity ($P = 0.002$), and outdoor relative humidity ($P = 0.045$). At the same time, it was not affected by indoor and outdoor temperature, wind speed and precipitation. The outdoor concentrations were influenced by the ambient air temperature ($P = 0.0005$) and precipitation ($P = 0.013$) but not by relative humidity, season, or wind speed. The indoor concentrations showed lognormal distribution but the outdoor concentration showed neither lognormal nor normal distribution. In accordance with the Spearman correlation analysis, indoor and outdoor concentrations had positive relationship ($r = 0.633$, $P < 0.0001$). Neither indoor nor outdoor concentrations between houses (nos. 1–6) were significantly different.

The total fungal spore concentrations measured in the spring campaign in the six houses ranged from 148 spores per cubic meter (house 4) to 6302 (house 2) spores per cubic meter indoors and from 106 spores per cubic meter (house 1) to 7704 (house 6) spores per cubic meter outdoors. The median spring concentration of indoor and outdoor total fungal spores was 405 spores per cubic meter and 1182 spores per cubic meter, respectively. In house no. 1, both indoor and outdoor concentrations were relatively low compared to the other houses possibly because of relatively low outdoor temperature (7.2 ± 1.7°C) during the measurement session (end of March). The indoor concentration in house no. 2 determined on the first day of the measurement session of the spring campaign showed peak value and consequently had high standard deviation because of the cleaning activities in the vicinity of sampling points. The indoor level of total fungal spores measured in the fall campaign was between 15 and 1473 spores per cubic meter, and the outdoor level was between 39 and 3187 spores per cubic meter. Interestingly, although the median outdoor concentration of total fungal spores measured in the fall (1372 spores/m^3) did not show a sizable difference with the outdoor level obtained in spring (1182 spores/m^3), the median indoor concentrations demonstrated considerable difference between the seasons: (120 spores/m^3 in fall versus 405 spores/m^3 in spring). The winter measurement campaign revealed the following total fungal spore concentration ranges: from 13 to 5528 spores/m^3 for indoor and from 24 to 3608 spores/m^3 for outdoor. The median concentrations were 106 spores/m^3 (indoor) and 277 (outdoor) spores/m^3. Only house no. 5 showed relatively high indoor and outdoor fungal spore concentrations. Overall indoor concentrations of fungal spores in the spring were significantly greater than those in the fall ($P = 0.007$), but spring vs. winter, and fall vs. winter were not statistically different. The outdoor fungal concentrations measured in spring and winter were close to a significant statistical difference at a confidence level of 5% ($P = 0.079$), but spring vs. fall, and fall vs. winter showed insignificant differences.

The Spearman correlation analysis was applied to determine the correlation coefficient between indoor and outdoor concentrations for fungal spores over six houses and three seasons. The results are shown in Table 2. Houses nos. 2, 4, 5, and 6 had strong positive correlations between indoor and outdoor concentrations over the three seasons, and these correlations are statistically

significant. Houses nos. 1 and 3 showed a positive but not statistically significant correlation. The indoor and outdoor fungal spore concentration obtained in each season over all six houses showed positive significant correlations.

The predominant indoor and outdoor genera of fungal spores are listed with their frequency of occurrence (%) as follows: *Aspergillus/Penicillium* (indoor = 98.9% and outdoor = 98.9%), ascospores (83.3% and 87.8%), *Cladosporium* (70.0% and 82.2%), and basidiospores (48.9% and 77.8%). The geometric mean and geometric standard deviation for indoor and outdoor concentrations calculated for the most prevalent fungal genera in each house over the three seasons are presented in Figures 2–5. The frequency of most other fungal genera was below 10%, and the geometric mean concentration was 5 fungal spores/m^3.

Aspergillus/Penicillium was the most prevalent fungal type over the three seasons both indoors and outdoors. The median indoor concentrations of *Aspergillus/Penicillium* in spring, fall, and winter were 243, 78, and 62 fungal spores/m^3, respectively, while the outdoor median concentrations were 539, 317, and 140 fungal spores/m^3, respectively. The spring indoor concentration levels of *Aspergillus/Penicillium* were greater compared to the fall levels (P = 0.003) but the spring and winter data showed no differences. Meanwhile, the indoor concentration measured in the fall and winter differed from each other (P = 0.046). With respect to outdoor concentration, only spring and fall showed differences (P = 0.038). A high positive correlation value was also obtained between indoor and outdoor concentration for *Aspergillus/Penicillium* (r = 0.683, P < 0.0001, n = 90).

Ascospores was the second most prevalent indoor and outdoor genera. The median indoor concentration of ascospores in spring, fall, and winter were 57, 16, and 16 fungal spores/m^3, respectively, while the respective median outdoor concentrations were 193, 99, and 32 fungal spores/m^3. The indoor concentrations of ascospores obtained in spring were significantly greater than those obtained in fall and winter (P = 0.014, P = 0.049) but no differences were observed between the fall and winter data. The comparison of ascospore outdoor concentration levels measured in spring and fall showed significant difference (P = 0.045). No difference was found between the spring and winter as well as the fall and winter data. The indoor and outdoor concentrations of ascospores showed positive correlation (r = 0.616, P < 0.0001, n = 90).

The median indoor concentration of *Cladosporium* measured in spring, fall, and winter were 40, 24, and 8 fungal spores/m^3, respectively. The respective outdoor median concentrations were 160, 161, and 24 fungal spores/m^3. The indoor concentrations of *Cladosporium* obtained in spring were significantly greater than the fall and winter levels (P = 0.036 and P = 0.0002, respectively) and fall levels were significantly greater than winter data (P = 0.014). The outdoor levels measured in spring and fall were greater than those recorded in winter (P = 0.002 and 0.007, respectively). At the same time, no difference between outdoor *Cladosporium* levels was found when the spring and fall data were compared. The indoor and outdoor concentration showed positive correlation (r = 0.733, P < 0.0001, n = 90).

The median indoor concentration of basidiospores obtained in spring, fall, and winter were 18, 32, and 8 fungal spores/m^3, respectively. The respective median outdoor values were 95, 105, and 8 fungal spores/m^3. The indoor and outdoor concentrations of basidiospores did not show any differences between seasons. Similar to other prevalent genera, basidiospores showed positive correlation (r = 0.552, P < 0.0001, n = 90) between indoor and outdoor levels.

The day-to-day scatter plot of I/O ratios for fungal spores are shown in Figure 6. Most of the ratios were laid between 0.1 and 1. The average I/O ratios for total fungal spores and prevalent fungal genera determined for the three seasons are presented in Figure 7. The I/O ratios for spring were between 0.034 and 2.698 with a median of 0.385. For fall, the median level I/O

ratio was 0.191, with a range from 0.014 to 0.692. For winter, the ratios were between 0.09 and 61.779, and the median was 0.534. The median value of I/O ratio for fungal spores for all seasons was 0.345. The I/O ratios for fungal spores were significantly different among seasons ($P = 0.016$) but not different among houses. The spring samples revealed significantly greater I/O ratios than the fall samples ($P = 0.023$) but the spring and winter I/O-databases were statistically the same. The I/O ratios obtained in winter were significantly greater than those obtained in fall ($P = 0.007$).

With respect to the I/O ratios determined for specific fungal genera, the ratios for *Aspergillus/Penicillium* were greater than those for basidiospores ($P = 0.0004$) and *Cladosporium* ($P = 0.0006$). The median of I/O ratio for *Aspergillus/Penicillium*, ascospores, *Cladosporium*, basidiospores were 0.422, 0.338, 0.195, and 0.222, respectively.

The indoor and outdoor concentrations and the I/O ratio for airborne pollen

Pollen were observed most frequently in indoor and outdoor air in spring season. This finding is consistent with results of Adhikari et al. (2003) who reported that the outdoor pollen counts were higher in spring than in summer and fall because many plants in Cincinnati area pollinated during that season. The outdoor pollen levels were much greater than the indoor ones. The spring indoor concentrations were found to range from 1 to 5 pollen grains/m^3 while the outdoor ranged from 1 to 1234 pollen grains/m^3. The indoor and outdoor median concentration for pollen in spring were 1 and 71 pollen grains/m^3, respectively. The result of Poisson regression analysis found that temperature ($P = 0.015$) had a significant effect on indoor concentration of pollen and relative humidity ($P = 0.019$) had an effect on outdoor concentration of pollen. A positive correlation was found between indoor and outdoor pollen concentrations ($r = 0.411$, $P = 0.027$). The outdoor predominant genera of pollen in spring were *Quercus*, *Morus*, Pinaceae, and *Platanus. Quercus*, *Acer*, Pinaceae, and Poaceae were the prevailing pollen in indoor environments. During the spring measurement campaign house no. 1 had particularly low outdoor concentration of pollen when compared to other houses. The indoor and outdoor concentrations and the I/O ratios of pollen in each house for spring measurement are presented in Table 3. The results from fall and winter measurement campaigns are not presented because of the lack of sampling points. In fall, outdoor pollen was detected only in house no. 4 but the levels were lower than in spring. The outdoor pollen levels measured in fall lay between 5 and 22 pollen grains/m^3. *Ambrosia* was the prevalent pollen genera in outdoor air during fall measurement. In winter, pollen grains were observed only in four of 60 samples (indoor and outdoor) during which the seasonal monitoring campaign was conducted (six homes × 5 days/home), and the outdoor concentration ranged from 1 to 5 pollen grains/m^3.

The I/O ratios for pollen were only calculable for the spring campaign. They ranged from 0.001 to 0.194, with a median of 0.025.

Discussion

The indoor and outdoor concentrations and the I/O ratios of three different types of bioaerosols were determined in three seasons in six houses. Actinomycetes represent the smallest biological particle type in this study. Reponen et al. (2001) reported that the aerodynamic size of *Streptomyces albus* and *Thermoactinomyces vulgaris* were 0.9 and 0.6 μm, respectively. Actinomycetes were not detected in sufficient quantities to perform reliable statistical analysis. It might be because of low prevalence of actinomycetes or the desiccation stress on actinomycetes occurred during a long-term filter collection period (24-h). Actinomycetes were detected more frequently indoors than outdoors because the former provides more favorable environmental conditions (e.g. temperature, humidity) for bacteria. The samples collected in house no. 5 had more actinomycetes colonies than those obtained in other houses. The ambient environment can affect this result as house no. 5 was surrounded by many trees and had small

creek in the vicinity. House no. 5 was also found to have minor water damage as described below. This supports the value of actinomycetes as an indicator microorganism for water/mold damage. The limited data available on the I/O ratio of actinomycetes (only four data pairs) show that actinomycetes had the highest I/O ratio among the three biological particle types.

Garrett et al. (1997) who investigated indoor and outdoor fungal spore concentrations using Burkard spore trap in 80 homes in Latrobe Valley, Australia, showed higher levels of indoor and outdoor total fungal spores than those recorded in the present study. The median indoor and outdoor levels of total spores reported by Garrett et al. for 1 year were 7778 and 8889 spores/m^3, respectively. The Canadian study (Li and Kendrick, 1995) also recorded higher levels of fungal spores (2307 and 3480 spores/m^3, indoors and outdoors, respectively). It should be noted that the referred measurements have been performed in the geographic regions that differ from southwest Ohio in terms of climate characteristics and vegetation. Furthermore, in contrast to our investigation, the Australian and Canadian studies presented the annual averages, which included the summer data. Both above-mentioned studies did not report the data on I/O ratio and did not account for indoor sources. In contrast to Garrett et al. and Li and Kendrick, two studies conducted in Texas (Sterling and Lewis, 1998; Stock and Morandi, 1988) with the RotoRod sampler reported the indoor and outdoor levels of fungal spores and pollen somewhat lower than those obtained in our study. The I/O ratios for total fungi determined in the Texas studies and in our investigation are close to each other. Their I/O ratios for pollen, however, showed considerable differences compared with our data. For example, Sterling and Lewis reported that the geometric mean of the I/O ratios determined for pollen was as high as 0.91, which is even greater than those the authors found for fungal spores. The information published in that study was not sufficient for us to interpret the differences. Generally, it is often impossible to make an adequate comparison of I/O ratios obtained in different field studies because of differences in sampling methods and strategy, variability of sampled areas, and specific climate characteristics of different geographic regions.

Although the statistical comparison revealed no differences between I/O ratios for total airborne fungi collected in spring and winter, the median I/O ratios in winter were highest among three seasons. The outdoor concentrations of fungal spores obtained in the winter campaign were lower than in the other two seasons, which made the winter I/O ratios higher. In contrast, the outdoor concentration of fungal spores in spring and fall displayed little differences. Meanwhile, the indoor concentrations measured in spring were higher than those in fall. This finding may be attributed to the fact that the residents were more likely to use their air conditioning systems (and, therefore, keep their windows closed) in September or early October, when the ambient air in the Ohio valley is often hot and humid, than in the generally cooler months of March, April, and even May. It should be noted that although the median values for indoor and outdoor fungal spores were lowest in winter, the ranges were comparable with those measured in spring and fall. Considerable variation in the fungal spore concentration in winter was attributed to changes in weather conditions. However, ambient bioaerosol levels are not usually measured in winter and thus have not been available to previous studies about relationship between ambient bioaerosols and health outcome in children (Dales et al., 2000; Lierl and Hornung, 2003). This finding calls for continuous monitoring of outdoor fungal spores throughout the winter by the outdoor aerobiological stations.

Only spring data were available for comparison of I/O ratios between different types of bioaerosols because of lack of data of pollen grains in fall and winter seasons. A paired *t*-test found that the I/O ratios for fungal spores were significantly greater than for pollen ($P < 0.001$). There are two possible reasons for the low I/O ratios obtained for pollen during the peak ambient pollination season. The first is that only a small fraction of pollen grains penetrate from outdoor air into the homes. The second is attributed to a relatively large size of pollen grains and consequently high gravitational settling velocity, which decreases the pollen aerosol

concentration indoors. Our previous studies show that the aerodynamic size of most airborne fungal spores is below 10 μm whereas it is above 10 μm for pollen. For example, the following aerodynamic sizes have been reported for fungal spores: 3.7 μm for *Aspergillus/Penicillium*, 5.6 μm for ascospores, 6.8 μm for basidiospores, and 8.1 μm for *Cladosporium* (Lee et al., 2005). Adhikari et al. (2003) calculated the aerodynamic size of several pollen types using their physical size. The aerodynamic size of *Ambrosia* and *Pinaceae* were 24 and 65 μm, respectively. Higher indoor concentration levels and variability in the I/O ratio observed for airborne fungal spores suggest that the spores have much higher penetration efficiency compared with the pollen grains, which reflects the difference in the particle sizes. The effect of particle size was also already seen in the I/O ratios of the four most common fungal spore types: the smaller the spores size, the higher the I/O ratios. Thus indoor exposure to airborne fungal spores and pollen grains are strongly dependent on the size of biological particles penetrating from the ambient air inside homes. The ranges of the I/O ratio of fungal spores and pollen grains demonstrate seasonal variation between spring and fall, which can be attributed to the different patterns of the ventilation, such as the frequency and duration of keeping windows open and the usage of the HVAC system.

There was an insignificant variation in indoor concentration of fungal spores among houses. However, house no. 4 showed strongest positive correlation among houses in indoor and outdoor concentration of total airborne fungi. As mentioned in the materials and methods section, house no. 4 was usually unoccupied by residents during sampling and, therefore, there was little human activity during the 24-h measurement. The possibility of aerosolization of the fungal spores inside the house was small and indoor concentrations were mainly determined by outdoor environment. Also this house was the oldest one from our cohort which suggests a higher air exchange rate because of a relatively ‘loose’ building structure. According to the record of residents of house no. 5, they usually opened their windows during the daytime, which explains high correlation value. The indoor and outdoor concentrations measured in house no. 5 during the winter campaign were higher than in other houses because of a high peak concentration of 2 days indoor and 1 day outdoors. The median concentrations obtained in fall and winter were almost the same. Two bathrooms in house no. 5 were remodeled during the sampling campaign, and one of the bathrooms had minor water damage. This might have caused an increment of indoor fungal spores as well as the increase in average values. The high outdoor concentration possibly occurred because of the high outdoor temperature (16°C) after rain and because of favorable outdoor relative humidity. Houses nos. 2 and 3 had relatively smaller correlation value compared with other houses. House no. 3 is the newest one that was expected to have a ‘tight’ building structure. In addition, the residents usually kept windows closed.

Overall, the presented pilot database on the inhalable levels of indoor and outdoor bioaerosols can help apportion and better characterize the inhalation exposure to these bioaerosols. Furthermore, the data can be incorporated into existing models to quantify the penetration of biological particles into indoor environments from outdoors.

Acknowledgements

This study was partially supported by the U.S. National Institute for Environmental Health Sciences through grant no. R01ES11170 and by the U.S. Department of Housing and Urban Development through grant no. OHLHH0099–01. The investigators appreciate the extramural programs of these agencies and thank residents for providing access to their homes.

References

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

Bornehag C-G, Blomquist G, Gyntelberg F, Järvholm B, Malmberg P, Nordvall L, Nielsen A, Pershagen G, Sundell J. Dampness in buildings and health. Indoor Air 2001;11:72–86. [PubMed: 11394014]

Burge HA, Rogers CA. Outdoor allergens. Environ Health Perspect 2000;108:653–659. [PubMed: 10931783]

Cole, EC.; Foarde, KK.; Leese, KE.; Green, DA.; Franke, DL.; Berry, MA. Assessment of fungi in carpeted environments. In: Samson, RA.; Flanningan, B.; Flanningan, ME.; Verhoeff, AP.; Adan, OCG.; Hoekstra, ES., editors. Health Implications of Fungi in Indoor Environments Air Quality Monographs. 2. Amsterdam, The Netherlands: Elsevier; 1994. p. 103-128.

Dales RE, Cakmak S, Burnett RT, Judek S, Coates F, Brook RJ. Influence of ambient fungal spores on emergency visits for asthma to a regional children's hospital. Am J Respir Crit Care Med 2000;162:2087–2090. [PubMed: 11112119]

Douwes J, Thorene P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: progress and prospects. Ann Occup Hyg 2003;47:187–200. [PubMed: 12639832]

Eudey, L.; Su, HJ.; Burge, HA. Biostatistics and Bioaerosols. In: Burge, HA., editor. Bioaerosols. Boca Raton, Florida: CRC Press, Inc; 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.

Gent JF, Ren P, Belanger K, Triche E, Bracken MB, Holford TR, Leaderer BP. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. Environ Health Perspect 2002;110:A781–A786. [PubMed: 12460818]

Gots RE, Layton NJ, Pirages SW. Indoor health: background levels of fungi. Am Ind Hyg Assoc J 2003;64:427–438.

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. J Aerosol Sci 1998;29:1137–1138.

Institute of Medicine (IOM). Damp Indoor Spaces and Health. Washington DC: National Academy of Sciences; 2004.

Jones NC, Thornton CA, Mark D, Harrison RM. Indoor/outdoor relationships of particulate matter in domestic homes with roadside, urban and rural locations. Atmos Environ 2000;34:2603–2612.

Klepeis NE, Nelson WC, Ott WR, Robinson JP, Tsang AM, Switzer P, Behar JV, Hern SC, Engelmann WH. The national human activity pattern survey (NHAPS): a resource for assessing exposure to environmental pollutants. J Expo Anal Environ Epidemiol 2001;11:231–252. [PubMed: 11477521]

Lacey J, Dutkiewicz J. Bioaerosols and occupational lung disease. J Aerosol Sci 1994;25:1371–1404.

Lee S, Adhikari A, Grinshpun SA, McKay R, Shukla R, Li H, Reponen T. Respiratory protection provided by N95 respirators against airborne microorganisms and dust in agricultural farms. J Occup Environ Hyg. 2005in press

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

Lierl MB, Hornung RW. Relationship of outdoor air quality to pediatric asthma exacerbations. Ann Allergy Asthma Immunol 2003;90:28–33. [PubMed: 12546334]

Neas LM, Dockery DW, Burge H, Koutrakis P, Speizer FE. Fungus spores, air pollutants, and other determinants of peak expiratory flow rate in children. Am J Epidemiol 1996;143:797–807. [PubMed: 8610690]

Nevalainen A, Pasanen AL, Niininen M, Reponen T, Jantunen MJ, Kalliokoski P. The indoor air quality in Finnish homes with mold problems. Environ Int 1991;17:299–1302.

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

Reponen T, Grinshpun SA, Conwell KL, Wiest J, Anderson WJ. Aerodynamic versus physical size of spores: measurement and implication for respiratory deposition. Grana 2001;40:119–125.

Samson, RA.; Flanningan, B.; Flanningan, ME.; Verhoeff, AP.; Adan, OCG.; Hoekstra, ES. Health Implications of Fungi in Indoor Environments Air Quality Monographs. 2. Amsterdam, The Netherlands: Elsevier; 1994.

Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol 2002;68:1743–1753. [PubMed: 11916692]

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

Stark PC, Burge HA, Ryan LM, Milton DK, Gold DR. Fungal levels in the home and lower respiratory tract illnesses in the first year of life. Am J Respir Crit Care Med 2003;168:232–237. [PubMed: 12724122]

Sterling DA, Lewis RD. Pollen and fungal spores indoor and outdoor of mobile homes. Ann Allergy Asthma Immunol 1998;80:279–285. [PubMed: 9532979]

Stock TH, Morandi MT. A characterization of indoor and outdoor microenvironmental concentrations of pollen and spores in two Houston neighborhoods. Environ Int 1988;14:1–9.

Thatcher TL, Lunden MM, Revzan KL, Sextro RG, Brown NJ. A concentration rebound method for measuring particle penetration and deposition in the indoor environment. Aerosol Sci Technol 2003;37:847–864.

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]

Verhoeff AP, Burge HA. Health risk assessment of fungi in home environments. Ann Allergy Asthma Immunol 1997;78:544–554. [PubMed: 9207717]

Vette AF, Rea AW, Lawless PA, Rodes CE, Evans G, Highsmith VR, Sheldon L. Characterization of indoor-outdoor aerosol concentration relationship during the Fresno PM exposure studies. Aerosol Sci Technol 2001;34:118–126.

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

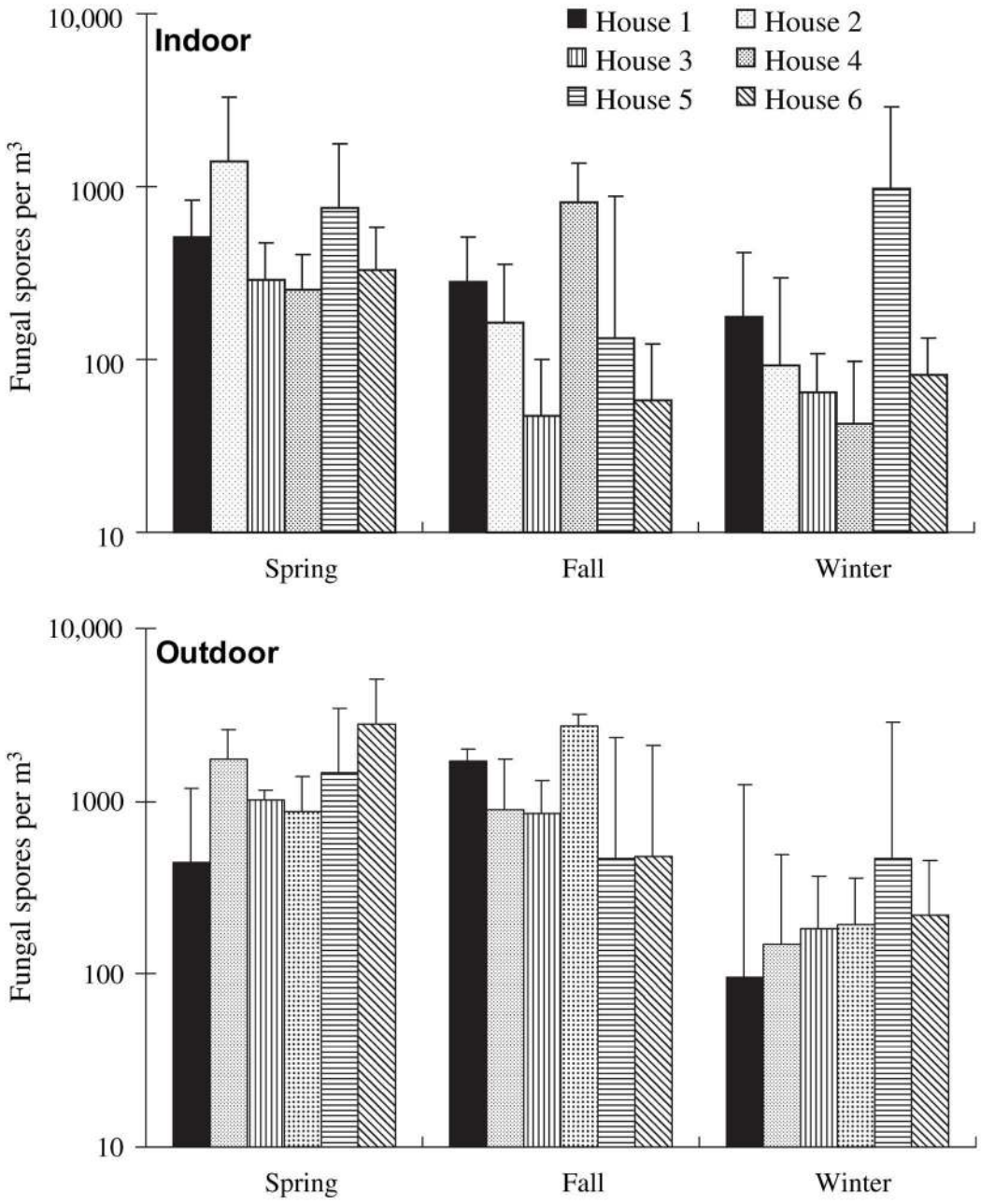

Fig. 1.

Indoor and outdoor concentrations of total fungal spores in three seasons (each column and error bar represents geometric mean and geometric standard deviation of five 24-h samples)

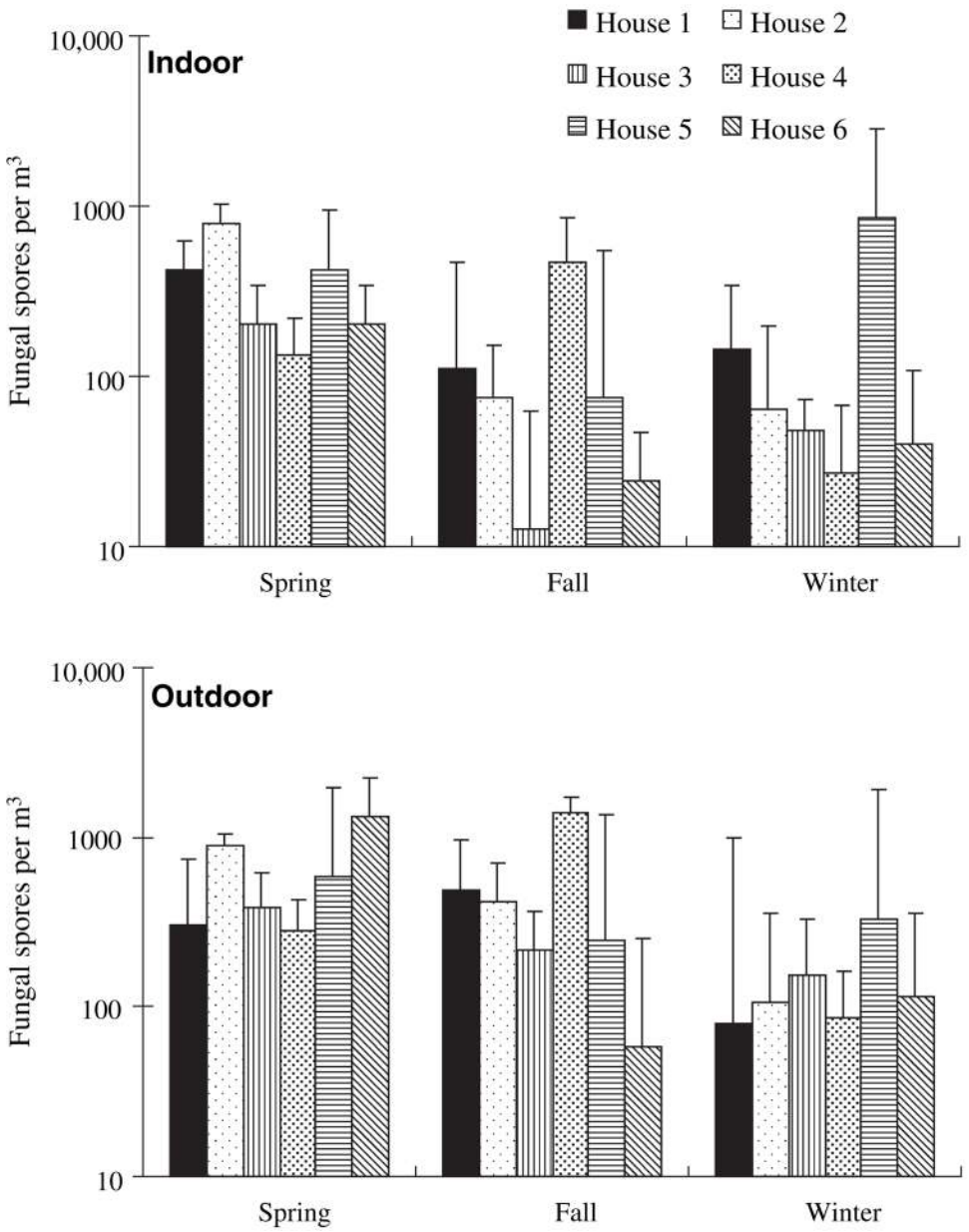

Fig. 2.
Indoor and outdoor concentrations of *Aspergillus/Penicillium* in three seasons (each column and error bar are same as in Figure 1.)

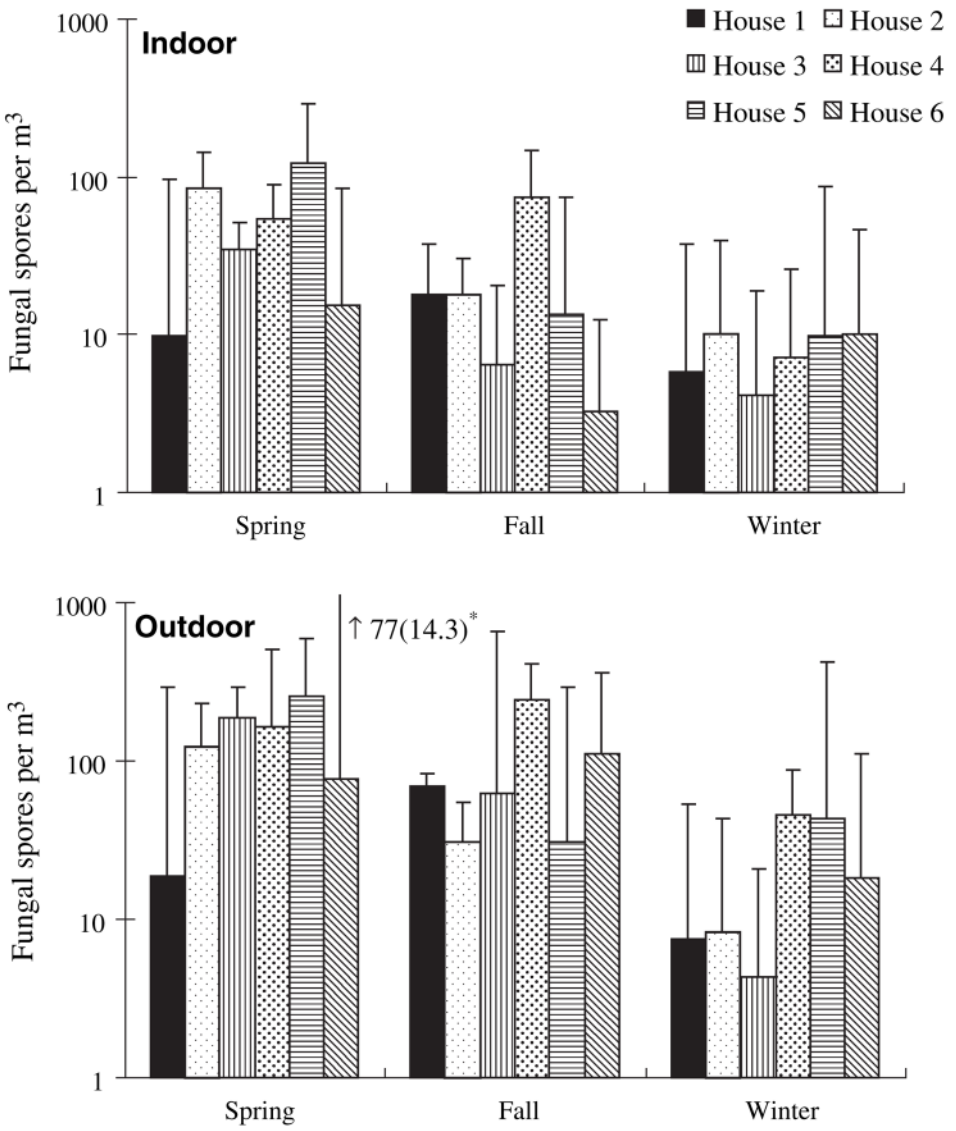

Fig. 3.

Indoor and outdoor concentrations of ascospores in three seasons (each column and error bar are same as in Figure 1). *Geometric mean (geometric standard deviation)

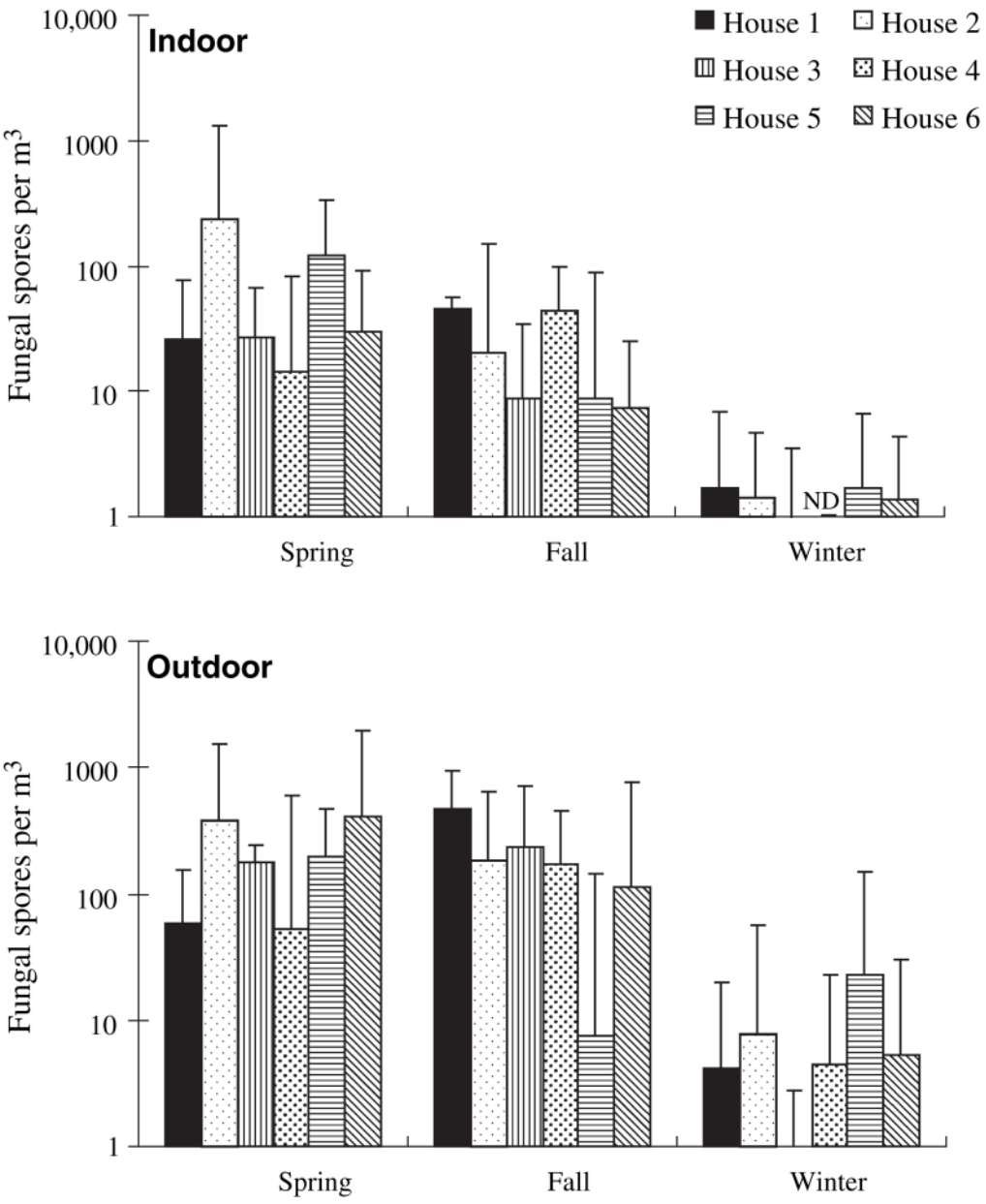

Fig. 4.
Indoor and outdoor concentrations of *Cladosporium* in three seasons (each column and error bar are same as in Figure 1. ND is not detected.).

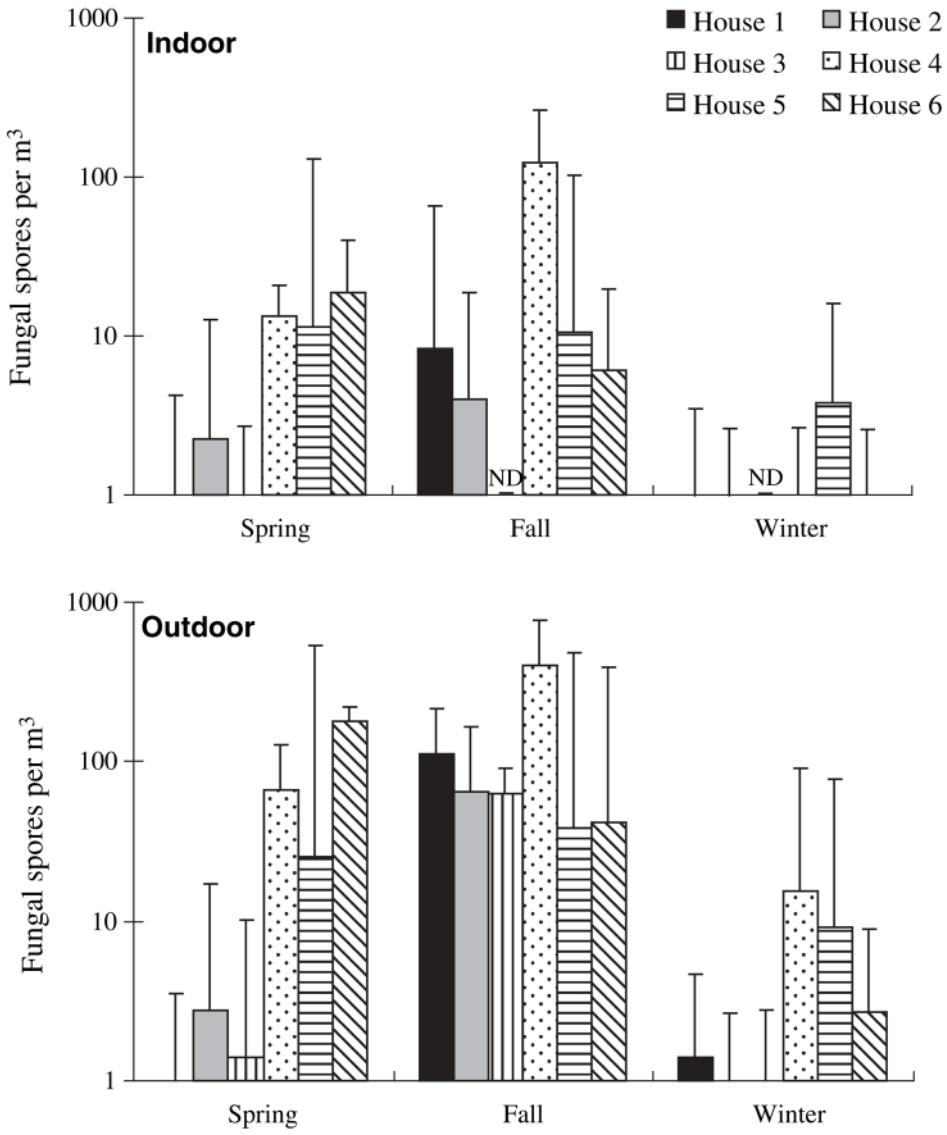

Fig. 5.
Indoor and outdoor concentrations of basidiospores in three seasons (each column and error bar are same as in Figure 1. ND is not detected)

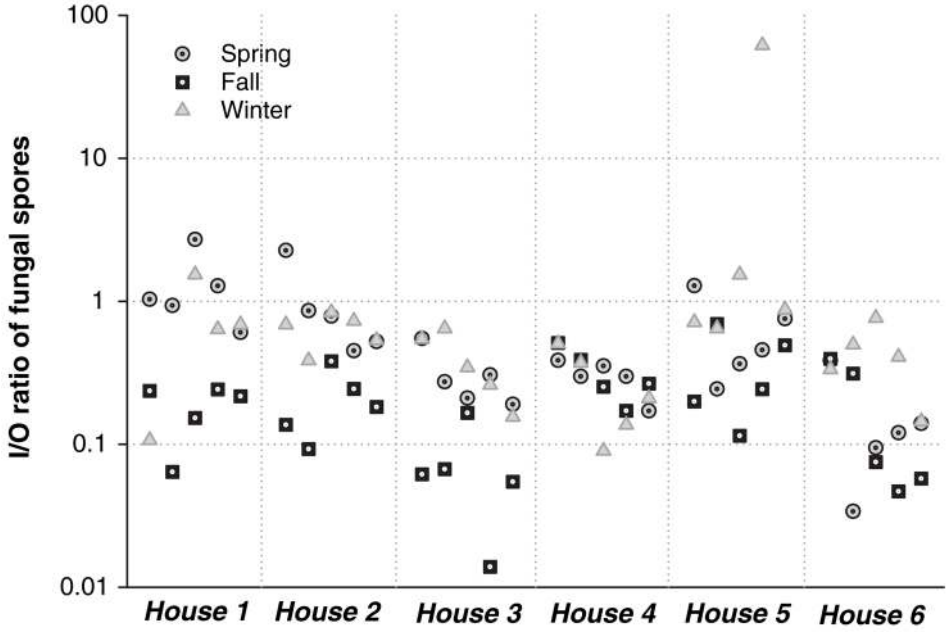

Fig. 6.
The day-to-day scatter plot of I/O ratios for total fungal spores in each home during three seasons

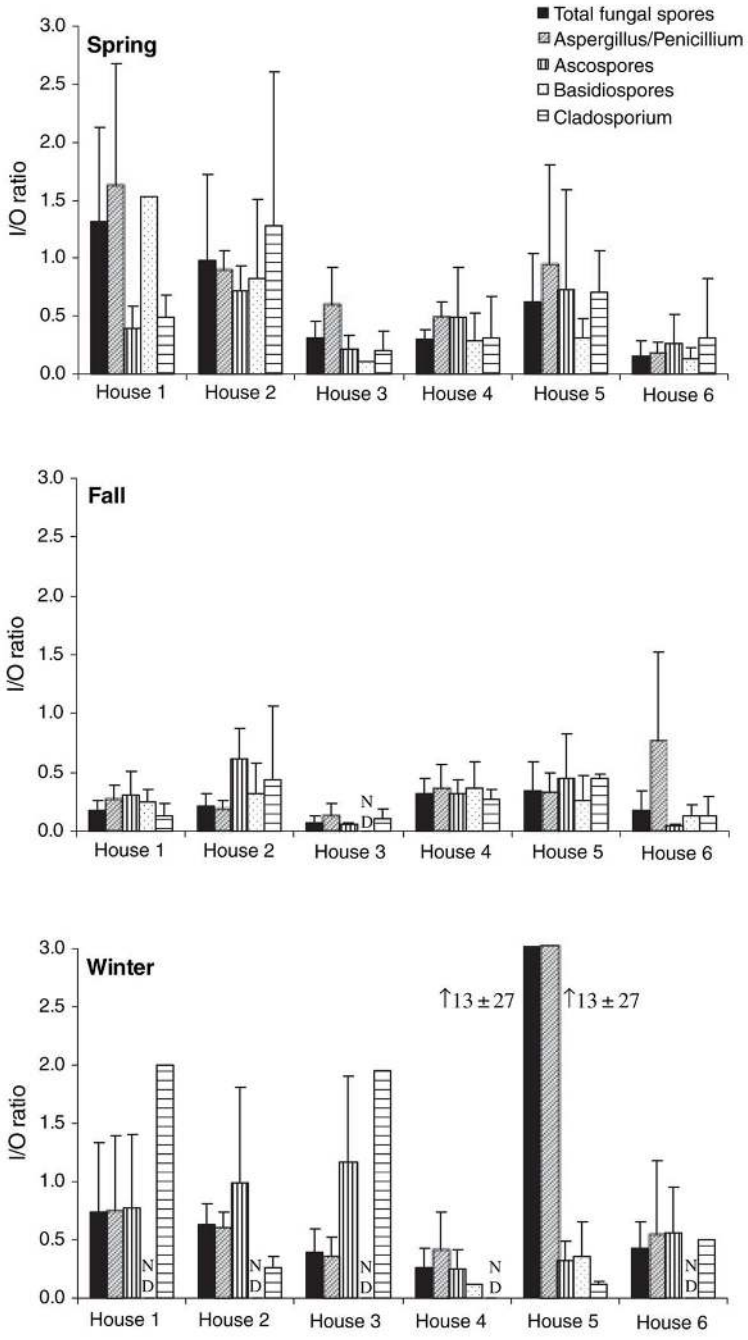

Fig. 7.

I/O ratio for fungal spores in three seasons (each column and error bar represents arithmetic mean and standard deviations. ND, not detected)

Table 1

Summary of characteristics of the selected homes

House characteristics	House 1	House 2	House 3	House 4	House 5	House 6
Age	56	24	48	60	115	33
Total area (m^2)	71	150	190	580	150	190
House material	Plaster walls, plankboard and vinyl siding	Brick with plaster walls	Wood siding	Brick	Brick and wood	Brick
Window type	Thermo-pane	Single pane, Wood	Double pane, Wood	Double pane, Aluminum	Single pane, Aluminum	Double pane, Aluminum
Water damage	No	No	No	No	Only in winter	No
Visible mold	No	No	No	No	No	No
HVAC filter type		Metal filter	Space guard cleaner		Bryant mechanical air cleaner	3M standard

Table 2

Spearman correlation coefficient between indoor and outdoor fungal spore concentrations in each house over three seasons and in each season over all six houses

	Sample number	Spearman correlation coefficient	*P*-value
House number			
1	15	0.214	0.443
2	15	0.846	<0.0001
3	15	0.421	0.1177
4	15	0.954	<0.0001
5	15	0.629	0.0121
6	15	0.789	0.0005
Season			
Spring	30	0.398	0.029
Fall	30	0.845	<0.0001
Winter	30	0.440	0.015

Table 3

Indoor and outdoor average concentrations (number of pollen/m^3) and the I/O ratio for pollen obtained in spring (the arithmetic mean and standard deviation, $n = 5$ for indoor and outdoor in each house)

House	1	2	3	4	5	6
Indoor	1	1 ± 0.3	1 ± 0.45	0	3 ± 1	2 ± 2
Outdoor	6 ± 6	157 ± 104	531 ± 503	71 ± 6	48 ± 35	73 ± 49
I/O Ratio	0.05	0.004 ± 0.004	0.04 ± 0.08	N/A	0.12 ± 0.10	0.05 ± .005

N/A, not applicable.