

Temporal and spatial variation of indoor and outdoor airborne fungal spores, pollen, and (1→3)- β -D-glucan

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Abstract Characterizing the variation in bioaerosol concentrations is important for the estimation of health effects associated with bioaerosols and planning exposure assessment strategies. This investigation was conducted in order to develop a better understanding of exposure to fungal spores, pollen, and (1→3)- β -D-glucan, by determining the variations of their concentrations between and within homes. In the study, 24-h air sampling was performed on five consecutive days in four Cincinnati area homes. The samples (a total of 160) were taken simultaneously in four different rooms inside each home and at four different outside locations near the home using Button Personal Inhalable Aerosol Samplers. The relative sizes of the between- and within-home variability to the total variability were calculated for each outcome. The relative sizes of the between- and within-home variability in indoor air ranged from 0.10 to 0.52 and 0.09 to 0.10, respectively. For outdoor air, the between- and within-home variability ranged from 0.27 to 0.50 and 0.09 to 0.10, respectively. Thus, the ranges of within-home variability, both indoors and outdoors, were much less than the variability between different homes. The results

suggest that, if long-term sampling methods are employed to characterize the bioaerosol exposure for a population, the sampling should be repeated in a larger number of homes as an alternative to replicate sampling in a fewer number of homes. When characterizing exposure within one home, the sampling should be repeated in different rooms, rather than repeating it on different days.

Keywords Between-home variation · Within-home variation · Intraclass correlation coefficient · Mold · Fungal component · Bioaerosols

1 Introduction

Bioaerosols comprise a diverse group of particles, ranging from bacteria, fungal spores, and pollen to cell-wall components, such as (1→3)- β -D-glucan, herein referred to as beta-glucan. Exposure to bioaerosols is common in indoor and outdoor environments and may be related to various adverse health effects. Dampness and mold in buildings have been associated with asthma, respiratory symptoms, and allergic effects in residential and occupational settings (IOM 2004; Dangman et al. 2005; Cho et al. 2006; Lignell et al. 2007). Similarly, elevated outdoor pollen concentrations were associated with asthma exacerbations (Lierl and Hornung 2003) and asthma-related

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hospital visits for children (Zhong et al. 2006). Pollen has also been implicated in contact dermatitis and occupational respiratory allergy (Yokozeki et al. 2007; Anguita et al. 2007). Beta-glucan, a cell-wall component of pollen, fungi, and some bacteria (Stone and Clarke 1992), has been used as a surrogate measure for exposure to fungi (Chew et al. 2001; Schram-Bijkerk et al. 2005; Douwes et al. 2006). Indoor as well as occupational exposures to beta-glucans have been associated with respiratory symptoms and airway inflammation (Douwes 2005; Douwes et al. 2006; Rylander et al. 1998). However, high ($>60 \mu\text{g/g}$) levels of beta-glucan have also been reported to reduce the risk for wheezing in infants (Iossifova et al. 2007).

Bioaerosol concentrations are associated with meteorological conditions and household characteristics, including ventilation (Li and Kendrick 1995). They also appear to vary by geographic location and season. Several studies reported considerable spatial (within-home) and temporal variation in the indoor concentration of viable fungi (Verhoeff et al. 1990; Pasanen et al. 1992; Hyvärinen et al. 2001), whereas others have shown almost non-existent variation (Ren et al. 1999, 2001; Ross et al. 2000). Greater variability in exposures measured over time introduces bias in the correlation of exposures to health outcomes (Brunekreef et al. 1987), therefore, the sampling method and time is important in order to estimate the true exposure to bioaerosols and its relationship to health effects in epidemiological studies.

Hyvärinen et al. (2001) investigated the spatial and temporal variation of fungi in the indoor air of two single-family homes, one with and one without mold problems. They recommended that sampling be conducted over an 11-day campaign for accurate characterization of airborne concentrations of fungi, and within-home sampling be conducted in two different rooms in the same day. However, this study only included indoor air and employed only the culture-based method. Sampling for culturable fungi concentrations is the method usually selected for the quantification of fungi, but this method has several limitations (Dillon et al. 1996). This method usually allows only short-term sampling and can underestimate the true exposure to fungi, because it does not account for non-culturable spores. Therefore, it is often advantageous to use a method accounting for both culturable and non-culturable fungi. One alternative is the total count method that can be combined with, e.g.,

spore trap or filter collection. The latter allows for long-term sampling, which may reduce the variation in measured concentrations. One option to collect a sample on a filter is by using the Button Personal Inhalable Aerosol Sampler (SKC Inc., Eighty Four, PA, USA). The Button Sampler has been reported as being efficient for collecting outdoor pollen and fungal spores (Adhikari et al. 2003). In addition, it has also been used for stationary, personal, and microenvironmental measurement of fungal spores and bacteria (Lee et al. 2006; Toivola et al. 2002; Adhikari et al. 2004).

Alternatively, several investigators have used dust sampling as a surrogate for long-term air sampling. Chew et al. (2001) found that, during one year, the within-home variability for culturable fungi in dust samples exceeded the between-home variability. Consequently, Chew et al. (2001), and later Heinrich et al. (2003) and Cho et al. (2008), reported that a single measurement of culturable fungi in dust is not a good proxy for the long-term exposure and recommended further detailed studies of the fungal spore counts over time.

Several investigators have studied the spatial variation of outdoor pollen (Gonzalo-Garijo et al. 2006; Arobba et al. 2000; Frenz et al. 1997), but did not arrive at the same conclusion as to whether pollen exhibits spatial variation when measured at different locations in the same city. While Arobba et al. (2000) and Frenz et al. (1997) concluded that little variation exists between pollen measured at different monitoring sites, Gonzalo-Garijo et al. (2006) suggested that pollen counts in one area of a city would not accurately represent the exposure of an allergy patient in another area of the same city. There appears to be no previous studies that compared outdoor to indoor variation in the concentrations of pollen.

Thorn (2001) repeated bi-weekly outdoor air sampling for measuring beta-glucan exposures of waste collectors over several seasons and showed a relationship between temperature and beta-glucan concentrations. The investigators concluded that the levels of beta-glucans are higher during the warm seasons, which may be attributed to the seasonal variation of pollen and fungal spores. Miller et al. (2007), who measured airborne beta-glucan, ergosterol, and endotoxin in living rooms and bedrooms in 20 Canadian homes using long-term air sampling, reported no difference between the two room types in any of the measured biocontaminants.

Chew et al. (2001) analyzed beta-glucan in dust samples collected in 23 Dutch homes over a period of a year and found similar variation of its levels within and between homes. Giovannangelo et al. (2007) analyzed the variance in the levels of beta-glucan, endotoxin, extracellular polysaccharide, and indoor allergens in duplicate house dust samples collected in 61 homes. They found that the within-home variance was less than half of the between-home variance in most cases. The latter study, however, did not address temporal variation, included only dust samples, and was limited to indoor samples. Bioaerosol concentrations may vary both spatially and temporally, and it is important to know the magnitude of this variation when developing a representative sampling strategy.

The objective of this research was to investigate the indoor and outdoor spatial and temporal variation of fungal spores, pollen, and beta-glucan within and between homes. The information generated in this study is expected to assist in developing strategies for monitoring bioaerosol exposure through a long-term air sampling.

2 Methods

2.1 Selection of sampling sites

Indoor and outdoor ambient air sampling for bioaerosols was conducted in four single-family homes. The house selection criteria included: no signs of visible mold, no recent history of water damage, no visible water damage, and no moldy odor present. All of the homes were located in the Cincinnati (Ohio) metropolitan area and were between 7 and 37 years old. The finished area ranged from 153 to 231 m². All homes had wood and brick frames; additionally, two of the homes had aluminum/vinyl siding. All homes had central forced-air HVAC systems, one with electrostatic, one with pleated fiberglass, and two with HEPA filtration. Three homes had double-pane windows and only one had single-pane windows. The homes in this study are labeled as A, B, C, and D, respectively. Indoor sampling was performed in four different rooms (living room, kitchen, bedroom, basement) for 24 h on five consecutive days, from Monday morning to Saturday morning. The outdoor samples were taken on four different sides of the home [front, back, left (Side 1), and right (Side 2)] for

24 h on five consecutive days simultaneously with indoor sampling. The sampling was conducted in the fall over 4 weeks (September–October 2005), the period when the outdoor fungal spore concentration in Cincinnati is at its maximum (Adhikari et al. 2006). All homes had low daytime human and animal activity, except House C, which had a cat and a dog and two people staying at home during the sampling campaign.

2.2 Measurement of indoor and outdoor fungal spores, pollen, and beta-glucan

Ambient indoor and outdoor fungal spores, pollen, and beta-glucan were collected using a Button Personal Inhalable Aerosol Sampler (SKC Inc., Eighty Four, PA, USA), as described in detail by Lee et al. (2006). For the collection of fungal spores and pollen, the samplers were loaded with 25-mm mixed cellulose ester (MCE) filters (1.2- μ m pore size, GE Osmonics Inc., Minnetonka, MN, USA). In parallel with the MCE filters, polycarbonate filters (0.4- μ m pore size, GE Osmonics Inc.) were utilized to collect beta-glucan. All samplers were operated at a flow rate of 4.0 L/min, maintained with a precision of 5%. The flow rate was verified with a DryCal[®] DC-Lite Calibrator (Bios International Corporation, Butler, NJ, USA) before and after each 24-h sampling session.

2.3 Fungal spore and pollen analysis

The MCE filters which captured and contained the pollen and fungal spores were made transparent using a modified acetone vaporizer unit (Quixfix, Environmental Monitoring System, Charleston, SC, USA). Each filter was stained with glycerin jelly mixed with Calberla's stain, as described by Lee et al. (2006). Pollen grain totals were counted by randomly selecting 40 microscopic fields under 100 $\times$ magnification using a high-resolution light microscope (Labophot 2, Nikon Corp., Tokyo, Japan). Fungal spores were enumerated using the same method under 400 $\times$ magnification. For both fungi and pollen, 40 microscopic fields were randomly selected for analysis. Fungal spores and pollen grains were categorized and grouped by genus, family, or class level based on their morphological characteristics, as described by Adhikari et al. (2004).

2.4 (1→3)-β-D-glucan analysis

Beta-glucan concentration both indoors and outdoors was determined by the *Limulus* amoebocyte lysate (LAL) (Associates of Cape Cod, East Falmouth, MA, USA) analysis method, as described by Lee et al. (2006). Briefly, particles were extracted from the polycarbonate filters using a touch mixer (Model 231, Fisher Scientific, Pittsburgh, PA, USA) for 2 min, followed by a 10-min ultrasonic bath (FS20, Fisher Scientific) agitation. NaOH (0.6 M) was added at room temperature (20°C) in order to extract the beta-glucan from fungal spores and pollen and to make it water-soluble. The samples were then shaken for 1 h (Wrist-Action Shaker, Burrell Scientific, Pittsburgh, PA, USA), and an aliquot of 25 µl was analyzed for beta-glucan. To avoid interference by endotoxin, the Glucatell modification of the LAL assay was used. The lower limit of detection was 5 pg ml⁻¹ and the beta-glucan concentration of blank filters ranged from 0.09 to 0.22 ng ml⁻¹, which was found to be negligible when compared to the beta-glucan concentration found in previously collected indoor and outdoor samples.

2.5 Statistical analysis

Statistical analysis was performed using the Windows-based SAS/Stat 9.1 (SAS Institute Inc., Cary, NC, USA) software package. Concentrations of fungal spores, pollen, and beta-glucan were logarithmically transformed for statistical analysis to approximate normality. Beta-glucan values that were below the detection limit were replaced by half of the detection limit. Because fungal spore and pollen data consist of concentrations of several types of fungi and pollen, a different approach was used. Initially, a zero value was assigned for fungal spore or pollen concentration if it was below the detection limit. In order to apply the log-transformation, a value of one was added to all concentration values before analysis. After analysis, the value one was deducted from the result obtained (Eudney et al. 1995).

Geometric means were obtained to describe the average indoor and outdoor bioaerosol concentrations for each outcome. Ninety-five percent confidence intervals of average values across four homes were based on estimates of between-home variability. Differences among mean concentrations between pairs of homes were evaluated by Tukey's adjustment

for multiple comparisons applied to confidence interval estimation. For each outcome, the geometric means of each area within each of the four homes were also calculated. Differences among mean concentrations between pairs of areas within the same home were based on estimates of day-to-day variability within homes, with Tukey's adjustment applied to confidence interval estimation. The variability of bioaerosol concentrations was estimated using variance component analyses, in which the total variability (σ^2_{total}) was partitioned as a sum of independent sources of variation, including: (1) between homes ($\sigma^2_{\text{bet home}}$), (2) between rooms within the same home (spatial variability) ($\sigma^2_{\text{bet room}}$), (3) between days within the same room (temporal variability) ($\sigma^2_{\text{bet day}}$), and (4) residual variability from all other sources (σ^2_{res}). Thus, $\sigma^2_{\text{total}} = \sigma^2_{\text{bet home}} + \sigma^2_{\text{bet room}} + \sigma^2_{\text{bet day}} + \sigma^2_{\text{res}}$. The intraclass correlation (ICC) was calculated to indicate the relative magnitude of spatial and temporal variation, where the ICC is a measure of reliability. Hyvärinen et al. (2006) have previously used the ICC to explore the reliability of endotoxin measurement using different sampling methods. An ICC value <0.04 is considered to be small, whereas an ICC value >0.64 is considered to be large (Cohen 1988).

The relative sizes of the between- and within-home variability were assessed. For example, if the between-home variability is large, there is likely to be a high correlation between measurements on the same home due to this source of variation. Similarly, if the between-home variation is small, then observations in the same home would be expected to be no more alike than observations in different homes. The ICC values, measuring the component sources of variability, were calculated as follows:

$$\begin{aligned}
 \text{Between-home: } ICC_{\text{bet home}} &= \sigma^2_{\text{bet home}} / \sigma^2_{\text{total}} \\
 \text{Within-home: } ICC_{\text{within home}} &= \sigma^2_{\text{bet room}} + \sigma^2_{\text{bet day}} \\
 \text{Day-to-day: } ICC_{\text{bet day}} &= \sigma^2_{\text{bet day}} / \sigma^2_{\text{total}} \\
 \text{Room-to-room: } ICC_{\text{bet room}} &= \sigma^2_{\text{bet room}} / \sigma^2_{\text{total}}
 \end{aligned} \tag{1}$$

3 Results

3.1 Overall trends

Table 1 shows the geometric mean (GM) concentrations and 95% confidence intervals for indoor and

Table 1 Geometric mean (GM), geometric standard deviation (GSD), and 95% confidence interval (CI) for indoor and outdoor data ($n = 160$)

Variable	Indoor data		Outdoor data	
	GM (GSD)	95% CI	GM (GSD)	95% CI
Total fungi (spores m^{-3})	214 (4.4)	(154, 297)	5,286 (1.7)	(4,695, 5,953)
Ascospores	3.0 (38)	(1.3, 6.9)	381 (2.4)	(314, 463)
Basidiospores	3.2 (52)	(1.2, 7.2)	1,134 (1.9)	(986, 1,303)
<i>Cladosporium</i>	2.8 (79)	(1.0, 7.4)	328 (19)	(170, 631)
<i>Penicillium/Aspergillus</i>	73 (7.8)	(46, 115)	1,691 (2.2)	(1,423, 2,010)
Total pollen (grains m^{-3})	0.04 (8.9)	(0.02, 0.06)	0.49 (20)	(0.25, 1.0)
<i>Ambrosia</i>	0.03 (6.6)	(0.02, 0.04)	0.21 (20)	(0.11, 0.41)
(1 $\rightarrow$ 3)- β -D-glucan (ng m^{-3})	1.0 (2.5)	(0.81, 1.2)	7.34 (2.3)	(6.1, 8.9)

outdoor fungi, pollen, and beta-glucan. The total indoor fungi collected over all homes had a GM of 214 spores m^{-3} , while the total outdoor fungal spores exhibited a much higher concentration of 5,286 spores m^{-3} . The most common spore genera/classes found in indoor and outdoor air were Ascospores, Basidiospores, *Cladosporium*, and *Penicillium/Aspergillus*. Among these, the *Penicillium/Aspergillus* group had the highest individual concentration for fungi indoors (GM = 73 spores m^{-3}) and outdoors (GM = 1,691 spores m^{-3}), while *Cladosporium* had the lowest GM concentrations indoors and outdoors (2.8 spores m^{-3} and 328 spores m^{-3} , respectively).

For all homes, the total pollen concentrations were lower than the total fungal spore concentrations. Indoor pollen had a GM of 0.04 grains m^{-3} , while the GM for total outdoor pollen grains was 0.49 grains m^{-3} . The majority of total pollen was *Ambrosia*, which had GM concentrations of 0.03 grains m^{-3} and 0.21 grains m^{-3} for indoor and outdoor concentrations, respectively. The GM concentrations of beta-glucan were 1.0 ng m^{-3} for indoor and 7.4 ng m^{-3} for outdoor air. House C had the highest overall concentrations of indoor fungi and beta-glucan, although the difference in average concentrations between houses was not significant.

3.2 Indoor and outdoor fungal spores

Indoor and outdoor averages (GM) of the total fungi are presented in Fig. 1 for different areas within each home (Fig. 1a) and for different outdoor sampling locations (Fig. 1b). Indoor and outdoor variability (ICC) between and within homes for the total fungi are

presented in Table 2. In indoor air, the total fungi had only slight variation from room to room within each home. This is reflected in the small relative size of the within-home variability ($\text{ICC}_{\text{within home}} = 0.10$). The between-home variability was much larger ($\text{ICC}_{\text{bet home}} = 0.52$), indicating that the variation between homes was the dominant source of the total variability in indoor air. House C had the highest overall concentrations of total fungi, while the lowest concentrations were observed in House B. The kitchen in House B had a significantly lower GM concentration as compared to the other kitchens. House B also showed lower concentration in the bedroom than House C. In all homes, the living room consistently yielded the highest fungal spore concentrations, but this difference was not statistically significant. The ICC for day-to-day variation was negligible for the indoor total fungi.

The outdoor concentrations of total fungi had relatively low within-home variability ($\text{ICC}_{\text{within home}} = 0.20$) and only moderate between-home variability ($\text{ICC}_{\text{bet home}} = 0.33$). Figure 1b shows that the variation of outdoor fungal spore concentrations for House D was slightly lower than for the other three homes. There were no significant differences between the average outdoor concentrations of total fungi at the four sides of the same home. In addition, day-to-day variation in the outdoor air was minimal ($\text{ICC}_{\text{bet day}} = 0.03$).

With respect to the relative sizes of between-home variability, *Penicillium/Aspergillus* had the largest variation ($\text{ICC}_{\text{bet home}} = 0.49$) among genera identified in indoor air, while Ascospores had the largest variation for outdoor air ($\text{ICC}_{\text{bet home}} = 0.37$).

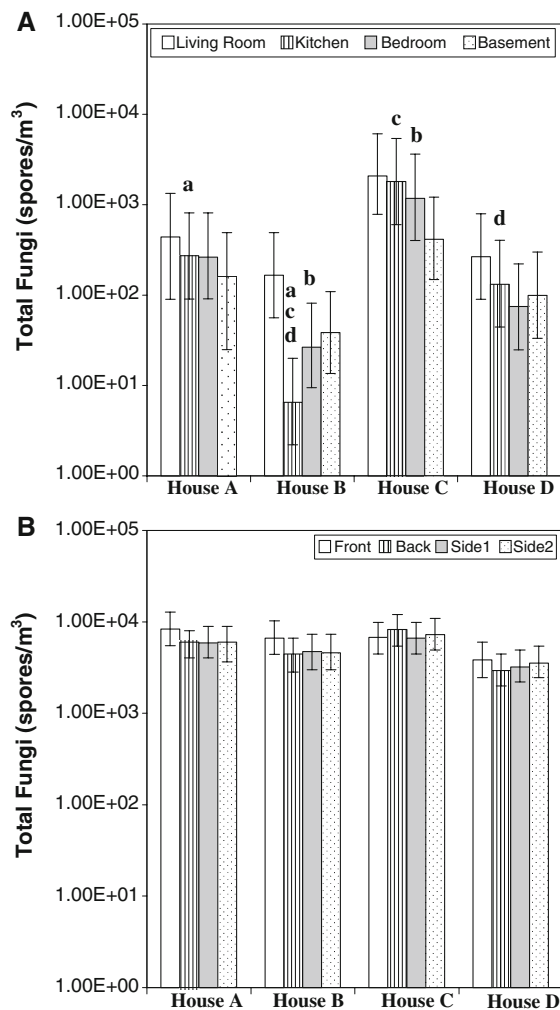


Fig. 1 Geometric means of the total fungal spore concentrations in indoor (a) and outdoor (b) air of the four houses. The error bars represent 95% confidence intervals for five consecutive 24-h samples. The means with the same lowercase letters represent significant difference ($P < 0.05$)

Interestingly, while showing the largest relative between-home variability in outdoor air, *Ascospores* had the lowest variation for indoor air ($ICC_{\text{bet home}} = 0.13$) and actually exhibited higher within-home variability ($ICC_{\text{within home}} = 0.20$). *Basidiospores* and *Cladosporium* had negligibly low relative between-home variability. Similar to indoor *Ascospores*, they both yielded higher within-home variability in outdoor air ($ICC_{\text{within home}} = 0.08$) as compared to the between-home variability ($ICC_{\text{bet home}} \sim 0$). Across all genera and classes, the within-home relative variability was below 0.22 for indoor or

outdoor air. Furthermore, day-to-day indoor variation across all fungal variables was minimal, with only *Cladosporium* showing significant daily variation ($ICC_{\text{bet day}} = 0.16$). The outdoor daily variation was small for all outcomes.

3.3 Indoor and outdoor pollen

The GM concentrations for indoor and outdoor total pollen grains for areas within each home are shown in Fig. 2. The total pollen concentrations seem to follow the same general trends as the total fungi. Although the mean concentrations were not significantly different, the living room had the highest concentrations in all homes except House C. Table 2 shows that the total pollen concentrations in indoor air had relatively low within- and between-home variability ($ICC_{\text{within home}} = 0.10$ and $ICC_{\text{bet home}} = 0.08$); however, this was not the case for outdoor air. The relative sizes of the within- and between-home variability for outdoor air were 0.09 and 0.50, respectively. The most dominant genera, *Ambrosia*, showed greater within-home variability ($ICC_{\text{within home}} = 0.17$) as compared to the variability between homes ($ICC_{\text{bet home}} = 0.02$) in indoor air, while having similar relative variability as the total pollen when measured in outdoor air. Concentrations measured in the back of House A were significantly higher than those recorded in the back of House D. House A also had significantly higher concentrations on Side 2 as compared to Houses C and D. Day-to-day variations for indoor and outdoor pollen and *Ambrosia* were small.

3.4 Indoor and outdoor beta-glucan

Figure 3 presents the indoor and outdoor daily averages (GM) of beta-glucan concentrations for each home and area within homes. House C showed significantly higher concentrations in the bedroom and kitchen than House B. Furthermore, within House C, the mean beta-glucan concentration was significantly higher in the kitchen than in the bedroom. Houses A and B had significantly different concentrations in their bedrooms. The relative amounts of between- and within-home variation for beta-glucan measured in indoor air were 0.41 and 0.09, respectively. For outdoor air data, the respective variations were 0.27 and approximately zero.

Table 2 Sources of between-home^a and within-home^b variability of indoor and outdoor data

Variable	ICC for indoor data			ICC for outdoor data		
	Between-home	Day-to-day	Within-home	Between-home	Day-to-day	Within-home
Total fungi	0.52	0*	0.10	0.33	0.03	0.20
Ascospores	0.13	0*	0.20	0.37	0*	0*
Basidiospores	0.47	0.07	0.11	0*	0*	0.08
<i>Cladosporium</i>	0.23	0.16	0.21	0*	0.02	0.08
<i>Penicillium/Aspergillus</i>	0.49	0.04	0.14	0.06	0*	0*
Total pollen	0.10	0.07	0.08	0.50	0*	0.09
<i>Ambrosia</i>	0.02	0.17	0.17	0.50	0.01	0.10
(1→3)- β -D-glucan	0.41	0*	0.09	0.27	0*	0*

^a Between-home intraclass correlations (ICC) measure the proportion of variability due to the variability among homes relative to the total variability of indoor (outdoor) measurements in all homes, determined from the variance component analysis

^b Within-home intraclass correlations (ICC) measure the proportion of variability due to the sum of the day-to-day and room-to-room (side-to-side) variability relative to the total variability of indoor (outdoor) measurements in all homes, determined from the variance component analysis

* Indicates that the variation for this source was negligible

Beta-glucan exhibited negligible day-to-day variation both indoors and outdoors. There were no significant within- or between-home differences between outdoor concentrations measured at different sides of the homes.

4 Discussion

In general, concentrations of all bioaerosols measured outdoors were higher than those measured indoors. This difference (previously reported by Lee et al. 2006) may be contributed by several factors, including high seasonal level of bioaerosols in outdoor air, lack of indoor contamination sources, and low outdoor to indoor penetration because of efficient HVAC systems in the homes. Indoor concentrations of the total airborne fungi and pollen were highest in living room areas, apparently because these were characterized by higher traffic that tends to enhance the resuspension of settled fungal spores and pollen. Buttner and Stetzenbach (1993) conducted trials to determine the effect of human activity on airborne fungal spore concentrations and reported that concentrations were significantly higher during periods of human activity than they were when no activity was taking place in the same room. It was also the primary entryway to all of the homes, so it may have had the highest amount of transport of fungal spores

and pollen from outdoors to indoors. Beta-glucan, however, did not follow this pattern. This may be due to the fact that, as a cell-wall component, beta-glucan is released when cells are destroyed. If the living room is the primary point of entry, these cells are probably less likely to be destroyed by mechanical or other means, such as desiccation. According to the review of Ruiz-Herrera (1991), some glucans are secreted in the form of slimy material from surface, which protects fungal cells from desiccation. Therefore, desiccation may lead to higher amounts of beta-glucan in the environment. House C had higher average living room and kitchen concentrations for the total fungi and beta-glucan than the other homes. This can be attributed to a higher level of occupants' activity in this house. In contrast to the other houses, the residents of House C stayed at home during daytime hours (8 am to 5 pm) and also had two pets (a cat and a dog). This increased activity, as well as the frequent opening of windows and doors in House C, led to greater resuspension of bioaerosol particles and enhanced their transport from outdoors to indoors.

4.1 Fungal spores

The total fungal spores exhibited high between-home variation, and this was more pronounced for indoor than for outdoor aerosolized fungi. This variation was observed due to the room differences. An extreme

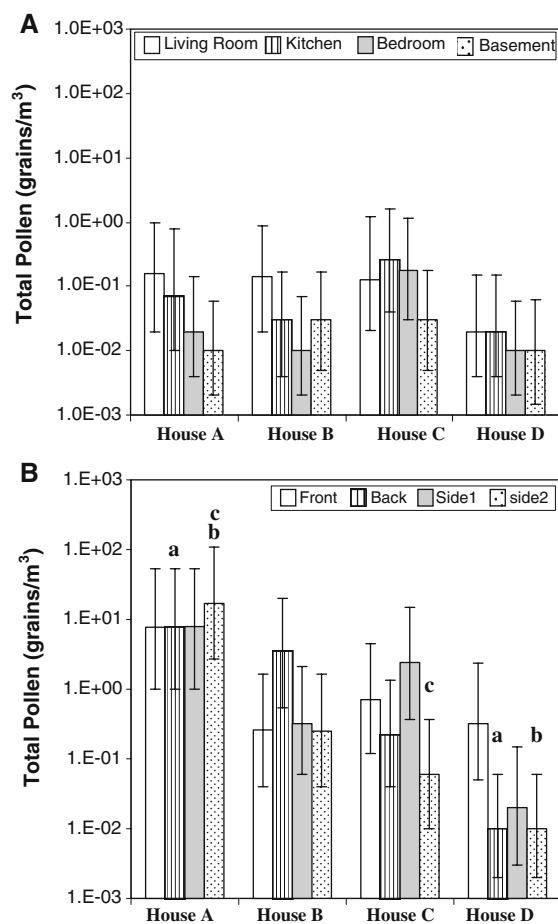


Fig. 2 Geometric means of the total pollen grain concentrations in indoor (a) and outdoor (b) air of the four houses. The error bars represent 95% confidence intervals for five consecutive 24-h samples. The means with the same lowercase letters represent significant difference ($P < 0.05$)

example of this is the difference between the GM concentrations of kitchens in House B (GM = 6.5 spores m⁻³) and House C (GM = 1,800 spores m⁻³). These differences may be attributed to a higher level of human activities in House C, as described above. Unlike Heinrich et al. (2003) and Cho et al. (2008), who observed higher variability within homes than between homes and Chew et al. (2001), who reported similar within-home and between-home variability in culturable fungi measurements, we found that the between-home variability exceeded the within-home variability. The main difference between the studies was that we used air sampling, whereas the three other studies used dust sampling. Studies have shown that within-home variances for biological contaminants

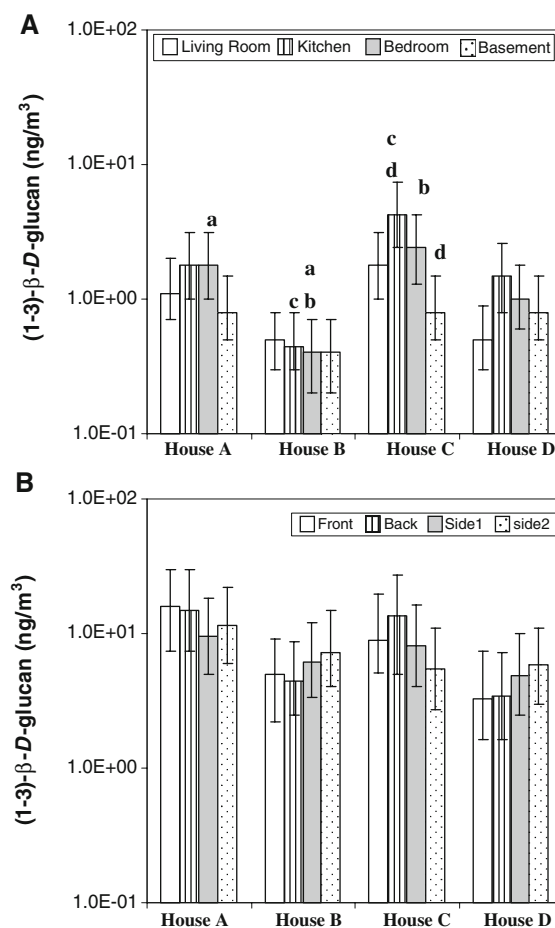


Fig. 3 Geometric means of (1→3)-β-D-glucan concentrations in indoor (a) and outdoor (b) air of the four houses. The error bars represent 95% confidence intervals for five consecutive 24-h samples. The means with the same lowercase letters represent significant difference ($P < 0.05$)

when measured in dust samples are relatively inflated. This is mainly driven by the variability in floor dust mass per unit area from one location to another location within each home (Cho et al. 2008; Heinrich et al. 2003). Therefore, the long-term air sampling of biological contaminants can be more advantageous than dust sampling, especially when attempting to establish a correlation between exposure and health outcomes.

Within the fungal types, indoor *Ascospores* and outdoor *Cladosporium* and *Basidiospores* were the only groups that had greater within-home than between-home variation. This difference is likely associated with room-to-room variation, and not to day-to-day variation, which was negligible for these

groups. Day-to-day variation was a major factor in indoor within-home variation only for *Cladosporium* and Basidiospores, which indicates that temporal variation is more important than spatial variation for these fungal types. This finding may be explained by the relatively large size of these spores that causes their rapid gravitational settling, thus, increasing temporal variation. The temporal variation is also caused by the seasonal variation of the fungal concentrations in the outdoor air (Kuo and Li 1994; Reponen et al. 1992; Ren et al. 1999) and by the activity of the occupants (Lehtonen et al. 1993).

4.2 Pollen

Pollen concentrations were relatively low because in the fall, the majority of pollen is released by weeds only, and not by trees and grasses. This point is also supported by the observed high levels of *Ambrosia* (short ragweed), which contributed to 75% of the total pollen count in indoor air. Although indoor total pollen had greater between-home variation as compared to within-home variation, both variations were lower than those obtained for the total fungi in indoor air. *Ambrosia* showed greater within-home variation, which was attributed to day-to-day differences. Thus, while all homes had overall similar indoor pollen levels, the concentration depended on the day when the sampling took place. Again, like for fungal spores, the findings are explained through the large size of pollen particles.

In the outdoor air, the between-home variation was much greater than the within-home variation and the sampling day was of no importance for the total pollen or *Ambrosia*. The large between-home variation in outdoor pollen concentrations can be attributed to the differences in the vegetation outside of the homes. For example, House A was located near a field several acres in length that had high grass and weeds, while House D was in an area with trees which were not in pollination at that time of year. House A also shows a significant difference with House C on one side of the home. Although not significant, the average pollen concentration for House D was about half that of House B and C as well. These results tend to agree with Gonzalo-Garijo et al. (2006), who concluded that exposure to pollen by allergy patients varies spatially and the concentrations depend on nearby vegetation and trees.

These factors contributed to the between-home variation, but did not affect the concentration variation between the sides (within-home) of the home because there were no barriers to separate or filter pollen grains from one side to the next of a given house. Interestingly, while showing a high significant difference for outdoor air, Houses A and D showed no significant difference for indoor air concentrations.

4.3 (1→3)- β -D-glucan

For beta-glucan, the between-home variation was the most important source of variation in both indoor and outdoor air. The within-home variation was low for indoor and negligible for outdoor air. The above findings are similar to those reported for beta-glucan measured in dust: Chew et al. (2001) found comparable within-home and between-home variations, and Giovannangelo et al. (2007) reported within-home variances to be close to or less than the between-home variances. For all sources of indoor and outdoor within-home variation, none was due to day-to-day variances. The variation of beta-glucan may be compared with the results presented by Hyvärinen et al. (2006) for the variation of indoor airborne endotoxin in five homes. They reported an ICC value of 0.36 for the variation of between-home airborne endotoxin. Our respective value for airborne indoor beta-glucan was 0.41. This suggests that the repeatability of the measurements of beta-glucan was consistent with the data published for endotoxin. Abraham et al. (2005) also reported that room-to-room and home-to-home variation was much greater than the same room temporal variation for endotoxin in house dust.

Within each house, there were no significant differences in beta-glucan concentrations between living rooms and bedrooms. This finding agrees with the results by Miller et al. (2007). Considerable differences, however, were found in average indoor room concentrations between Homes A to C; particularly large differences were seen in beta-glucan concentrations measured in the kitchens. Thorn (2001) showed that temperature difference can have an effect on airborne beta-glucan, which could be explained by seasonal variation in outdoor air fungal spores and pollen in their study. Lee et al. (2006) reported a significant positive correlation between indoor temperature and concentration of beta-glucan.

Therefore, one may speculate that higher temperatures can lead to the desiccation of fungal spores and increased release of beta-glucan from fungal spores and pollen grains. Consequently, differences in temperatures within the homes could have affected the levels in each home, causing varying levels in the air. Some homes may have used air conditioning units on certain days, while others did not, which would result in temperature differences between homes. The high beta-glucan concentration measured in House C may be due to frequent use of heat-generating appliances when preparing food.

4.4 Strengths and limitations

The strength of the study was the use of continuous long-term sampling, since it provides for a more precise estimate when dealing with spatial and temporal variations of bioaerosol data. Unlike studies designed to assess bio-contaminants in dust samples, we measured these in air, thus, eliminating the influence of dust mass on the between-home variance. A limitation of this study is the small number of homes investigated. Furthermore, we collected samples only in the fall. If the samplings were conducted during spring or summer, because of contributions from trees and flowers, greater variability and mean concentrations of pollen would be expected. However, Chew et al. (2001) have shown that the sampling season was not predictive for indoor measures of glucan and fungi. Nevertheless, geographical and climatic differences may cause differences in seasonal variations. Therefore, the results obtained in this study for temporal variation are representative to the Greater Cincinnati area and may not be generalized to other geographical areas.

5 Conclusions

The results from this study assist in developing strategies for monitoring population bioaerosol exposures through long-term air sampling. In conclusion, the between-home variation was high for the total fungi and beta-glucan in indoor air. The between-home variation was lower for the total indoor pollen. In outdoor air, the total pollen and beta-glucan showed higher between-home variation as compared to within-home variation. Day-to-day variation was

negligible in most cases, except for larger bioaerosol particles, including Basidiospores, *Cladosporium*, and *Ambrosia*.

These results show that the extent of variation is much greater between homes than it is within one home. The data analysis allows the conclusion that the majority of within-home variation is caused by differences between areas and not daily variation within any single area. This suggests that, when long-term sampling techniques are employed for characterizing bioaerosol exposure for populations, it is more beneficial to conduct limited sampling in a larger number of homes than to carry out extensive repeat sampling within a smaller number of homes. Within one home, the more advantageous strategy is to sample different rooms for indoor air or different sides of the home for outdoor air, instead of repeating sampling on different days in the same season.

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