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Spring is associated with increased total and allergenic fungal concentrations in house dust from a pediatric asthma cohort in New York City

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Abstract

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.buildenv.2022.109711.

Introduction: Asthma and allergy symptoms vary seasonally due to exposure to environmental sources of allergen, including fungi. However, we need an improved understanding of seasonal influence on fungal exposures in the indoor environment. We hypothesized that concentrations of total fungi and allergenic species in vacuumed dust vary significantly by season.

Objective: Assess seasonal variation of indoor fungi with greater implications related to seasonal asthma control.

Methods: We combined next-generation sequencing with quantitative polymerase chain reaction (qPCR) to measure concentrations of fungal DNA in indoor floor dust samples (n = 298) collected from homes participating in the New York City Neighborhood Asthma and Allergy Study (NAAS).

Results: Total fungal concentration in spring was significantly higher than the other three seasons ($p \leq 0.005$). Mean concentrations for 78% of fungal species were elevated in the spring (26% were significantly highest in spring, $p < 0.05$). Concentrations of 8 allergenic fungal species were significantly ($p < 0.5$) higher in spring compared to at least two other seasons. Indoor relative humidity and temperature were significantly highest in spring ($p < 0.05$) and were associated with total fungal concentration ($R^2 = 0.049$, $R^2 = 0.11$, respectively).

Conclusion: There is significant seasonal variation in total fungal concentration and concentration of select allergenic species. Indoor relative humidity and temperature may underlie these associations.

Keywords

Spring; Fungi; Dust; Indoor; qPCR; Asthma

1. Introduction

Asthma exacerbations which result in emergency department (ED) visits and hospitalizations account for 15% of the total direct costs of pediatric asthma and 17% of the total direct costs of asthma overall [1–3]. These exacerbations contribute to the overall asthma burden and vary with season. Exacerbations for children increase dramatically in autumn in the northern hemisphere, particularly in early September [4, 5]. Some studies have also observed a peak for pediatric exacerbations in spring [6–8]. A randomized, double-blind trial examining efficacy of the bronchodilator tiotropium in reducing seasonal exacerbations observed autumn and springtime peaks only among the placebo group [9]. Another study observed similar results examining the humanized monoclonal anti-IgE antibody omalizumab in inner-city children. Both autumn and springtime peaks among the placebo group were controlled in the treatment group by this IgE-targeting medication [10]. In contrast, exacerbations for adults seem to increase in winter months, namely December and January [11,12]. It is hypothesized that these trends in asthma exacerbations are influenced by variation in exposure to respiratory viruses as well as outdoor and indoor allergens. Increases in autumn exacerbations correlate to increases in autumn respiratory virus infections which result from children returning to school and spending increased time indoors [4,11]. Autumn exacerbations have also been associated with sensitization to common indoor allergens (e.g. dust mite, rodent, and cockroach) resulting from increased

time spent indoors [13]. Springtime increases in pediatric exacerbations are associated with increased abundance of outdoor allergens, especially tree allergen [7,14–16]. The seasonal effects of exposure to viruses and allergens are likely not independent. One study found that the combination of sensitization, recent exposure to allergens, and current viral infection significantly increased the risk of hospital admissions among asthmatics when compared to the risk from any one of the factors alone [17]. However, the risk of independent exposures should not be underestimated. Another study indicated that exposure to outdoor total fungi and certain species in the order *Pleosporales* (including species in the genus *Alternaria*) were significantly associated with increased risk of hospitalization among pediatric asthmatics, independent of rhinovirus infection [18].

We need to better understand how seasonal variation in indoor fungi may play a role in seasonal asthma exacerbations. Previous research has assessed seasonal trends in total fungi and individual fungal species. However, some studies have utilized culture-based methods to detect viable fungi. These methods are susceptible to culture-dependent biases and leave a portion of fungi undetected [19–21]. Other studies have measured seasonal fluctuations using fungal aerosols [19,20,22], which may provide a more transient and less time-integrated perspective of fungi indoors [23–25]. Alternatively, DNA-based methods and floor dust samples may better capture total fungi indoors, thereby better representing potential exposures which stay relatively stable over a single season [23,26]. Many of these studies also examine trends in outdoor fungi [19,20,22,27], which may not correlate with measures of indoor fungal concentration [20,28,29]. It is also important to note that seasonal trends in indoor and outdoor fungi are not conserved across geographies. Distributions of fungal species are shown to vary significantly in floor dust across the United States [27]. Further, degree of urbanization in sample sites affects fungal diversity [30]. Seasonal trends in one region are therefore not necessarily generalizable elsewhere, necessitating studies which characterize fungal communities in all regions of interest. Seasonal trends in indoor environmental conditions may also produce unexpected results. Changes in indoor temperature and relative humidity (which affect indoor fungi) are not necessarily correlated with changes to their outdoor counterparts, partly due to occupant behavior, including air-conditioning use.

The goal of this work is to assess seasonal variation of indoor fungi in New York City with greater implications related to seasonal asthma control. We utilized DNA-based methods to evaluate floor dust samples from homes of asthmatic children in NYC. We hypothesized that total fungal concentration and individual allergenic fungal species would vary significantly by season. We further hypothesized that seasonal trends in indoor temperature and relative humidity underlie seasonal trends in indoor fungi and predict that seasonal patterns for these variables will align.

2. Methods

2.1. Overview

Floor dust samples were collected from a starting pool of 349 eligible participants. We obtained species-rank data from next-generation sequencing. We later performed an additional round of DNA extraction on the respective dust samples and measured total

fungal concentration using quantitative polymerase chain reaction (qPCR). We evaluated statistical significance in seasonal variation compared with fungal concentration and species using one-way ANOVA analysis and multi-comparison t-tests (adjusted with the positive false discovery rate), respectively.

2.2. Participants and environmental data collection

We recruited participants aged 7–8 years old through the Health Insurance Plan of New York (HIP) between 2008 and 2009 [31]. Indoor environmental characteristics, including building type, were recorded by a field technician. Questionnaires also captured clinically relevant data. Hourly relative temperature and humidity data were collected using HOBO loggers (Onset Computer Corporation, Bourne, MA) which were placed in each site for approximately 1 week beginning at the time dust samples were initially collected. Participants were asked about general air-conditioning use. However, participants were not asked about monthly or seasonal differences in air-conditioning use. We collected floor dust from each home at a single time point and allowed the fungal community in each sample to be representative of the season in which the home was sampled.

2.3. Total fungal quantification and species rank identification

We collected one sample of bedroom floor dust per household. Samples were collected from an approximately 2 m^2 area surrounding each child's bed using a Dustream® collector (Indoor Biotechnologies, Charlottesville, VA), operated for 3 min. Samples were collected between March 2008 and June 2011 and stored at −20 °C until delivery to the following institutions. Assured Bio LLC (Oak Ridge, Tennessee) performed fungal taxonomic analysis on aliquots of dust sample. The Ohio State university analyzed total fungal concentration in separate dust aliquots. Dust sample processing preceded analysis. Dust was homogenized using 250 μm sieves (no. 60, 0.0098 inches) (Dual MFG Co., Franklin Park, Illinois). A hierarchal protocol determined the dust mass from each sample to be used in DNA extraction (Table SI–1). We excluded 44 samples from DNA extraction which had less than 1 mg of dust (Figure SI–1). We set this threshold to ensure quantitative reliability from low biomass samples. We extracted DNA from the remaining samples using QIAGEN DNEasy PowerLyzer PowerSoil kits (Qiagen; Hilden, Germany), with a modified bead-beating protocol for fungal cell lysis [22]: 1 g, 2.0 mm diameter garnet beads; 0.1 g, 0.5 mm diameter glass beads; and 0.3 g, 0.1 mm diameter glass beads. All samples were diluted 10-fold in Tris-EDTA (TE) buffer prior to DNA quantitation. Extracted DNA was used to obtain total fungal concentration and fungal taxonomic identification [32].

We measured total fungal concentration in our samples using quantitative polymerase chain reaction (qPCR) and an assay which targeted the fungal 18S rRNA region. The forward and reverse primers are respectively FF2 (5′-GGTTCTATTTTGTTGGTTTCTA-3′) and FR1 (5′-CTCTCAATCTGTCAATCCTTATT-3') [33]. All reactions contained 12.5 μL SYBR green; 0.75 μL universal fungal forward primer (FF2); 0.75 μL universal fungal reverse primer (FR1); 10 μL deionized, molecular-grade water; and 2 μL DNA template. Instrument cycling conditions were as follows: a single 10-min denaturation step at 95 °C, followed by 40 cycles of denaturation (15-s at 95 °C) and annealing/extension (1-min at 60 °C). DNA extracted from *Aspergillus fumigatus* (AF293 strain) served as standards. Subsequently, we

reported all results in spore-equivalents, representing the concentration of genetic material in our experimental samples relative to the known number of spores in our standard. We then normalized all samples by the mass of dust from which they were extracted to obtain spore-equivalents per milligram dust.

Fungal identification at species rank and relative abundance was determined using high-throughput next-generation sequencing. Illumina MiSeq 2×300 bp technology (Research and Testing Laboratory, Lubbock, Texas) and ITS1 primers were chosen to accomplish this. QIIME 1.9 (fastq-join) was used to trim reads and pair ends. Reads were further quality-trimmed to Q20 using UCLUST default settings. A minimum threshold of 10,163 reads/sample standardized the dataset. At this stage, 7 sample sites were excluded from statistical analysis involving species-rank and diversity data, as they did not meet this threshold (Figure SI–1). BLAST and UNITE databases provided taxonomic identification and FHiTINGS 1.5 parsed using the lowest common ancestor method [32]. The Ohio Super Computer provided a platform for this analysis (Ohio Super Computer. 2018. Pitzer Supercomputer. Columbus, OH: Ohio Supercomputer Center. http://osc.edu/ark:19495/hpc56htp). Sequence data was submitted to the European Nucleotide Archive under accession number PRJEB52192.

2.4. Statistical analyses

The goal of this analysis was to evaluate concentrations of total fungal species and allergenic species (Table SI–2) for statistically significant variance across four seasons. Quantitative data from qPCR complemented our sequencing results to provide absolute concentrations for each fungal taxon [26]. We adjusted these data using the inverse hyperbolic sine transformation, which approximates a log transformation, but better performs with values between zero and one [34]. This adjustment was performed prior to any averages, thus effectively generating the geometric mean [35]. We used the natural-logarithmic transformation in only one instance, to normalize the percent of allergenic species compared to total fungal concentration (Fig. 1c). All statistical tests were performed on the transformed data. Back-transformations were only performed to provide ease of understanding and context for results.

We defined four seasons in this study, each composing three-month intervals as following; winter (January/February/March); spring (April/May/June); summer (July/August/September); autumn (October/November/December). The number of dust samples collected in each season were as follows: winter = 78; spring = 98; summer = 106; autumn = 63. We did not adjust sample sizes as the variance in total fungal concentration was not significantly different between groups. We averaged concentrations of total fungi across sites according to the season in which each sample was collected. Then, we used ANOVA tests (STATA 16) to examine significant differences in total fungal concentration between seasons. Multi-comparison t-tests (SAS 9.4) examined significant differences in concentration of individual fungal species between seasons. For individual fungal species, we restricted our analyses to only those species present in at least 20% of homes in this study. We did this to reduce the influence of relatively rare fungal species during multiple-comparison analyses, which may otherwise obscure trends in more common species [36].

Our multiple-comparison t-tests included a positive false discovery rate (pFDR) adjustment. This produces q-values which are adjusted to control for effects of multiple comparisons. Significance for this term was still defined at $q < 0.05$ [37, 38]. Multiple comparison tests for season as a nominal independent variable required a modification to include six contrast levels. This produced direct comparisons between concentrations in each season to each other season for every individual fungal species. We also used ANOVA and Tukey post-hoc to examine significant differences in temperature and relative humidity by season. Welch's ANOVA and Games-Howell post-hoc was used (SPSS Statistics) when the assumption of equal variance was not met (e.g., examining mean relative humidity between seasons).

3. Results

We excluded seven samples from our analysis as they did not reach our sequencing quality threshold (10,000 reads) (Figure SI–1). Sequencing produced 21,423,819 high-quality reads, resulting in a mean 62,742 reads per sample for 342 samples. We identified 6 phyla, 10 subphyla, 23 classes, 20 subclasses, 64 orders, 128 families, 208 genera, and 291 species belonging to the kingdom fungi and present in 20% or more of homes in this cohort. *Ascomycota* and *Basidiomycota* dominated the fungal phyla in this cohort. The 10 species with the greatest prevalence averaged across sites included *Ambiguous* sp. (unclassified at the rank of species), *Alternaria alternata, Cyberlindnera jadinii, Aspergillus sydowii, Cladosporium delicatulum, Candida parapsilosis, Epicoccum nigrum, Penicillium chrysogenum, Aureobasidium namibiae,* and *Cladosporium halotolerans.*

3.1. Total fungal concentration varies seasonally

Mean total fungal concentration (in spore equivalents per mg of dust) was highest for spring, followed by summer, winter, and autumn (Fig. 1a). Furthermore, the springtime mean concentration was significantly elevated over the other three seasons (respectively, $p = 0.005$; $p < 0.001$; $p < 0.001$).

We observed a similar trend for summed allergenic fungal species, which we calculated by summing the concentrations of species known to contain allergens [39]. These allergenic species included fungi such as *E. nigrum, P. chrysogenum,* and *Rhodotorula mucilaginosa* (Table SI–2). We averaged the summed concentration of all allergenic species for all sites sampled within each season (Fig. 1b). Mean concentration of summed allergenic species was highest in the springtime and significantly greater than in autumn ($p < 0.001$) and winter ($p < 0.001$), but not in summer (p 0.244). Concentrations of summed allergenic species concentrations demonstrated similar seasonal trends as total fungal concentrations (Fig. 1b). However, the percent summed allergenic species of total fungi demonstrated a different trend, with summer representing the season the highest relative abundance of allergenic species (Fig. 1c). The similarity in trend between total fungi and allergenic fungi is partially explained by a high degree of correlation ($R^2 = 0.69$) (Fig. 1d).

3.2. Concentration varies seasonally at species rank

Concentrations of individual fungal species also varied by season. Concentrations of all the 291 species identified in this study were independently averaged across sample-sites. We

further separated species rank concentrations by season in order to assess fluctuations in each species' mean concentrations throughout the year. We found that mean concentrations of 227 fungal species were increased in the spring compared to each species' respective concentrations during the rest of the year (Fig. 2b). Mean concentrations were highest in the winter for 26 species (Fig. 2a); highest in the summer for 36 species (Fig. 2c); and highest in the autumn for only 2 species (Fig. 2d). We also found that fungal species with the highest yearly concentrations experienced their highest concentrations in the spring (e.g., *A. alternata, C. delicatulum,* and *C. parapsilosis*) (Fig. 2b). Moreover, the range of maximum springtime concentrations is much greater than the ranges we observed for the other three seasons (Fig. 2). Mean springtime concentration was also significantly highest for 77 species ($p < 0.05$). In comparison, the mean summertime concentration was significantly highest for just 3 species ($p < 0.05$). No species had mean concentrations that were significantly highest in the winter when compared to all seasons and no species had mean concentrations that were significantly highest in the in the autumn when compared to all seasons.

3.3. Concentration varies seasonally for established allergenic species

We further focused our analysis of significant associations to species known to contain allergens [39]. We identified 18 established, allergenic fungal species present in >20% of homes sampled (Table SI–2). Year-round concentrations for 12 of these species peaked in spring; 3 peaked in summer; 2 peaked in winter; and one peaked in autumn. We found 10 allergenic species to be significantly associated with season ($p < 0.05$) (Fig. 3). *A. alternata, Alternaria brassicae, Aspergillus niger, Candida albicans, E. nigrum, P. chrysogenum, R. mucilaginosa,* and *Saccharomyces cerevisiae* were significantly increased in spring compared to at least two other seasons (Fig. 3a). Meanwhile, *Alternaria metachromatica* was significantly increased in all seasons compared to autumn and *Curvularia lunata* was significantly increased in both summer and autumn compared to both winter and spring (Fig. 3b). *A. alternata* dominated allergenic fungal concentrations throughout the year. *A. alternata* concentrations were increased in the spring and summer; however, mean autumn and winter concentrations for this species were still greater than those for any other allergenic species. In fact, *A. alternata* is one of the most abundant fungal species identified in this cohort overall.

3.4. Indoor temperature and humidity associated with total fungi and season

Welch's ANOVA revealed mean indoor temperature (Fig. 4a) and mean indoor relative humidity (Fig. 4b) were significantly highest in spring compared to any other season. We also observed significant associations between total fungal concentration and these two environmental factors. Relative humidity was significantly and positively associated with total fungal concentration ($p < 0.001$, $R^2 = 0.0474$) (Fig. 4 c). Temperature was also positively and significantly associated with total fungal concentration ($p < 0.001$, $R^2 = 0.1192$) (Fig. 4 d).

3.5. Seasonal trends in fungal diversity were not clearly resolved

We observed fewer associations for fungal diversity compared to total fungal concentration. Shannon fungal diversity was highest in spring ($\mu = 5.92$), followed by winter ($\mu = 5.87$), summer ($\mu = 5.41$), and finally autumn ($\mu = 5.02$). Welch's ANOVA showed a significant

difference in Shannon fungal diversity between seasons ($p < 0.001$). Games-Howell post hoc analysis showed that Shannon diversity in spring was significantly greater than autumn ($p = 0.003$) but not summer ($p = 0.07$) or winter ($p = 0.99$). Shannon fungal diversity in winter was also significantly greater than autumn ($p = 0.006$). We did observe weakly positive correlations between Shannon fungal diversity and mean indoor temperature ($\beta = 0.08$, $p < 0.027$, $R^2 = 0.018$), and with mean indoor relative humidity ($\beta = 0.2$, $p < 0.005$, $R^2 = 0.0242$).

4. Discussion

Our results indicate there is potential for increased total and allergenic fungal exposures in spring in NYC. Season has previously been associated with asthma and variation in asthma symptoms [13]. This could be explained by seasonal changes in viral infection rates and airborne allergen levels [11,13]. Season was additionally associated with indoor fungal concentrations in this cohort of bedroom dust samples ($n = 298$) collected from homes in NYC. Total fungal concentration was significantly increased in the spring ($p < 0.001$). Furthermore, 77 fungal species were significantly increased in concentration in the spring ($p < 0.05$). Of the 10 allergenic fungal species significantly ($p < 0.05$) associated with season, eight were significantly increased in mean concentration in the spring when compared to at least two other seasons.

4.1. Geography and humidity may drive springtime associations

Fungal communities measured in dust are influenced by outdoor species brought indoors and any indoor sources such as people, pets, and growth from damp conditions [30,40]. Season may influence both outdoor fungal species as well as indoor building conditions, ventilation, and air conditioning use. We found total fungal concentration to be significantly increased in the spring compared to mean totals in winter ($p < 0.001$), summer ($p = 0.005$), and autumn ($p < 0.001$). Previous studies have observed seasonal variation in total fungal measures, but directions of these associations vary. One study observed a peak in total allergenic fungal species in the autumn (outdoor; air; New Haven, USA; DNA-based methods) [22]. Another found total fungi were increased in the summer (indoor and outdoor; air; Craców, Poland; culture-based methods) while a third found no significant difference in total fungi between seasons (indoor; dust; New Haven, USA; culture-based methods) [19,41]. The differences in these findings could be explained by factors such as indoor versus outdoor sampling and collection of dustborne versus airborne fungi. Variation in fungal enumeration methods (i.e., DNA-based or culture-based) and geography could also explain some key differences.

Differing results between other studies and the current work could arise from indoor and outdoor environmental characteristics as a factor of geography. Distributions of fungal species in floor dust are shown to vary significantly by geographic region [27]. This could explain how a study in Finland (indoor; dust; Kuopio, Finland; DNA-based method) instead found a significant increase in total species in the summer [42]. Additionally, samples in that study originated from an urban environment far less populous than NYC, which may also explain differences in our findings. Microbial richness is observed to be greater in sub-

urban indoor environments compared to urban ones [30]. Variable atmospheric conditions between study latitudes may also influence occupant behavior, especially air-conditioner use, which was not captured in the Finland study. The findings in this study demonstrate correlations, and future research is necessary to examine causality and the degree to which geography influences indoor microbial communities, specifically mediated by indoor and outdoor environmental conditions.

Additionally, our findings may be partially explained by seasonal changes in indoor temperature and relative humidity. Associations between indoor moisture levels and increased total fungal concentration have been observed [34,41,43,44]. If relative humidity becomes elevated, fungal growth can also occur in house dust [45,46]. Data collected from this study identified spring as the season with the highest indoor relative humidity and temperature. Indoor relative humidity and temperature were also associated with total fungal concentration, however the coefficient of determination for both was relatively low (Fig. 4.; $R^2 = 0.0474$; $R^2 = 0.1192$). This suggests that neither temperature nor relative humidity alone explain the variability in total fungal concentration which we observe. There may be additional mechanisms influencing these trends in fungal growth, including pets in the home, presence of carpet, and cleaning or other occupant habits [23,47,48]. One such behavior, seasonal differences in air conditioner use, could explain why we observed highest indoor temperature and relative humidity in spring while outdoor temperatures and relative humidity were greatest in summer months according to ambient data collected during study duration.

Differences in methods for sample collection and fungal quantitation may be responsible for some of the differences seen between studies as well. For instance, spring represented the greatest concentration of total fungi in our cohort. However, previous culture-based studies of aerosolized fungi found total fungal concentration to be highest in summer (indoor and outdoor; air; Craców, Poland; culture-based methods) (indoor; air; Copenhagen, Denmark; culture-based methods) [19,20]. These previous studies measured airborne fungi whereas we measured fungi in floor dust. This could explain differences in our findings as dominate fungal taxa measured in dust samples have not been found to reflect those in air samples when accounting for season (indoor; dust; New Haven; USA; culture-based methods) [41]. Additionally, air sampling can produce results with high variability, even within a short timeframe [49]. We deemed dust to be a suitable matrix for measuring potential fungal exposures because it is readily resuspended by indoor occupants [50]. It also acts as a reservoir for microbial growth and for airborne microbial deposition [44,47]. Ultimately, air sampling and floor dust likely offer separate insights to dynamic indoor fungal communities. Fungal quantification may vary between culture-based and DNA-based methods as well. Previous research relied upon culture-based methods for fungal detection (indoor and outdoor; air; Craców, Poland; culture-based methods) (indoor; air; Copenhagen, Denmark; culture-based methods) [19,20]. These methods are susceptible to culture-dependent bias in which fungal species not suited to grow on selected media remain undetected [21,51]. In comparison, DNA-based methods provide a much more comprehensive measurement of the fungal community. These methods do not distinguish cellular viability. However, it is also beneficial to measure non-viable and fragmented cells, which may also represent health impacts [26]. The differences in the breadth of fungal detection between these culture-based

and DNA-based methodologies may also be responsible for the differences in seasonal trends we observe between studies.

4.2. Concentrations of most-dominant fungal species increased in spring

Alternaria alternata was the most abundant classified fungal taxa in this study, representing the second greatest percent in all seasons except spring. This could result from qPCR quantification methodology, wherein a standard curve prepared from *A. fumigatus* spores produces results in terms of spore equivalents relative to the *A. fumigatus* 18S copy-number. *A. alternata* dictyspores are multicellular and may therefore appear in greater concentration compared to cells with fewer copy numbers [52]. *C. parapsilosis* was the most dominant fungal species in the spring, though its springtime concentration was near equal to that observed for *A. alternata*. The genus *Candida* is associated with the human skin microbiome, which could explain why we observed such a high concentration of *C. parapsilosis* in these samples [53,54]. These findings are counter to other studies which found that the genus *Cladosporium* dominated either across all seasons or in the season representing the greatest mean total fungal concentration [19,26,42]. Again, this could be a result of measurement methods or geography affecting indoor environmental factors.

We did observe significant seasonal variation for at least one *Cladosporium* species. *Cladosporium delicatulum* was the fourth most prevalent species (absolute concentration) on average across all seasons. Mean concentration of *C. delicatulum* was significantly higher in the spring compared to winter ($p = 0.019$) and autumn ($p = 0.002$) but not compared to summer ($p = 0.37$). The non-significant difference between spring and summer means that this *Cladosporium* species is increased in warmer months, which is consistent with trends in previous work [19, 42].

The most abundant fungal species for summer in our study was *A. alternata*, which was significantly increased in mean concentration in both spring and summer compared to both winter and autumn. Another study conducted in the Northeast United States also found concentrations of the genus *Alternaria* to be highest in summer [41]. Relatively large conidia (10 μm diameter) leading to increased deposition rates could explain why we observed such high concentrations of *A. alternata* in our floor dust samples [52,55]. It is important to note that these observations are likely affected by indoor environmental factors as studies conducted in other geographies found increased *Alternaria* concentrations associated with autumn [19,26].

4.3. Majority of allergenic species associations observed in spring

We identified 10 allergenic fungal species in this cohort that were significantly increased in concentration during one or more seasons [39]. *A. alternata, A. brassicae, A. niger, C. albicans, E. nigrum, P. chrysogenum, R. mucilaginosa,* and *S. cerevisiae* were significantly increased in spring compared to at least two other seasons (Fig. 3a). Overall, spring represents a potentially important season for NYC asthmatics sensitized to fungi due to the number of allergenic species which are increased in concentration within the indoor environment (Fig. 3).

Growth of the allergenic species identified in this study might be explained by their relative hydrophilicity and that indoor relative humidity is seen to increase in the spring. The hydrophilic yeast, *R. mucilaginosa* has been associated with growth in humid indoor environments including bathrooms [29,49,56,57]. Other yeasts, including taxa in the genera *Candida* and *Saccharomyces,* have also been associated with increased moisture conditions indoors [49,57]. *Candida* is also associated with the human skin microbiome, which may be another source for concentrations of this genus [53,54]. Similar trends have been observed for some mesophilic fungi, including *E. nigrum* and *A. alternata* [56,57]. This may explain increased fungal concentrations in the spring, during which time indoor relative humidity was higher compared to all other seasons. It is possible that the period of increasing relative humidity and indoor temperatures which we observed during the spring provides optimal growth conditions for a broad range of fungi. This could explain an increase in springtime total fungal concentration, and specifically hydrophilic fungi, some of which have been identified as allergenic [39].

Further research is necessary to understand the influence of indoor environmental characteristics on diverse fungal communities. We observed that seasonal variation has a profound effect on the indoor environment and thus should be considered when evaluating fungal exposures in future epidemiological studies. Overall, spring was associated with high concentrations of total fungi, allergenic fungi, and in- door environmental conditions which could explain fungal growth.

4.4. Diversity

We observed significant associations between season and fungal concentration, but we did not observe the same associations between season and fungal diversity. Shannon fungal diversity in winter and spring was significantly increased over autumn, but not summer. Further, fungal diversity was weakly associated with mean indoor temperature and relative humidity. Our findings suggest that these indoor environmental conditions, and season overall, may have a greater impact on concentrations of total fungi compared to richness and evenness of specific fungal taxa. This position is supported by a previous study which demonstrated that composition of fungal taxa in carpets was less influenced by moisture conditions compared to site-specific characteristics, which could include number of occupants, presence of pets, and other factors [46]. In some cases, ‘moldy’ homes or homes with a history of water damage have even been associated with lower fungal diversity than homes with no such history [58]. However, diversity remains a measure of interest in understanding the role of fungal exposures in asthma outcomes. Exposure to low fungal diversity indoors has been associated with increased risk of sensitization and asthma development, though the underlying mechanisms remain unclear [34,59,60]. Thus, further research is needed to understand the role of season as well as temperature and humidity on total fungal growth and fungal diversity.

4.5. Limitations and future considerations

DNA-based methods are susceptible to mis-quantification bias due to primers preferentially amplifying G-C rich regions in select species [61–63]. Further, it is difficult to resolve fungal taxa at rank of species beyond 97% agreement as the ITS1 region can vary even

within a fungal species [63]. Mis-quantification can also occur due to differences in 18S copy-number. For instance, when using qPCR, we may observe higher concentrations of *A. alternata* due to the multicellularity of its dictyspores [52]. Yet, this potential limitation avoids equivocating environmental fungi. It is true that an *A. alternata* spore is larger (and multicellular) compared to a single spore of *A. fumigatus*; however, it is not known whether larger spores could generate more allergens resulting in greater potential exposures. We can also reasonably assume that multicellularity for a single fungal species is more consistent between certain environmental conditions (e.g., seasons) than is copy-number between disparate fungal species. By capturing seasonal trends in concentration of individual species, we provide a more robust analysis than comparing different fungal species within a season.

We also recognize limitations in indoor temperature and relative humidity measures without consideration for patterns in air conditioner use, which may very well explain unexpected decreases for both these variables in the summertime. Additionally, there are limitations in the strength of the associations we found between seasons due to our study design. Temperature and relative humidity data were recorded after the dust sample was collected as necessitated by study logistics. This reduced the required number of home visits and allowed for data collection from an unprecedented number of homes. However, we make assumptions regarding the temporality of environmental conditions and their effect on fungal concentrations which can be avoided in future research.

We did not sample each site during each season, rather we sampled each site once and allowed the fungal community in each site to be representative of the season in which it was sampled. Additionally, we did not adjust for potential effects from the distribution of samples within a season. Collection dates for dust samples were distributed approximately evenly (Figure SI–2) across each season; however, we did not exclude or adjust data collected from sites at the beginning of each season, which could be strongly influenced by environmental conditions in the season prior. Future research should attempt to reproduce these findings by sampling in each home during four seasonal time points.

Future studies should also seek to examine these findings in relation to asthma outcomes as we collected twelve-month, and not seasonal, health data to examine alongside our findings. Previous research has identified September as a critical month for asthma control, citing dramatic increases in ED visits and hospitalizations related to asthma during this time [4]. It is believed that this results from renewed exposure to respiratory viruses when children and adolescents return to school [11]. Importantly, spring represents a secondary increase in rhinovirus prevalence [4,64]. Further research should measure the seasonal variability in fungal species alongside respiratory viruses, such as rhino- virus, to assess possible synergistic effects related to asthma exacerbations. Special attention should be given to spring, as implicated in this study.

5. Conclusion

Our findings implicate spring as an important season for asthma control among asthmatics sensitized to fungi in NYC. We found that total fungal concentration and concentration of individual allergenic species were significantly increased in the spring. This pattern

in indoor fungal exposure may partially explain the springtime increase in pediatric asthma exacerbations observed in some studies [6,7,9]. Such exacerbations represent a substantial percent of the global pediatric asthma burden [1–3,65,66]. Our goal to reduce this burden requires implementation of intervention strategies and design for healthier indoor environments, both of which must first consider the profound effect season has on potential fungal exposure. Here we present a study that uses novel DNA-based methods to characterize dynamic fungal communities in accordance with this aim. However, the role of fungal exposures in health outcomes remains understudied and further research is required to understand the associations to improve asthma control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The research protocol under which this study was conducted was approved by The Ohio State University and Columbia University Institutional Review Boards.

Data availability

Sequence data was submitted to the European Nucleotide Archive under accession number PRJEB52192.

Abbreviations

ADONIS	Permutational analysis of variance of dissimilarities
ANOVA	Analysis of variance
BLAST	Basic Local Alignment Search Tool
FHiTINGS	Fungal high-throughput taxonomic identification tool for use with next-generation sequencing
HAPN	High Asthma Prevalence Neighborhoods
HIP	Health Insurance Plan of New York City
LAPN	Low Asthma Prevalence Neighborhoods

NGS	Next-generation sequencing
QIIME	Quantitative Insights Into Microbial Ecology
qPCR	Quantitative polymerase chain reaction

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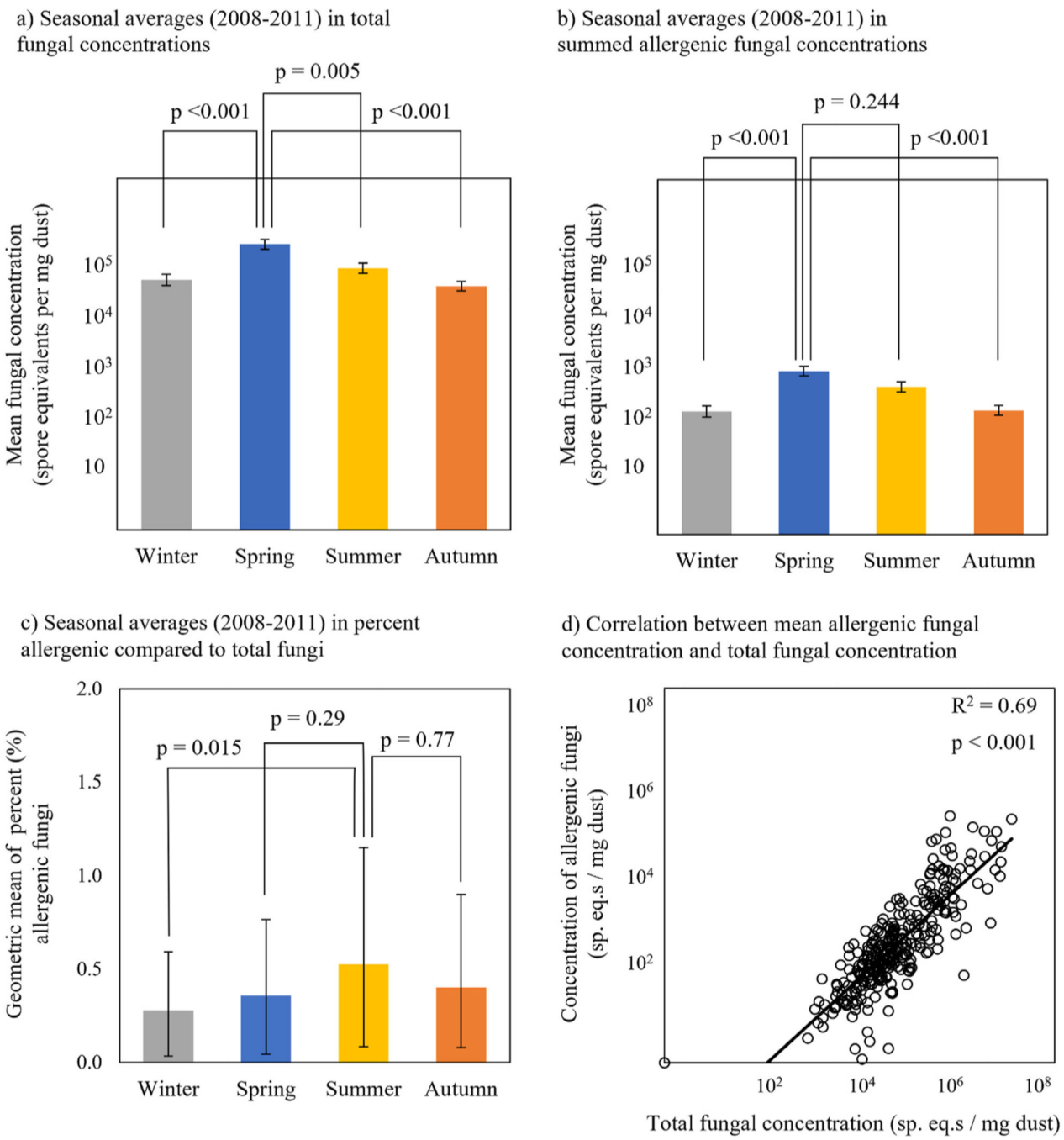

Fig. 1.

Effect of season on total and allergenic fungi. a) Mean concentration (spore equivalents per mg of dust) of total fungal species per each season (concentration was adjusted using the inverse hyperbolic sine, which approximates a log-normal transformation, in order to compare significant differences between seasons); b) summed allergenic fungal species in each season, (concentration was adjusted using the inverse hyperbolic sine, which approximates a log-normal transformation, in order to compare significant differences between seasons); c) percent of total fungal concentration represented by allergenic species,

(percentages were normalized using the natural logarithmic transformation, averaged by season, then back-transformed to obtain the geometric mean); d) linear regression of allergenic fungal concentrations and total fungal concentrations, R^2 is unadjusted.

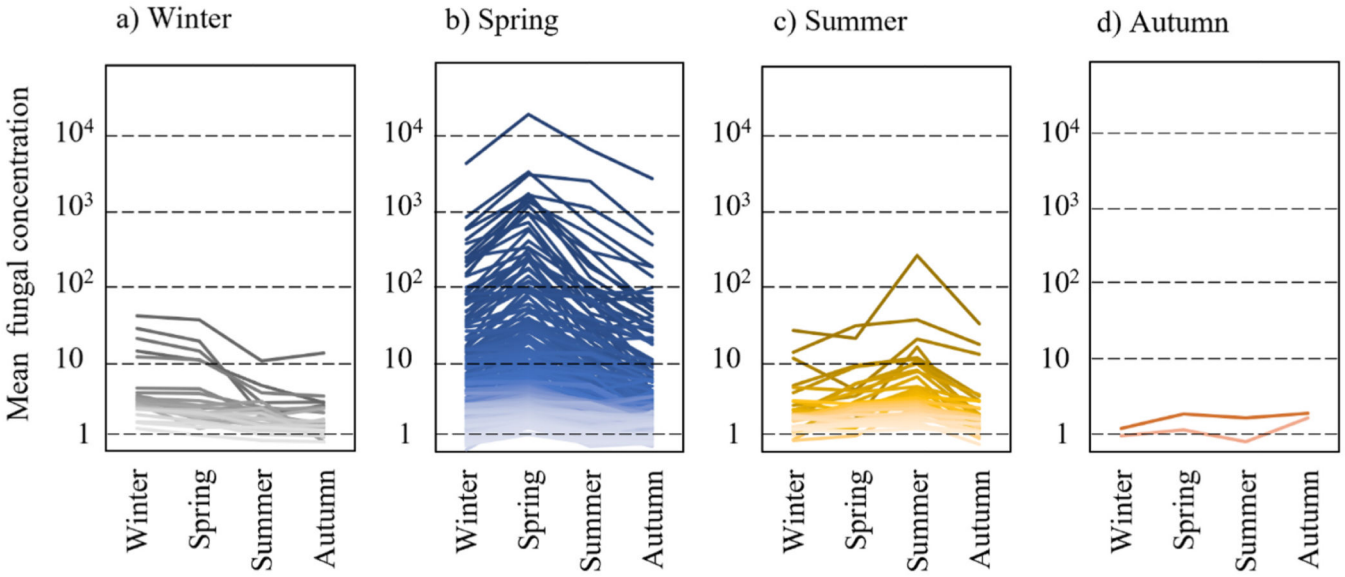

Fig. 2.

Mean concentration of individual fungal species per each season.

Mean concentration (spore equivalents per mg of dust) of individual fungal species per each season. Concentration was adjusted using the inverse hyperbolic sine, which approximates a log-normal transformation, in order to compare significant differences between seasons. A total of 291 fungal species are represented as separate lines on the four graphs; species appear on the graph matching the season in which their highest concentration was observed; a) seasonal concentrations of fungal species which peaked in the winter compared to the other three seasons; b) seasonal concentrations of fungal species which peaked in the spring; c) seasonal concentrations of fungal species which peaked in the summer; d) seasonal concentrations of fungal species which peaked in the autumn.

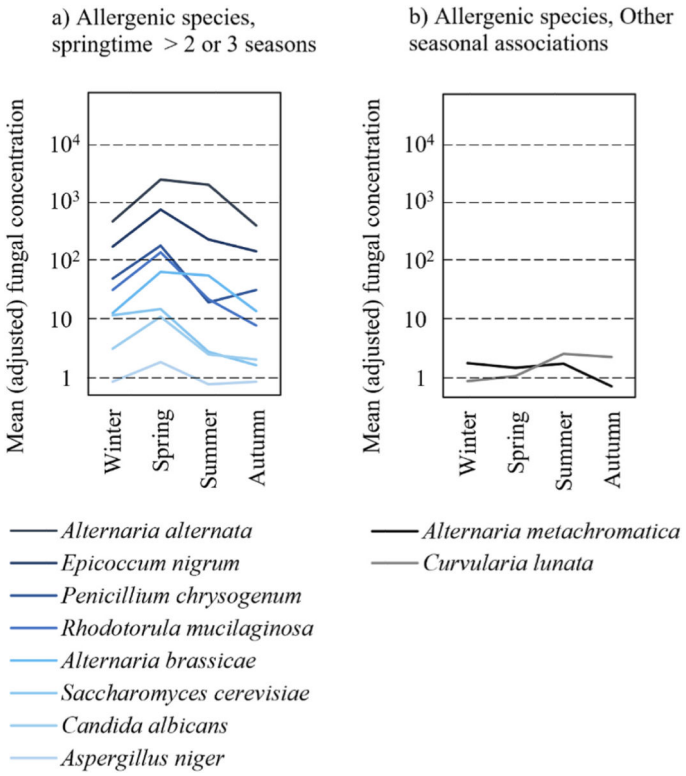

Fig. 3.

Mean concentration of individual, allergenic fungal species per each season.

Mean concentration (spore equivalents per mg of dust) of individual, allergenic fungal species per each season. Concentration was adjusted using the inverse hyperbolic sine, which approximates a log-normal transformation, in order to compare significant differences between seasons; a) 10 allergenic fungal species were significantly associated with seasonality; 8 of these allergenic species demonstrated mean springtime concentrations that were significantly higher than observed concentrations for at least two other seasons; c) only two species were significantly associated with season without demonstrating a significantly elevated springtime concentration. Fungal species listed in the legend are ordered according to descending springtime concentrations observable in the above chart.

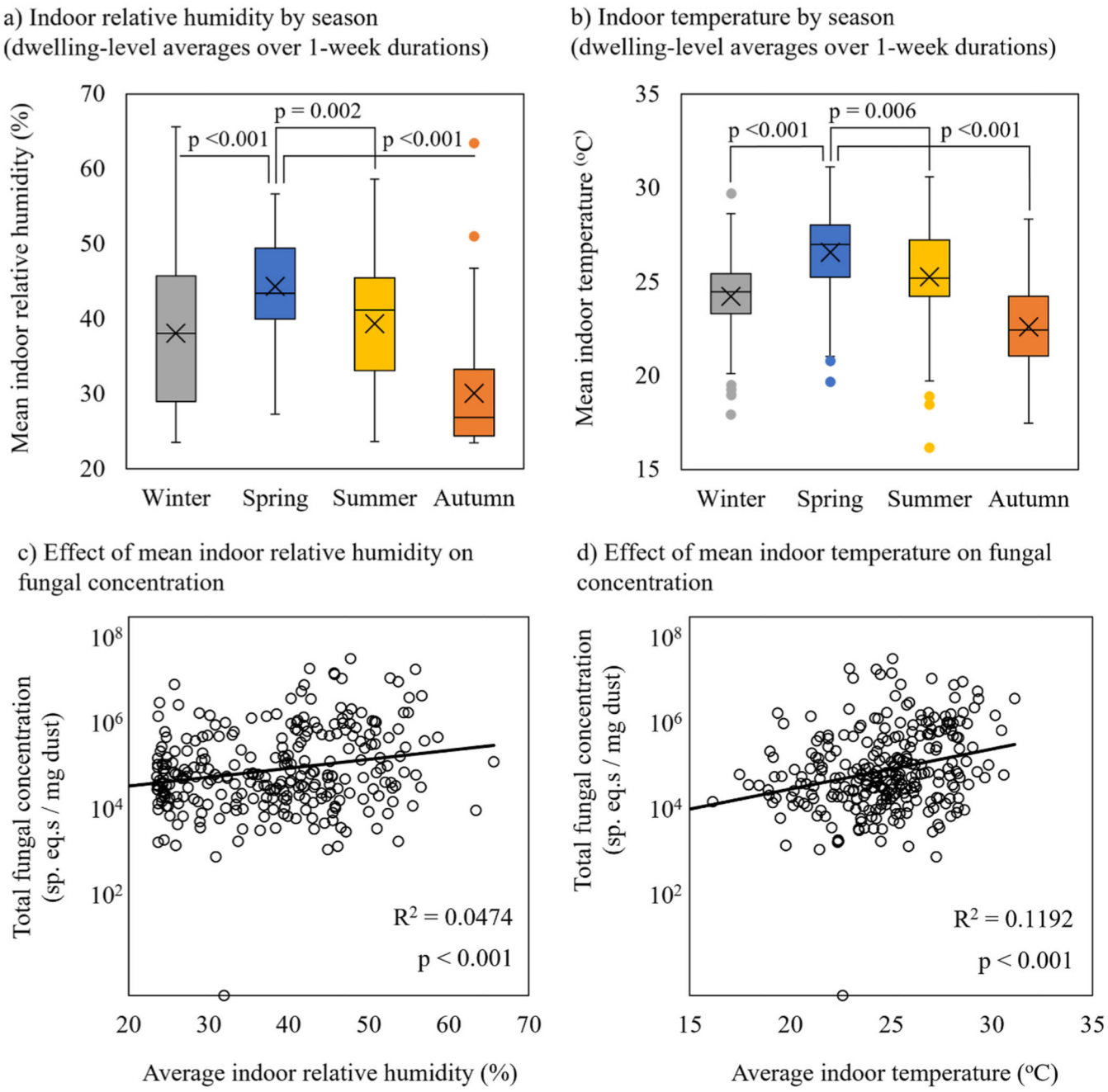

Fig. 4.

Trends for mean indoor temperature and mean indoor relative humidity.

Trends between two environmental factors, mean indoor temperature and mean indoor relative humidity with season and total fungal concentration. Temperature and RH were averaged over 1-week sampling durations for each dwelling. a) indoor relative humidity averaged across all sites is indicated by crosses on the graph and was used to determine significant differences between seasons; indoor RH in spring was significantly greater than that in winter, summer, and autumn); b) indoor temperature aver- aged across all sites is indicated by crosses on the graph and was used to determine significant differences between

seasons; indoor temperature in spring was significantly greater than that in winter, summer, and autumn; c) mean indoor relative humidity was significantly and positively associated with total fungal concentration, d) mean indoor temperature was significantly and positively associated with total fungal concentration.