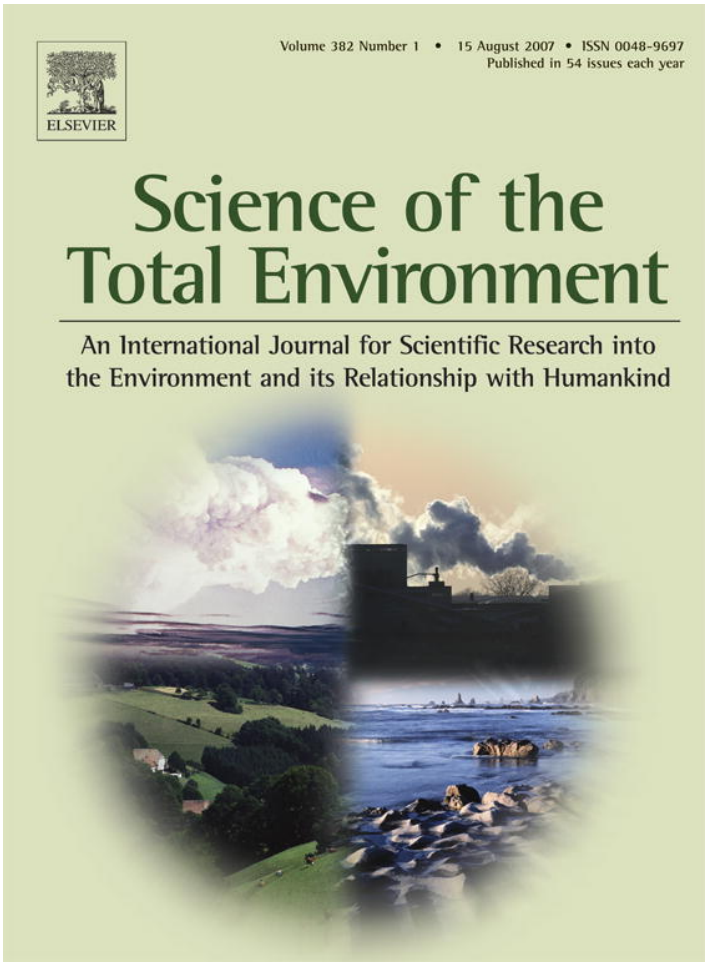

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Short communication

Comparison of mold concentrations quantified by MSQPCR in indoor and outdoor air sampled simultaneously

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Abstract

Mold specific quantitative PCR (MSQPCR) was used to measure the concentrations of the 36 mold species in indoor and outdoor air samples that were taken simultaneously for 48 h in and around 17 homes in Cincinnati, Ohio. The total spore concentrations of 353 per m^3 of indoor air and 827 per m^3 of outdoor air samples were significantly different ($p \leq 0.05$). However, only the concentrations of *Aspergillus penicillioides*, *Cladosporium cladosporioides* types 1 and 2 and *Cladosporium herbarum* were correlated in indoor and outdoor air samples (p-value ≤ 0.05 and sufficient data for estimate and absolute value rho estimate ≥ 0.5). These results suggest that interpretation of the meaning of short-term (<48 h) mold measurements in indoor and outdoor air samples must be made with caution.

Keywords: Mold; Indoor air; Outdoor air; Mold specific quantitative PCR

1. Introduction

One common procedure used to determine if a building has an abnormal mold condition is the comparison of indoor and outdoor mold concentrations in air samples (Gots et al., 2003). Often the comparison is based on the ratio of the total number of spores, or totals for a few genera, quantified by either spore counting or culturing on one or two media. These methods, however, ignore three basic problems. First, not all species grow on the same media and/or at the same rate. Second, not identifying molds at the species level reduces the relevance of mold diversity. Third, air sampling times are usually short, often less than 5 min, because of overgrowth on culture plates or masking in spore traps. In this study, longer sampling times and a DNA-based method for identifying and quantifying species of molds were tested to examine the relationship between indoor and outdoor mold populations in air samples.

2. Materials and methods

2.1. Selection of homes

Seventeen homes were selected as part of the Cincinnati Childhood Allergy and Air Pollution Study

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(CCAAPS) (Ryan et al., 2005). The homes were inspected for visible mold/water damage and classified groups: 1) no mold or water damage, 2) minor mold or water damage, and 3) major mold damage (Cho et al., 2006). Among the seventeen homes, six had no mold or water damage and eleven had minor mold–water damage that appeared as visible signs of water and /or mold damage <0.2 m^2, moldy odor or water mold damage history reported by the occupants (Cho et al., 2006). The homes were built between 1928 and 1998. Sixteen homes had central air conditioning. Fibrous HVAC filters were used in seven homes and an electrostatic filter in four homes. No filters were found in the other homes.

2.2. Indoor air and outdoor air sample collection

All homes were sampled in the March to May period of 2003. Indoor and outdoor air samples were taken simultaneously using Button Personal Inhalable Aerosol Samplers (SKC Inc., Eighty Four, PA) loaded with 25 mm polycarbonate filters (1 μm pore size, Osmonics Inc., Minnetonka, MN, USA). Each sampling device was operated at a sampling flow rate of 4 l per min, which was maintained by a small vacuum pump (Model 224-PCXR4; SKC Inc.) and the flow rate verified with dryCal® DC-Lite Calibrator (Bios International Corporation, Butler, NJ, USA) before and after each 48-h measurement. The outdoor samplers were fixed on a tripod with a rain shield at a height of 1.5 m. Each indoor sampler was placed in the room with the sampler pumps housed inside a noise-insulated enclosure. The residents stayed at home and carried-on with their normal activities.

2.3. Mold specific quantitative PCR (MSQPCR) analysis of air samples

Methods have been reported previously for extracting DNA and performing MSQPCR analyses (Haugland et al., 2002; Brinkman et al., 2003; Haugland et al., 2004). All primer and probe sequences, as well as known species comprising the assay groups, were published at the website: http://www.epa.gov/microbes/moldtech.htm.

2.4. Statistical analyses

Mold concentration data having a minimum detection limit of 1 cell per m^3 for air samples were treated as left-censored data with appropriate statistical methods applied (Helsel, 2005). Procedurally, non-detections were set at 1/2 the limit of detection (LOD). Thus, non-detections were given equal and lowest rank for non-parametric rank-based analyses (Helsel, 2005). The Wilcoxon Signed Rank Test was used for comparisons of mold spore concentrations between paired samples taken at each of the seventeen homes with *p*-values adjusted for multiple comparisons using the method of Benjamini and Hochberg (1995). Correlation between mold species in the different sample types was tested using the rank based Spearman's Rho test. An agreement ratio reflecting the number of identical

Table 1
Averages of the number of spores per m^3 of indoor or outdoor air followed by percent of homes with mold concentrations above the limit of detection (LOD) of 1 cell per m^3 of air

	Averages (% above LOD of 17 homes)	
	Indoor	Outdoor
Group 1 molds		
Aspergillus flavus	<LOD (12%)	1 (35%)
Aspergillus fumigatus	1 (29%)	3 (82%)
Aspergillus niger	1 (35%)	1 (59%)
Aspergillus ochraceus	1 (18%)	<LOD (24%)
Aspergillus penicillioides	7 (94%)	9 (82%)
Aspergillus restrictus	1 (47%)	3 (41%)
Aspergillus sclerotiorum	1 (24%)	<LOD (6%)
Aspergillus sydowii	1 (12%)	35 (18%)
Aspergillus unguis	<LOD (12%)	<LOD (6%)
Chaetomium globosum	<LOD (0%)	<LOD (6%)
Aspergillus versicolor	3 (35%)	5 (65%)
Eurotium chevalieri	6 (94%)	34 (100%)
Penicillium brevicompactum	20 (65%)	40 (71%)
Cladosporium sphaerospermum	2 (71%)	2 (47%)
Penicillium corylophilum	3 (24%)	<LOD (12%)
Penicillium purpurogenum	<LOD (6%)	<LOD (6%)
Penicillium spinulosum	4 (47%)	10 (65%)
Penicillium variabile	2 (29%)	5 (41%)
Paecilomyces variotii	1 (29%)	1 (41%)
Penicillium crustosum	2 (12%)	4 (41%)
Aureobasidium pullulans	104 (94%)	264 (100%)
Scopulariopsis brevicaulis	<LOD (6%)	<LOD (18%)
Scopulariopsis chartarum	<LOD (6%)	1 (53%)
Stachybotrys chartarum	<LOD (0%)	<LOD (0%)
Trichoderma viride	<LOD (0%)	<LOD (12%)
Wallemia sebi	4 (82%)	13 (100%)
Group 2 molds		
Acremonium strictum	21 (59%)	17 (53%)
Alternaria alternata	1 (47%)	3 (94%)
Penicillium chrysogenum Type 2	1 (59%)	1 (12%)
Aspergillus ustus	<LOD (6%)	<LOD (0%)
Cladosporium cladosporioides Type 1	103 (100%)	165 (100%)
Cladosporium cladosporioides Type 2	2 (59%)	13 (77%)
Cladosporium herbarum	37 (94%)	114 (100%)
Epicoccum nigrum	25 (88%)	84 (100%)
Mucor racemosus	<LOD (24%)	<LOD (6%)
Rhizopus stolonifer	<LOD (0%)	<LOD (0%)

Table 2
Evaluation of the difference in each mold species' concentration in indoor or outdoor air are based on Wilcoxon Signed Rank Test (significant in bold)

	Wilcoxon test *p*-values [a]		Spearman's Rho test		Proportion >LOD	
	In vs out doors	*I*/*O* ratios	Rho	*p*-values	Indoor	Outdoor
Group 1						
A. flavus	0.424	NA	0.177	0.49	0.118	0.35
A. fumigatus	0.093	0.33	0.412	0.10	0.294	0.82
A. niger	0.067	1.00	−0.083	0.75	0.353	0.59
A. ochraceus	0.231	NA	0.463	0.06	0.176	0.24
A. penicillioides	**0.003**	**0.78**	**0.544**	**0.03**	**0.941**	**0.82**
A. restrictus	**0.030**	**0.33**	0.373	0.14	0.471	0.41
A. sclerotiorum	0.140	NA	−0.137	0.60	0.235	0.06
A. sydowii	0.424	0.03	0.336	0.19	0.118	0.18
A. unguis	0.424	NA	−0.091	0.73	0.118	0.06
C. globosum	NA [b]	NA	NA	NA	0.000	0.06
A. versicolor	0.067	0.60	0.656	0.01	0.353	0.65
E. chevalieri	**0.003**	**0.18**	0.350	0.17	0.941	1.00
P. brevicompactum	**0.013**	**0.50**	0.363	0.15	0.647	0.71
C. sphaerospermum	**0.010**	**1.00**	0.176	0.50	0.706	0.47
P. corylophilum	0.140	NA	0.176	0.49	0.235	0.12
P. purpurogenum	1.000	NA	−0.062	0.81	0.059	0.06
P. spinulosum	**0.030**	**0.40**	0.073	0.78	0.471	0.65
P. variabile	0.093	0.40	0.096	0.71	0.294	0.41
P. variotii	0.093	1.00	0.451	0.07	0.294	0.41
P. crustosum	0.424	0.50	−0.292	0.26	0.118	0.41
A. pullulans	**0.003**	**0.39**	0.432	0.08	0.941	1.00
S. brevicaulis	1.000	NA	0.576	0.02	0.059	0.18
S. chartarum	1.000	NA	0.168	0.52	0.059	0.53
S. chartarum	NA	NA	NA	NA	0.000	0.00
T. viride	NA	NA	NA	NA	0.000	0.12
W. sebi	**0.005**	**0.31**	0.273	0.29	0.824	1.00
Group 2						
A. strictum	**0.014**	**1.23**	−0.136	0.60	0.588	0.53
A. alternata	**0.021**	**0.33**	0.447	0.07	0.471	0.94
P. chrysogenum 2	**0.015**	**1.00**	−0.143	0.58	0.588	0.12
A. ustus	1.000	NA	NA	NA	0.059	0.00
C. cladosporioides 1	**0.003**	**0.62**	**0.616**	**0.01**	**1.000**	**1.00**
C. cladosporioides 2	**0.015**	**0.15**	**0.585**	**0.02**	**0.588**	**0.77**
C. herbarum	**0.003**	**0.32**	**0.531**	**0.03**	**0.941**	**1.00**
E. nigrum	**0.004**	**0.30**	0.279	0.28	0.882	1.00
M. racemosus	0.110	NA	−0.139	0.60	0.235	0.06
R. stolonifer	NA	NA	NA	NA	0.000	0.00

Correlations in concentrations are based on Spearman's Rho test. Species in bold have *p*-value ≤0.5 and sufficient data for estimate of absolute value Rho ≥0.5.
[a] Adjusted for multiple comparisons.
[b] NA = All values below limit of detection.

species isolated from both of two sample types and relative to the total number of species identified in both sample types was calculated by equation $R=2W/(A+B)$,where W=number of species both samples have in common, A=total number of species in sample 1 and B=total number of species in sample 2. Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC) and the R Software environment for statistical computing and graphics (http://www.r-project.org/).

3. Results

Table 1 shows the average concentrations of the 36 mold species measured by MSQPCR in the indoor (*I*) or outdoor (*O*) air samples. The total spore concentrations of 353 per m^3 of indoor air and 827 per m^3 of outdoor air samples were significantly different ($p \leq 0.05$). Comparison of the concentration of specific molds in indoor and outdoor air samples showed that many were statistically significantly different in concentrations

($p \leq 0.05$ based on the Wilcoxon Signed Rank Test) (Table 2). The *I*/*O* ratios for the species varied from 0.15 to 1.23 (Table 2) compared to the total spore *I*/*O* of 0.43. The correlation between mold species in indoor and outdoor air samples was evaluated with the Spearman's Rho test (Table 2). Only *Aspergillus penicillioides*, *Cladosporium cladosporioides* types 1 and 2 and *Cladosporium herbarum* were correlated between indoor air and outdoor air samples.

4. Discussion

Spearman's Rho analysis showed that the concentrations of only four individual species in indoor and outdoor air samples were correlated with each other. This is consistent with our previous results in which the fungal genera clusters *Aspergillus/Penicillium* and *Cladosporium* were correlated in indoor and outdoor air samples (Lee et al., 2006). This may be explained by the fact that *A. penicillioides* is the dominant member of the *Aspergillus/Penicillium* genus cluster and *C. cladosporioides* types 1 and 2 and *C. herbarum* are the dominant members of the *Cladosporium* genus cluster (Vesper et al., 2004; Meklin et al., 2004; Vesper et al., 2006). The limitations of air samples have been observed by others.

O'Connor et al. (2004) noted that air samples have often been limited to 5 min or less with the resulting limitations in understanding the environmental source of the mold. Verhoeff et al. (1992) used N6-Andersen samplers in combination with DG-18 agar with a sampling time of 5 min to compare indoor and outdoor air samples. They stated that "the number of CFU/m^3 in the indoor and outdoor air varied widely" and the "low predictive value … limits their use in epidemiological studies." Spicer and Gangloff (2005) showed that "the levels of fungi in the outdoor air varied significantly between morning and afternoon … with no pattern by species, time of day or location."

In our study, the lack of species specific concentration correlations may be due to the relatively small number of homes, most without significant water damage. Also, most homes had central air conditioning with either a fibrous or electrostatic filter. However, our results suggest that evaluating the mold burden indoors by a simple genus level comparison to the outdoors may be misleading.

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Notice

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References

Benjamini Y, Hochberg Y. Controlling the false-discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 1995;57:289–300.

Brinkman NE, Haugland RA, Wymer LJ, Byappanahalli M, Whitman RL, Vesper SJ. Evaluation of a rapid, quantitative real-time PCR method for cellular enumeration of pathogenic *Candida* species in water. Appl Environ Microbiol 2003;69:1775–82.

Cho S-H, Reponen T, LeMasters G, Levin L, Huang J, Meklin T, et al. Mold damage in homes and wheezing in infants. Ann Allergy Asthma Immun 2006;97:539–45.

Gots RE, Layton NJ, Pirages SW. Indoor health: background levels of fungi. AIHAJ 2003;64:427–38.

Haugland RA, Brinkman NE, Vesper SJ. Evaluation of rapid DNA extraction methods for the quantitative detection of fungal cells using real time PCR analysis. J Microbiol Methods 2002;50:319–23.

Haugland RA, Varma M, Wymer LJ, Vesper SJ. Quantitative PCR of selected *Aspergillus*, *Penicillium* and *Paecilomyces* species. Syst Appl Microbiol 2004;27:198–210.

Helsel DR. Nondetects and Data Analysis, Statistics for Censored Environmental Data. Wiley and Sons, Inc. NY, NY. Environmental data. Wiley and Sons Inc. Hoboken, NJ. 2005.

Lee T, Grinshpun SA, Martuzevicius D, Adhikari A, Crawford CM, Luo J, et al. Relationship between indoor and outdoor bio-aerosols collected with a Button inhalable aerosol sampler in urban homes. Indoor Air 2006;16:37–47.

Meklin T, Haugland RA, Reponen T, Varma M, Lummus Z, Bernstein D, et al. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. J Environ Monit 2004;6:615–20.

O'Connor GT, Walter M, Mitchell H, Kattan M, Morgan WJ, Gruchalla RS, et al. Airborne fungi in the homes of children with asthma in low-income urban communities: The Inner-City asthma study. J Allergy Clin Immunol 2004;114:599–606.

Ryan PH, LeMasters G, Biagini J, Bernstein D, Grinshpun SA, Shukla R, et al. Is it traffic type, volume, or distance? Wheezing in infants

living near truck and bus traffic. J Allergy Clin Immunol 2005;116:279–84.
Spicer R, Gangloff H. Establishing site specific reference levels for fungi in outdoor air for building evaluation. J Occup Environ Hyg 2005;2:257–66.
Verhoeff AP, van Wijnen JH, Brunekreef B, Fischer P, van Reenen-Hoekstra ES, Samson RA. Presence of viable mould propagules in indoor air in relation to house damp and outdoor air. Allergy 1992;47:83–91.
Vesper SJ, Varma M, Wymer LJ, Dearborn DG, Sobolewski J, Haugland RA. Quantitative PCR analysis of fungi in dust from homes of infants who developed idiopathic pulmonary hemorrhaging. J Occup Environ Med 2004;46:596–601.
Vesper SJ, McKinstry C, Yang C, Haugland RA, Kercsmar CM, Yike I, et al. Specific molds associated with asthma. J Occup Environ Med 2006;48:852–8.