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Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American county[1]

PING REN, THOMAS M. JANKUN AND BRIAN P. LEADERER

Division of Environmental Health Sciences, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut

Fungi cause allergies and many other adverse health effects. In this study, we characterized the nature and seasonal variation of fungi inside and outside homes in the Greater New Haven, Connecticut area. Three indoor air samples (in the living room, bedroom, and basement) and one outdoor sample were collected by the Burkard portable air sampler. House dust samples were collected in the living room by a vacuum cleaner. The mold concentrations varied widely from house to house in both indoor and outdoor air. No significant difference ($p>0.05$) in concentration and type of fungi between living room and bedroom or by season was observed. Both concentration and type of fungi were significantly higher ($p<0.05$) in the basement than other indoor areas and outdoor air in winter. The type of fungi in living room, bedroom, and outdoor air were found to have significant changes among seasons, but there was no significant difference for the basement among seasons. *Cladosporium* spp. was dominant in both indoor and outdoor air in summer. *Penicillium* and *Aspergillus* were dominant in indoor air in winter, but neither was dominant in any season in outdoor air. The type of fungi and their concentrations in house dust samples were not representative of those isolated in indoor air. In dust samples, more *Mucor*, *Wallemia*, and *Alternaria* species, but less *Aspergillus*, *Cladosporium*, and *Penicillium* species were found in all seasons. Air sampling in spring or fall in every suspected house is suggested for year-round fungal exposure assessment.

Keywords: *house dust, indoor air, outdoor air, seasonal fungal prevalence.*

Introduction

Microbial aerosols in indoor and outdoor environments include a wide range of organisms (viruses, bacteria, mites, and their feces, insects such as moths and cockroaches, dander from mammalian pets and pests, and pollen, etc.). Many of these aerosols are associated with adverse health effects in humans. In recent years, a considerable amount of attention has focused on the role of aeroallergens in both the development and severity of asthma. While much of that attention has focused on allergens associated with dust mites, cockroach, cats, and dogs, there is an interest in the potential adverse respiratory effects associated with inhalation of fungi (NRC, 1981). Little, however, is known about the nature of human exposures to fungi in the residential environments. Fungal propagules are frequently encountered in both indoor and outdoor environments. Because of their resistance to dryness and their ability to survive and to germinate under more favorable environmental conditions, they have the capacity to colonize even in hostile environments. Therefore, owing to their ubiquitous presence in nature, fungi as an allergen source are almost inevitable in a range of enclosed environments and in community air. When attempts are made to determine the overall health impact of fungi in indoor environments, many studies have indicated that repeated exposure to spores or volatiles from fungi present in indoor air may result in type I allergy (asthma, rhinitis) (Beaumont, 1988; Wardlaw and Geddes, 1992), type III allergy (hypersensitivity pneumonitis) (Siersted and Gravesen, 1993), Sick Building Syndrome (SBS) (Holmberg, 1987), and Organic Dust Toxic Syndrome (Sigsgaard et al., 1990). Liebeskind (1971) has suggested a relation between the intensity of a given influence (i.e., molds) and the inhalation of allergens. The health effects of fungi are not limited to allergic diseases, but also might relate to a number of other health effects, including infections. The roles of inhalation of mycotoxins and fungal volatiles have not yet been clearly explained.

Understanding the nature and concentrations of indoor fungi may serve various purposes. Specifically, it can provide information on which fungi sensitive individuals

1. Abbreviations: ACGIH, American Conference of Government Industrial Hygienists; CFU/m^3, colony forming units per cubic meter; CFU/g, colony forming units per gram; DG-18, dichloran-glycerol agar; MEA, malt extract agar; SBS, sick building syndrome.

2. Address all correspondence to: Ping Ren, PhD, Division of Environmental Health Sciences, Department of Epidemiology and Public Health, Yale University School of Medicine, Room 400 LEPH, 60 College Street, P.O. Box 208034, New Haven, CT 06520-8034. Tel.: (203)737-6020. Fax: (203)737-6023. E-mail: airquality1@yale.edu

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are exposed to. Seasonal changes in the symptoms may be associated with the seasonal variations of the molds. Familiarity with the predominant airborne fungi and their occurrence throughout the year, both inside and outside the dwellings of symptomatic individuals, may contribute to a better knowledge and diagnosis of mold allergy and suggest effective mitigation strategies. A better qualitative and quantitative understanding of the indoor and outdoor flora of fungi, to which sensitive individuals are exposed is important.

Indoor and outdoor air concentrations of culturable fungal propagules as well as fungal propagules in house dust were studied in eleven residences. The purpose of this study was to explore the relationship between types and concentrations of airborne microfungi that can be found in indoor, outdoor air, and house dusts. This study also investigated if seasonal variations can be demonstrated in the indoor microflora to characterize the fungal exposure that may cause health effects. It is important to provide more information about the representative sampling period and sampling location so that cost-effective environmental monitoring protocols can be developed for epidemiologic studies of the health effects associated with exposure to residential fungi.

Materials and methods

Between November 1996 and October 1997, seasonal air sampling was carried out in 11 dwellings in the Greater New Haven, Connecticut area. These 11 homes (10 single-family residences and one apartment) were selected from colleagues and friends, which were located in a variety of locations (wooded lots, near water, or close to town or city). Air samples were collected in the living room, main bedroom, and basement of each home. No attempt was made to adjust the conditions prevailing in the homes prior to sampling. One air sample was also collected outside each residence and a house dust sample was collected from the living room of each home.

Culturable airborne propagules were collected using the Burkard portable air sampler (Burkard, Rickmansworth, England) in combination with dichloran-glycerol agar (DG-18) and malt extract agar (MEA). The sampling period was 1 min with air-flow rate at 20 l/min. After exposure, the plates were incubated at 25°C for 7 to 10 days, after which the resulting colonies were counted. Fungi were identified to genus and if possible to the species level by colony morphology or microscopic examination of the spore structure. Concentrations were reported as colony forming units per cubic meter (CFU/m^3) of air.

House dust samples were collected from a couch in the living room by a Eureka Mighty Mite II portable vacuum cleaner fitted with an adapter that holds a Whatman cellulose extraction thimble (single thickness, 19 mm×90 mm, 11 μm pore size). All vacuum cleaner attachments preceding the thimble adapters were cleaned with detergent, rinsed with deionized water, then given a final rinse with ethanol to limit contamination between samples. Materials were kept in clean Zip-loc bags until use. After sampling, contaminated vacuum attachments were replaced. Dust sampling was conducted for 5 min and over 1 m^2 area of couch. House dust samples were analyzed for the presence of fungal propagules by plating 30 mg of dust directly on either DG-18 or MEA and by suspending 100 mg of dust in a 1:50 (w/w) sucrose or peptone solution (Verhoeff et al., 1994).

Results

Comparison of Culturable Fungal Propagules among Locations

During the period November 1996 to October 1997, 342 air samples were taken from 11 houses. The number of colony forming units per cubic meter (CFU/m^3) in indoor and outdoor air varied widely. Table 1 presents the concentrations of culturable fungal propagules in CFU/m^3 (mean, standard deviation, standard error, and range) found in indoor and outdoor air by season. Approximately 50% of samples had total culturable fungal propagules lower than 575 CFU/m^3. The highest concentration measured was 4450 CFU/m^3. Ninety-seven percent of samples had fewer than 100 CFU/m^3 of genus *Alternaria*. Less than 28% of samples were found to have more than 50 CFU/m^3 of

Table 1. Concentration of culturable fungal propagules in CFU/m^3 (mean, S.D.[a], S.E.[b], and range) found in both indoor and outdoor in four seasons.

Seasons	Locations	*n*	Mean	S.D.	S.E.	Range
Winter	living room	22	431.8	327.7	69.9	0–1200
	bedroom	22	313.6	297.8	63.5	0–1200
	basement	20	1657.5	1249.4	279.4	0–3800
	outdoor	21	504.8	787.8	171.9	0–2400
Spring	living room	22	834.1	944.8	201.4	0–3500
	bedroom	22	790.9	1109.9	236.6	0–4450
	basement	20	1165	862.1	192.8	50–2950
	outdoor	22	829.5	538.7	114.9	50–2000
Summer	living room	22	1036.4	693.2	147.8	100–3000
	bedroom	22	970.5	698.8	149.0	150–2950
	basement	20	987.5	628.3	140.5	200–2950
	outdoor	22	1197.7	817.8	174.3	150–3600
Fall	living room	22	706.8	484.1	103.2	0–1850
	bedroom	22	704.5	704.0	150.1	100–3300
	basement	19	1242.1	759.2	174.2	150–3000
	outdoor	22	606.8	445.8	95.0	50–1600

[a]S.D.: standard deviation.
[b]S.E.: standard error.

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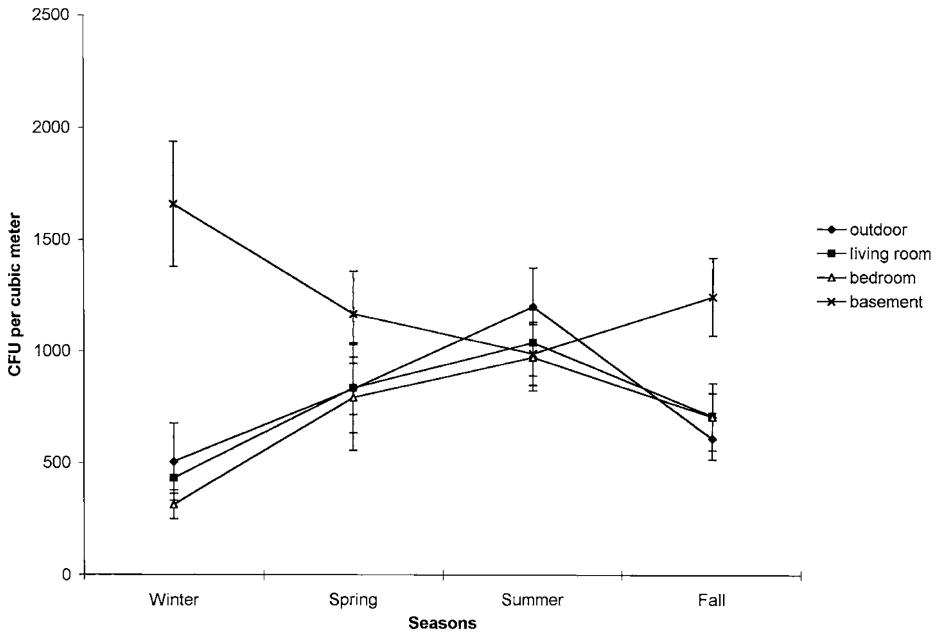

Figure 1. Seasonal changes of mean fungal concentrations in CFU/m^3.

Aspergillus. None of the samples contained the *Cladosporium* spp. over the 3000 CFU/m^3, the allergic threshold set by Gravesen (1979). Almost 90% of samples had genus *Penicillium* concentrations fewer than 250 CFU/m^3. The seasonal mean counts in CFU/m^3 are shown in Figure 1. The fungal count in the total number of propagules was significantly higher in the basement than other areas in winter ($p<0.001$) and fall ($p<0.005$). Significantly more types of fungi were collected in the basements than in all other locations in winter ($p<0.001$) and significantly less types of fungi were collected in outdoor than indoor in fall ($p<0.05$). In spring, the number of mold type was significantly higher outdoors than in the living rooms ($p<0.05$) and bedrooms ($p<0.1$). During summer, no significant difference in the type of fungi in any area was observed.

Spearman rank correlation coefficients were calculated between the numbers of CFU/m^3 found in the living rooms, bedrooms, basements, and outdoors in each season. High correlation coefficients were found between the concentrations in the living room and bedroom samples for all seasons (Table 2). Concentrations in the living room and outdoor air were highly and significantly correlated in both summer ($r=0.680$, $p<0.05$) and fall ($r=0.841$, $p<0.01$), as were bedroom and outdoor air (summer: $r=0.822$, $p<0.01$; fall: $r=0.746$, $p<0.05$). Low and nonsignificant correlation coefficients were found between fungal concentrations in basement and living room, basement and bedroom, and basement and outdoors for all seasons. Table 3 presents the comparisons of common fungi by location of measurement. High correlation coefficients between living room and bedroom in summer ($r=0.620$, $p<0.1$) and fall ($r=0.704$, $p<0.05$) for *Alternaria alternata* were observed. While for *Aspergillus* spp., living room and bedroom were highly correlated with each other in winter ($r=0.622$, $p<0.1$) and spring ($r=0.768$, $p<0.05$). High correlations between living room and bedroom occurred in spring ($r=0.592$, $p<0.1$) and fall (0.725, $p<0.05$) for *Cladosporium* spp. and in the fall ($r=0.739$, $p<0.05$) for *Penicillium* spp. There were strong correlations between living room and outdoor for *Penicillium* spp. in spring ($r=0.619$, $p<0.1$) and summer ($r=0.541$, $p<0.1$) and for *Cladosporium* spp. in winter ($r=0.608$, $p<0.1$). For

Table 2. Spearman rank correlation coefficients between the number of CFU/m^3 found in the living rooms, bedrooms, basements, and outdoors.

		Living room	Bedroom	Basement
Winter	bedroom	0.679*	–	–
	basement	0.293	0.448	–
	outdoor	0.511	0.648*	0.345
Spring	bedroom	0.755*	–	–
	basement	0.357	0.696*	–
	outdoor	0.411	0.067	0.146
Summer	bedroom	0.840**	–	–
	basement	0.299	0.324	–
	outdoor	0.680*	0.822**	0.434
Fall	bedroom	0.717*	–	–
	basement	0.638	0.642	–
	outdoor	0.841**	0.746*	0.220

*$p\leq0.05$, $p>0.01$.
**$p\leq0.01$.

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Table 3. Comparison of selected fungi by location of measurement (only the high correlation coefficients presented).

Fungi	Comparison	Correlation coefficient			
		Winter	Spring	Summer	Fall
A. alternata	living room vs. bedroom			0.620*	0.704**
Aspergillus spp.	living room vs. bedroom	0.622*	0.768**		
Penicillium spp.	living room vs. bedroom				0.739**
	living room vs. outdoor		0.619*	0.541*	
	basement vs. outdoor		0.786**		
Cladosporium spp.	living room vs. bedroom		0.592*		0.725**
	living room vs. outdoor	0.608*			
	living room vs. basement	0.589*	0.638*	0.728**	
	bedroom vs. outdoor			0.630**	
	basement vs. outdoor	0.625*			0.614*

*$p \leq 0.1$, $p > 0.05$.
**$p \leq 0.05$.

Cladosporium spp., there was a strong correlation between bedroom and outdoor levels in the summer ($r=0.630$, $p<0.05$) as well.

The 11 homes we selected in this study were located within three different environments — near water, in wood lots, and other areas. No significant difference was found between different home locations for concentrations of culturable fungal propagules in air by any seasons. There was one home near water which often had flooding in the basement. In this home, there were significantly higher concentrations of fungi than in the basement of other homes ($p<0.05$).

Comparison of Culturable Fungal Propagules among Seasons

The fungal count of the total number of propagules throughout the sampling period reached a peak in summer in both living room and bedroom, as well as outdoor air. The lowest count of culturable fungi in these three areas was in winter. In contrast, the count recorded in the basement was

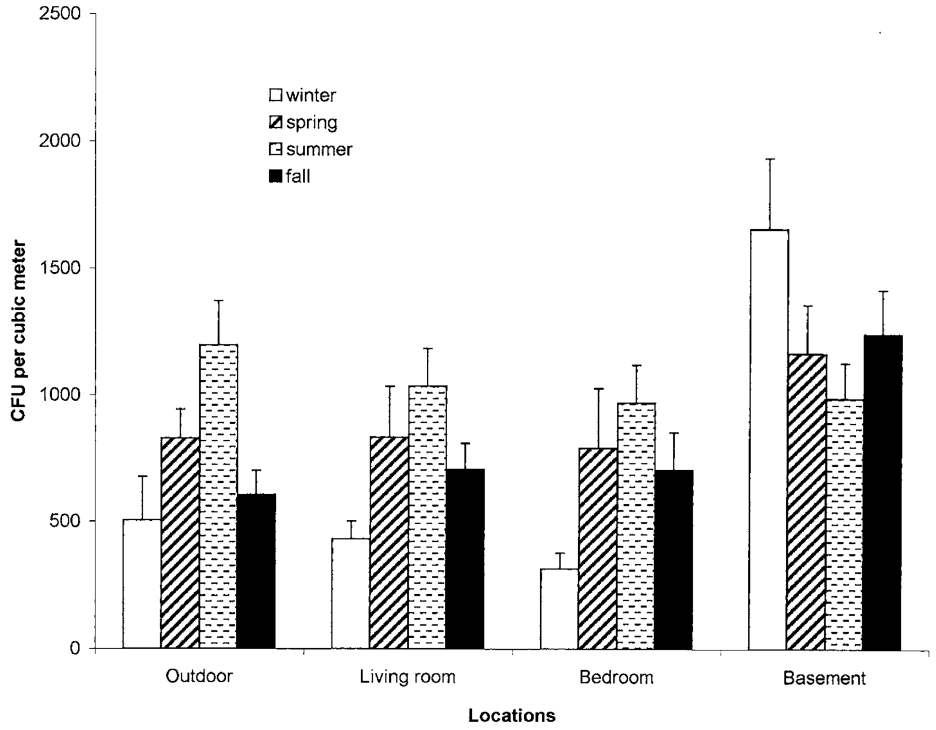

Figure 2. Mean seasonal fungal concentrations in CFU/m^3 in four locations.

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Table 4. Percentage of air samples yielding common fungi during November 1996 to October 1997.

Period	Year	% of indoor samples				% of outdoor samples			
		Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
No. of samples	(342)	(64)	(64)	(64)	(63)	(21)	(22)	(22)	(22)
Fungi	percentage frequency								
Cladosporium	69.0	39.1	62.5	93.8	81.0	23.8	77.3	95.5	77.3
Yeast	64.6	62.5	65.6	60.9	71.4	47.6	72.7	54.5	77.3
Penicillium	51.5	56.3	48.4	53.1	77.8	19.0	36.4	31.8	31.8
Aspergillus	39.2	54.7	54.7	31.3	50.8	4.8	27.3	9.1	13.6
Alternaria	14.9	0	1.6	39.1	14.3	0	4.5	54.5	13.6
Botrytis	11.1	1.6	6.3	26.6	3.2	0	18.2	31.8	13.6
Wallemia	10.2	10.9	9.4	7.8	23.8	4.8	0	0	4.5
Epicoccum	5.0	0	0	10.9	4.8	0	0	27.3	4.5
Fusarium	3.2	1.6	0	4.7	4.8	0	4.5	13.6	0
Curvularia	2.9	1.6	0	4.7	6.3	0	0	4.5	4.5
Ulocladium	1.8	6.3	0	0	1.6	0	4.5	0	0

the highest in winter and lowest in summer (Figure 1). Figure 2 shows the change of mean total CFU/m^3 by season in the four sampling locations. The mean CFU/m^3 in the living room, bedroom, and outdoor air in summer was significantly higher than in winter ($p<0.05$). For outdoor air, the culturable fungal concentration in summer was also higher than in fall ($p<0.1$). In contrast, fungal concentration in the basement in winter was significantly higher than summer ($p<0.1$). There was no significant differences between winter, spring, and fall as well as between spring, summer, and fall in the living room, bedroom, and basement. There was no significant difference between winter, spring, and fall or between spring and summer in outdoor air (Figure 2).

More types of fungi were typically found in fall than in winter ($p<0.01$) and spring ($p<0.05$) for the living room. Fewer types of fungi were found in winter than fall ($p<0.001$) and summer ($p<0.05$) for the bedroom. However, in outdoor air samples, a higher number of fungal types were found in spring than in winter ($p<0.001$) and fall ($p<0.005$). No highly significant difference in the number of types between seasons in basement samples was observed ($p>0.1$).

Fungal Genera and Species

In total, 113 different mold species belonging to 36 genera were found. Table 4 presents the most frequently isolated fungi in indoor and outdoor air samples. The most frequently isolated fungi were *Cladosporium* spp. which were present in 69.0% of air samples during the entire year. Yeasts, *Penicillium* spp., and *Aspergillus* spp. were isolated from 64.6%, 51.5%, and 39.2% of samples respectively. *A. alternata*, *Botrytis cinerea*, and *Wallemia sebi* were present in more than 10% of the samples taken. In summer, *A. alternata* and *B. cinerea* were isolated more often than in other seasons. *Cladosporium* spp. was isolated much less in winter than other seasons. Both *Aspergillus* and *Penicillium* had a relatively constant ability to be isolated throughout the whole year.

Influence of Time of Year on the Composition of Fungal Spores in Air Samples

A comparison between the CFU/m^3 concentrations of molds in indoor and outdoor air showed clear differences. For *Aspergillus* and *Penicillium*, the concentrations were higher indoors than outdoors. For *Cladosporium* and other genera, the concentrations in outdoor air were higher than in

Table 5. Seasonal variation in the mean percentage composition of fungi in indoor and outdoor air.

		Alternaria (%)	*Aspergillus* (%)	*Cladosporium* (%)	*Penicillium* (%)	*Wallemia* (%)	Yeast (%)	Other (%)
Indoor	Winter	0	47	7	30	2	6	8
	Spring	0	47	11	15	3	8	16
	Summer	4	13	54	11	1	7	10
	Fall	1	22	30	23	6	12	6
Outdoor	Winter	0	2	30	13	0	7	48
	Spring	0	9	25	10	0	23	33
	Summer	5	1	64	9	0	6	15
	Fall	4	5	38	1	1	32	19

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indoor air. Examination of the seasonal variation in the mean percentage composition of fungi in indoor air (Table 5) revealed that in winter *Aspergillus* and *Penicillium* predominated in the air spores, accounting for a total 77% of the fungi isolated, with *Cladosporium* accounting for only 7%. The total level of *Aspergillus* and *Penicillium* fell in the summer time to 24% whereas *Cladosporium* spp. increased to account for 54% of the total colonies. Genus *Alternaria* was practically absent in winter and spring in both indoor and outdoor air. It was found in summer and fall in both locations and reached 4% in indoor air and 5% in outdoor air in summer. In outdoor air, *Cladosporium* spp. showed predominance in summer similar to indoor air. In contrast, neither *Aspergillus* nor *Penicillium* were dominant in any season. *W. sebi* was present in all seasons in indoor air but only fall in outdoor air. Genera other than *Alternaria, Aspergillus, Cladosporium, Penicillium, Wallemia*, and yeasts were more often found in outdoor than indoor air in corresponding seasons.

Culturable Fungal Propagules in Dust Samples

The dominant fungal genera in house dusts did not represent the dominant fungi in indoor air and *vice versa* during any season. More often *Mucor* spp., *W. sebi*, and *Alternaria* spp., but fewer *Aspergillus, Cladosporium*, and *Penicillium*, were found in dust samples than indoor air samples. The concentrations of *Alternaria, Aspergillus, Cladosporium, Penicillium, Mucor*, and *Wallemia*, etc., in dust samples, in colony forming units per gram dust (CFU/g) were 2 to 55 times higher than those in air samples in CFU/m^3 ($p<0.005$) when they were present in both dust and air samples. No significant correlation coefficient between the concentrations of culturable fungi in dusts (CFU/g) and air (CFU/m^3) was found. There was no significant change of the fungal types and their concentrations in the dust samples among seasons either.

Discussion

In this study, 11 homes were sampled. Among these 11 homes, two of them were located near water (Long Island Sound). Three of them were located in wooded lots away from bodies of water. One residence was an apartment, one home was newly constructed, and one was newly occupied. Ten of 11 homes had basements. Two basements were finished and used more often than the unfinished basements. One basement flooded whenever there was heavy rain. Four basements were flooded once or twice a year. None of the families used a humidifier and two families used dehumidifiers in the basements. Because of the small sample size, no significant difference was found between different home locations for culturable fungal propagules in air by any seasons, except for the home near water, whose basement was most often flooded. This home had significantly higher concentrations of fungi than the other homes' basement samples. This result indicated that there was a relation between the concentration of airborne fungal propagules and home dampness.

This study has revealed that the seasonal trend of the concentration of fungal propagules in indoor environments (except basement) and outdoors were generally comparable with those found by Hunter and Lea (1994) in British homes, lowest in winter, increasing in spring, reaching highest in summer, and starting to decrease in fall. The counts were not, however, as high as the maximum during each season reported in the literature; for example, the research of Solomon (1976) in the US reported maxima of approximately 20,000 CFU/m^3 and Hunter and Lea (1994) found, in the study in Britain, maxima of 35,900 CFU/m^3 in living rooms and 22,900 CFU/m^3 in bedrooms. There are some guidelines for the levels of fungi in the indoor environment, which allow the determination of maximum acceptable levels within domestic dwellings. However, suggestions of maximum permissible burden vary greatly. Miller et al. (1988), after studying the air spores in Canadian homes during the winter, proposed that if counts obtained in a house were (a) >50 CFU/m^3 and comprising of only one species, or (b) >150 CFU/m^3 if containing a mixture of species, or (c) >300 CFU/m^3 if containing mainly phylloplane fungi, then they should be investigated with a view to remedial action. The American Conference of Government Industrial Hygienists (ACGIH) published a draft proposal for sampling airborne viable microorganisms in the indoor environment (Burge et al., 1987) which stated that the indoor level is normally less than one-third of the outdoor level. If the indoor/outdoor ratio exceeded this value then the draft proposal recommends that remedial action should be taken to identify the source of emission and methods to reduce the counts should be instigated. Reponen et al. (1990) proposed a maximum level of 500 CFU/m^3 in the wintertime in subarctic climates. These authors determined this maximum level of airborne fungi by applying a statistical formula used in biomedical research, which defines the normal range as that which encompasses 95% of the cases. Therefore 95% of the counts will fall below the value of [mean+2×(standard deviation)] specified by Reponen et al. (1990). If this formula is applied to the data obtained from our study, conducted in a more temperate climate, then the upper limit for fungi in the winter would be 1087 CFU/m^3 in living rooms, 909 CFU/m^3 in bedrooms, and 4156 CFU/m^3 in basements. In our samples, we found no substantial difference between concentrations in the living room and bedroom, but huge differences between living room and basement as well as bedroom and basement. This may indicate differences in room ventilation and other house construction and utilization characteristics. Indoor sources in basement could be

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another important factor. The values derived from our study are more than three times higher than the guideline set by Miller et al. (1988) or almost twice higher than the maximum set by Reponen et al. (1990) in living room and bedroom air. This is probably a result of the different climatic conditions prevailing both externally, which effect the outdoor fungal airborne burden and internally, which affect the conditions prevailing within the dwelling.

Because the upper limit for fungi in the winter in our study would be 2080 CFU/m^3 in outdoor air and 909 CFU/m^3 in indoor air (the lowest count in bedroom chosen) according to the above mentioned formula, the fungal concentration in indoor air was much more than one-third level of outdoor air, which is beyond the limit set by ACGIH (Burge et al., 1987). Mouilleseaux and Squinazi (1994) concluded that outdoor fungal counts were significantly higher than those found for all indoor mechanically ventilated environments. The average for the indoor/outdoor ratio found in their study was approximately 0.5 and in many cases they measured a ratio equal to or greater than one, indicating an indoor source and/or poor maintenance of the ventilation system. That may be due to inefficient filtration at the inlet. In our study, the fungal indoor/outdoor ratios were also close to or greater than one in all seasons, which was similar to the findings of Mouilleseaux and Squinazi (1994). Both results indicated that naturally ventilated interiors are more like (often generally higher than) outdoors than those with mechanical ventilation.

Our results, regarding the presence of culturable molds outdoors as well as indoors varying widely from house to house generally agree with those reported by others (e.g., Gravesen, 1972; Hunter et al., 1988). For two of the common molds, regarded as the main allergenic fungi, threshold concentrations for evoking allergic symptoms were estimated to be 100 *Alternaria* spores per cubic meter air and 3000 *Cladosporium* spores per cubic meter air (Gravesen, 1979). None of the homes in our studies reached threshold concentration of *Cladosporium*. Only 3% of samples collected from 11 homes had *Alternaria* spores over 100 per cubic meter air. *Alternaria* has been identified in one study as a major allergen associated with the development of asthma in children raised in a semiarid environment, but the exposure level of *Alternaria* was not reported (Halonen et al., 1997). Holmberg (1987) reported that *Aspergillus* spore concentrations above 50 CFU/m^3 were associated with a higher prevalence of Sick Building Syndrome (SBS). In our studies, more than one-fourth of the samples had *Aspergillus* concentrations over this level. However, more than half of them were collected from basements. *Penicillium* is one of the most commonly isolated fungi in both indoor and outdoor environments. Hiddlestone (1961) documented the seasonal variation of fungal spore counts in the air in Nelson, New Zealand and described a number of patients with asthma related to exposure to *Cladosporium*, *Penicillium*, and *Alternaria* species. A relationship between symptoms of asthma or peak expiratory flow rate and the presence of particular fungal types, especially *Aspergillus* was reported by Holst et al. (1983). In a recent case-control study, it was shown that asthmatics were more frequently exposed to indoor molds than controls, especially *Penicillium* (Burr et al., 1988).

Clear differences between the CFU/m^3 concentrations of molds in indoor and outdoor air were found. The concentrations of *Aspergillus* and *Penicillium* were higher indoors than outdoors, but the concentrations of *Cladosporium* and other genera were higher in outdoor air than indoor air. Correlation coefficients between indoor and outdoor samples were also present for some fungi in some seasons. These results support the current recommendations that outdoor air may influence the presence of culturable fungal propagules in indoor environments, but that the presence of culturable molds in indoor air may not be a reflection of the presence of molds in outdoor air, particularly in problem environments. Also, the high correlation found between the total numbers of CFU/m^3 in the living room and bedroom indicated that indoor sources of molds are present. In basements especially, low ventilation and human activity allow the types of fungi to have less variety than other indoor and outdoor areas. Thus, either living room or bedroom could be chosen for the indoor sampling. Basement samples could be measured for specific purposes. Since approximately 75% of an individual's time may be spent inside his home and a much higher percentage for susceptible individuals (old, young, and the ill) and a total of over 90% in all types of indoor environments (Moschandreas, 1981), where levels of certain airborne pollutants can be higher than outside (Jarke et al., 1981; Berglund et al., 1982; Yocom, 1982), there is a possibility for long-term effects on the health of the general population. Because no significant difference between fungal concentrations in indoor and outdoor air were found in this study, indoor air samples are recommended to be taken for assessing fungal exposure instead of outdoor air samples, even though differences between the type of fungi in indoor and outdoor air was present in spring and fall. The advantage of taking indoor air samples is that more directed and reliable information could be collected for exposure assessment for indoor than outdoor air samples. Regarding the sampling time and frequency, the winter or the summer is not the best season to collect the representative sample if only one sample can be obtained to represent year-round fungal concentration. Winter or summer samplings would likely capture the lowest or highest indoor air fungal concentrations respectively.

The fungi isolated in the present study are broadly the same as those found in some European studies (Beaumont et al., 1984, 1985; Verhoeff et al., 1988; Hunter and Lea,

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1994; Hunter et al., 1988). However, comparing the percentage composition of *Cladosporium* spp. in winter months of this study to that obtained by Hunter et al. (1988) from Scottish dwellings, it was found that *Cladosporium* spp. accounted for significantly less of the total composition indoors, but it is in agreement with the finding of Hunter and Lea (1994) from British homes. *Cladosporium* numbers also followed the expected trend of peaking during the summer as reported by Richards (1954). The reverse was found for *Aspergillus* spp. where nearly 10 times the level higher than that occurring in the British study by Hunter and Lea (1994). Certainly concentrations of *Aspergillus* and *Cladosporium* spores in moldy buildings were higher than in reference buildings (Nevalainen et al., 1994) and since our study results included the samples collected from basements, which were good sources for *Aspergillus*, this might explain this phenomenon. In contrast to most other common molds, *Penicillium* has relatively small seasonal variation both indoor and outdoor. This might suggest that *Penicillium* might not be the major factor for inducing the seasonal respiratory symptoms.

The fungal distribution of house dust in Japan was investigated from 1987 to 1991 by Takatori et al. (1994). They found no particular trends or seasonal changes in the fungal flora and number of colony forming units between years and within years. Similar results were obtained in our study about fungal propagules in dust samples. However, this was not the case for air samples. In our studies, no association was found between fungal types and concentrations in dust and air, suggesting the types of fungi and their concentrations in house dust samples did not represent those in indoor air. So house dust for the assessment of fungal propagules may not provide a reliable measure of the potential inhalation exposure to fungi in indoor environment. Air samples may provide a better measure of inhalation exposure.

Conclusions

The study reported here involved only a few houses. Although arguably typical, these houses cannot be considered as valid statistical samplings of the millions of houses in the country. Nor is the number and location of the measurements sufficient to represent the inherent variability in fungal population potentially exposed. Namely, the data are limited in terms of the number of samples, the number of homes, the type of homes, and the location in country. However, the results suggest three findings.

1. Although the data presented here are relatively few, incorporating them into a simple indoor exposure model adds further evidence that large variations of the concentration and type of fungi are present from home to home. It is important to sample every house to determine the concentrations and identification of fungi for risk assessment. At present, no standard threshold limit values for fungi have been set, but there are some suggestions of maximum permissible burden discussed above. Fungal concentrations in excess of those suggested guidelines were quite common in our study. Different organisms should be considered differently regarding the methods of dealing with their contamination, e.g., *Stachybotrys atra* is not allowable (Sudakin, 1998).

2. This study provides additional evidence of the sampling strategies for fungi related to sampling times and locations. Fungal concentrations do show substantial variations or trends among seasons. There was an increase in the number of CFU/m^3 from winter towards the end of summer. One sample could be taken in either spring or fall, which represents most median exposure for year-long study. Relating to sampling locations in home, either the living room or bedroom may be the most appropriate locations to sample.

3. This study also suggests that the air sampling is a more reliable analysis method for fungal-exposure assessment than dust sampling. More fungi could be identified in air samples than dust samples, especially the potentially pathogenic organisms; for example, *Aspergillus*, was more often to be found in air samples.

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