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Airborne Mould Levels and Related Environmental Factors in Australian Houses

Diana Godish[a]
Thad Godish[b]
Bev Hooper[c]
Martin Hooper[c]
Mary Cole[d]

[a] Department of Physiology and Health Science and
[b] Department of Natural Resources and Environmental Management, Ball State University, Muncie, Ind., USA;
[c] Centre for Environmental Science, Monash University – Gippsland, Churchill, Victoria, and
[d] Department of Ecology and Evolutionary Biology, Monash University – Clayton, Clayton, Victoria, Australia

Abstract

Airborne mould sampling studies were conducted in 40 selected Australian (Latrobe Valley, Victoria) houses. Both total indoor culturable and total mould spore levels were observed to be relatively high with 58% of houses with one or more rooms exceeding 1,000 cfu/m^3 and 48% exceeding 10,000 spores/m^3, respectively. Median indoor total mould spore levels exceeded total culturable levels by 14-fold in concurrent sampling. An evaluation of the indoor/outdoor ratios of selected genera indicated that 50% of indoor concentrations could be explained by outdoor mould levels. Applying a univariate analysis of variance, significant differences in mould levels associated with a variety of housing characteristics and environmental factors were observed. A house age >20 years and relative humidity ≥ 70% were observed to be significant independent contributing factors to elevated indoor culturable mould levels while similarly these factors with water intrusion through the building envelope gave elevated total indoor mould spore levels.

Key Words

Mould
Houses
Indoor air quality
Risk factors
Australia

Introduction

A number of epidemiological studies have implicated exposure to mould and/or dampness as factors potentially causally associated with respiratory symptoms such as chronic allergic rhinitis and/or asthma. These include the studies of Strachan and Elton [1] in Scotland; Platt et al. [2] in Glasgow, Edinburgh, and London; Wagemakers et al. [3] in the Netherlands; Jaakkola et al. [4] in Finland; Dekker et al. [5] and Dales et al. [6] in Canada, and Su et al. [7], Brunekreef et al. [8] and Spengler et al. [9] in the United States. These epidemiological studies suggest that exposure to airborne mould in residential environments may be a significant risk factor for both upper respiratory and pulmonary symptoms.

This study represents the results of the mould assessment phase of a 40-house study conducted during the austral winter of 1992. Major objectives included the determination of airborne culturable and total mould spore levels based on one-time sampling and evaluation of housing and environmental factors which may have been contributing factors to elevated airborne mould levels.

Methods

The study population of 40 single-family houses was located in the Latrobe Valley of Victoria, Australia. One or more occupants in each house had previously reported experiencing persistent upper or pulmonary (asthma) respiratory symptoms. All houses were located in small-town or rural sites. Mould sampling was conducted in June/July, 1992, the cool, rainy, winter season. Three or four living spaces

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Dr. Thad Godish
Department of Physiology and Health Science
Ball State University
Muncie, IN 47306 (USA)

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Table 1. Indoor and outdoor mould levels in selected Australian houses

	Culturable mould concentrations, cfu/m³		Total indoor mould spore concentrations, S/m³
	indoor	outdoor	
n	127	40	80
Geometric mean	495	584	10,488
Range	n.d. – 5,185	n.d. – 4,234	2,025–36,900
Frequency	>500 (61) >1,000 (31) >2,000 (10)	>500 (70) >1,000 (47.5) >2,000 (17.5)	>5000 (78) >10,000 (48) >15,000 (20)

n.d. = Not detectable; figures in parentheses are percentages.

including the bedroom of individuals who reported experiencing upper respiratory symptoms or asthma were sampled in each house for culturable mould (n = 127 rooms); two rooms in each house were sampled for total (viable and non-viable) airborne mould spore/particle levels (n = 80). Samples were collected at heights of 0.6–1.5 m reflecting normal breathing-zone levels during sleep and activities on awakening. All sampling was conducted under house closure conditions with a minimum closure period of 12 h before sampling. A sample of culturable mould was collected outside each house for comparison purposes. Concurrent measurements were made of indoor and outdoor temperature and relative humidity using a sling psychrometer (model 12-7013, Bacharach Instrument Co., Pittsburgh, Pa., USA). Absolute humidity (water vapour pressure, mm Hg) was calculated from temperature and relative humidity using a psychrometric chart.

Culturable mould spores/particles were collected with an Andersen N-6 single-stage sampler (Graesby-Andersen, Smyrna, GA 30082) operating at a flow rate of 28.3 litres/min for a 2-min sampling period. Samples of mould were collected and cultured on malt extract broth (2% w/v; Oxoid) solidified with agar. Total airborne mould spores/particles were collected on silicone-greased microscope slides using mini-Burkhard samplers (Burkhard Mfg. Co. Ltd., Rickmansworth, Herts., UK) operating at an air flow rate of approximately 9 litres/min for a sampling duration of 9 min.

Sample malt extract agar plates were incubated at room temperature under fluorescent lighting. Colonies were counted and identified to genus. Initial counts and identifications began on the 4th day and ended on the 7th day of incubation. Concentrations of culturable mould in each collected sample were expressed as colony-forming units per cubic meter (cfu/m³). Concentrations were adjusted for multiple impactions on the agar surface using the positive-hole correction method [10]. Total mould spore concentrations were determined microscopically by counting 5% of the 2 × 14 mm deposition area of sample slides. All counts were made using 1,000× magnification and oil immersion. Concentrations were expressed as spores per cubic meter (S/m³).

Air sampling was accompanied by an intensive visual inspection of each room for evidence of mould growth on surfaces and dampness parameters such as condensation and water intrusion into the building structure. The smell of mould odour was recorded. Data were also collected on house characteristics such as age, substructure, and cladding type.

Data collected from mould sampling were subjected to statistical analysis. This included comparisons of indoor and outdoor culturable mould levels and indoor culturable and total mould spore levels. It also included an evaluation of differences in concentrations associated with indoor climate factors (temperature, relative and absolute humidity); housing characteristics (age, substructure and cladding type); mould odour; and water intrusion through the building envelope. All concentrations were log-transformed since mould data were not normally distributed. Statistical procedures employed included Student's t-test, regression analysis, and univariate design analysis of variance. A probability value of 0.05 was accepted as significant.

Results

Total culturable mould concentrations (cfu/m³) for samples collected both inside and outside the 40-house study population are summarised in table 1. Indoor levels ranged from undetectable (n.d.; the limit of detection was 18 cfu/m³) to over 5,000 cfu/m³, with a geometric mean of 495 cfu/m³. Outdoor values had a similar range (n.d. – 4,234 cfu/m³) with a significantly higher geometric mean, 612 cfu/m³ ($p \leq 0.05$). Indoor culturable mould levels were observed to be relatively high (compared to a guideline value of 1,000 cfu/m³), with 25% of the houses surveyed having one or more rooms with mould concentrations exceeding 2,000 cfu/m³; 58% had one or more rooms exceeding 1,000 cfu/m³.

Mould genera identified in indoor and outdoor samples are reported with their frequency of occurrence in table 2. The most common mould genera/types observed in indoor samples included *Cladosporium* ssp., *Penicillium* ssp., yeasts and members of the class Basidiomycetes. Also commonly found (but in fewer numbers) were *Botrytis* spp., *Monocillium* spp., and *Phoma* spp. The genera *Penicillum* and *Aspergillus* were calculated to be significantly more prevalent in indoor as compared to outdoor samples.

Table 2. Indoor/outdoor occurrence of mould genera in culturable mould samples

Type	Indoors			Outdoors	
	% total cfu/m³	% indoor spaces from which recovered		% total cfu/m³	% outdoor samples from which recovered
		houses	rooms		
	(n = 5,220)	(n = 40)	(n = 127)	(n = 2,259)	(n = 40)
Cladsporium	32.6	100.0	87.4	34.4	82.5
Penicillium	22.4	100.0	85.8	4.9	65.0
Yeasts	15.8	100.0	87.4	15.8	92.5
Basidiomycetes	10.4	90.0	65.4	14.2	72.5
Botrytis	4.3	62.5	36.2	8.0	45.0
Monocillium	1.9	70.0	30.7	1.7	40.0
Phoma	1.2	57.5	24.4	2.2	42.5
Sporotrichum	0.6	45.0	16.5		
Oidiodendron	0.6	37.5	13.4		
Alternaria	0.5	25.0	11.0	1.8	20.0
Fam. Dematiaceae	0.2	22.5	7.1	0.2	7.5
Fusarium	0.2	15.0	5.5	0.8	22.5
Stysanus	0.2	15.0	4.7	0.1	7.5
Myrothecium	0.2	12.5	4.7		
Rhizopus	0.2	10.0	3.1	0.3	5.0
Melanconium	0.1	15.0	4.7		
Papularia	0.1	12.5	4.7	0.3	10.0
Stachylidium	0.1	12.5	3.9		
Aspergillus	0.1	10.0	3.9	<0.1	2.5
Epicoccum	0.1	7.5	2.4	0.7	10.0
Chaetomella	0.1	7.5	2.4		
Mucor	<0.1	2.5	0.8		
Stemphyllium	<0.1	2.5	0.8		
Fam. Stilbaceae	<0.1	2.5	0.8		
Order Moniliales[a]	4.6	87.5	57.5	10.9	10.0
Unknowns[b]	1.3	35.0	31.5	1.8	12.5
Gliocladium				<0.1	2.5

[a] 7 different genera of order Moniliales, non-sporulating.
[b] 14 different genera not identified as belonging to order Moniliales.

Outdoor total culturable airborne levels were calculated to be significantly higher than those sampled indoors. The relationship between indoor and outdoor culturable mould levels was evaluated by several different approaches. Application of simple linear regression revealed no significant correlation between total indoor and total outdoor culturable airborne mould levels. However, a significant but weak (r = 0.40) linear relationship was observed between indoor and outdoor *Botrytis* spp. levels, with the latter twice as high as the former. The potential relationship between indoor and outdoor mould was also evaluated by determining the ratio of the summed concentrations of two genera commonly associated with the indoor environment (*Penicillium* and *Aspergillus*) divided by the summed concentrations of genera commonly associated with the outdoor environment (*Cladosporium, Alternaria, Epicoccum,* and members of the class Basidiomycetes). The median value for this ratio determined for all 40 houses was 0.48.

Total airborne mould spore levels measured in two rooms in each of the 40 houses surveyed are reported in table 1. These levels were considerably higher than culturable concentrations, varying from 4–450 times higher (median 14×) when sampled in the same room concurrently. A significant but weak (r = 0.39) linear association was observed between mould concentrations determined by these two sampling methods.

Table 3. Differences in culturable and total mould spore levels associated with house and environmental factors determined from univariate analysis of variance

Constant	Environmental variable	Mould measurement	F	p
House age (>20 years)	cladding type	culturable	5.07	0.027*
		total	3.09	0.083
	substructure	total	5.76	0.019*
	absolute humidity	culturable	7.09	0.009**
		total	8.12	0.007**
	temperature	culturable	4.74	0.033*
		total	4.31	0.041*
	mould odour	culturable	4.50	0.037*
		total	3.77	0.056
Substructure (crawl)	house age	total	3.02	0.086
	cladding type	culturable	3.37	0.070
	relative humidity	total	3.01	0.087
	absolute humidity	culturable	3.75	0.062
	water intrusion	culturable	12.48	0.001**
Relative humidity (≥70%)	house age	total	3.26	0.075
	cladding type	culturable	4.60	0.035*
		total	4.26	0.042*
	substructure type	culturable	3.67	0.060
		total	8.46	0.005**
	absolute humidity	culturable	2.87	0.090
	water intrusion	culturable	10.20	0.002**
	temperature	total	3.91	0.052
	mould odour	culturable	4.63	0.034*
		total	4.68	0.034*
Water intrusion (present vs. absent)	cladding type	total	4.95	0.029*
	substructure type	total	3.04	0.086
	relative humidity	total	3.92	0.051
	temperature	total	5.87	0.018*
	mould odour	total	5.88	0.018*
Temperature (≤18°C)	house age	total	3.24	0.076
	substructure type	total	4.80	0.032*
	mould odour	total	2.83	0.097

*Significant at p = 0.05; **significant at p = 0.01.

Univariate analysis of variance (SPSS MANOVA) statistical procedures were applied to the data to determine whether there were significant differences in mean airborne mould concentrations associated with selected housing characteristics and environmental factors in study residences. These included house age (>20 vs. ≤20 years), cladding type (non-brick vs. brick), substructure type (crawlspace vs. slab), water intrusion through the building envelope, indoor relative humidity (≥70% vs. <70% RH), absolute humidity (≥10.5 mm Hg vs. <10.5 mm Hg), temperature (≤18°C vs. >18°C) and mould odour.

Data presented in table 3 summarise analysis of variance results (includes all p values ≤ 0.10). A number of housing characteristics and environmental factors appeared to contribute to significant differences among sample means for both total culturable and total mould spore levels. However, only relatively few of these were observed to be independent contributing factors to elevated mould levels when adjusted for other housing characteristics and environmental factors.

House age >20 years was observed to be a significant independent contributing factor to elevated total culturable and total mould spore levels. Significantly higher (p ≤ 0.05) culturable mould levels were observed in

houses >20 years when data were adjusted for cladding type, absolute humidity, temperature and mould odour. Significantly higher total mould spore levels were observed in houses >20 years when adjusted for substructure, absolute humidity and temperature, with $p \leq 0.10$ for cladding type and mould odour. Water intrusion could not be evaluated because of insufficient sample size.

Relative humidity was also observed to be an independent contributing factor for elevated mould levels. Significantly higher ($p \leq 0.05$) culturable mould levels were observed in houses with relative humidities $\geq 70\%$ when adjusted for cladding type, water intrusion, and mould odour, with p values ≤ 0.10 for substructure and absolute humidity. Total mould spore levels were observed to be significantly associated with relative humidity $\geq 70\%$ when data were adjusted for cladding type, substructure, and mould odour, with $p \leq 0.10$ for age and temperature. Water intrusion (indicated by stains on ceilings, walls, windows and doors not associated with condensation) was observed to show an interesting relationship with mould levels. There were no significant ($p \leq 0.05$) or near significant ($p \leq 0.10$) differences in the means of culturable mould levels related to the occurrence of water intrusion through the building envelope. However, total mould spore levels were significantly higher in houses with water intrusion when adjusted for cladding type, temperature and mould odour, with a p value of ≤ 0.10 for substructure and relative humidity. Data could not be adjusted for age and absolute humidity due to small sample size.

No significant differences in means of total culturable mould levels were observed for cladding type, room temperature, absolute humidity, and mould odour when the data were adjusted for all other variables. This was also true for substructure except for culturable mould levels adjusted for water intrusion. Similarly, no significant differences were observed in the means of total mould spore levels associated with cladding type, absolute humidity and mould odour. House temperature was only observed to be significant for total mould spore levels when adjusted for substructure, with p values ≤ 0.10 for cladding type, age and mould odour.

Discussion

Houses sampled in this study can be described as complaint houses because they were selected on the basis that one or more residents were experiencing respiratory health problems. Culturable mould levels reported here (geometric mean = 495 cfu/m^3) were in the same range as those reported in complaint California houses (mean = 480 cfu/m^3) [11], but somewhat lower than complaint houses (median = 624 cfu/m^3) sampled in Scotland [12]. Values for non-complaint houses have varied from low levels (150 cfu/m^3) for the sub-arctic climate of Finland [13], to somewhat higher levels (median = 271 cfu/m^3) in Scottish houses [12], to relatively high levels (geometric mean = 1,200 cfu/m^3) in Iowa houses [14].

Total mould spore levels (S/m^3) were observed to exceed culturable levels (cfu/m^3) by 4- to 450-fold with a median of 14-fold for all comparisons. Burge et al. [15] reported that total mould spore counts of the genus *Cladosporium* outdoors exceeded culturable levels by a factor of 2–20. In the indoor studies of Meldrum et al. [16] for specific taxa such as *Cladosporium* and the combination of aspergilli/penicilli, total mould spore levels exceeded culturable levels by only a factor of 2–4.

Differences between culturable and total airborne mould spore levels are to be expected since concentrations reported for the former are based on only viable mould propagules and hyphal fragments which germinate and grow on collection medium. These would not include mould propagules or particles which are viable but do not germinate because of collection medium limitations (or growing conditions), collection and mould growth problems associated with the use of culturable mould samplers [17], and the presence of non-viable propagules/particles in airborne mould aerosols. Despite significant differences observed between culturable and total mould spore levels, one would nevertheless expect to observe a relationship between the two. A significant ($r = 0.39$) but weak association was indeed observed. Because both viable and non-viable mould particles are allergenic, total mould spore levels are biologically more significant (assuming that levels of the more allergenic mould taxa are also higher in total mould spore counts than culturable mould concentrations) than culturable levels.

Mould concentrations determined from outdoor samples are in most reports significantly higher than those determined from indoor samples. This suggests that indoor concentrations may be affected by outdoor levels particularly when windows and/or doors are open immediately before and/or during sample collection. Meldrum et al. [16], in their studies of houses in the Sonoran desert (USA), have suggested (based on indoor/outdoor ratios) that infiltration under closure conditions accounted for 60% of indoor mould (excluding *Penicillium* and *Aspergillus*). However, indoor/outdoor ratios of aspergilli/penicilli were often observed to be greater than one, indicating that indoor sources in those cases were dominant.

In this study regression analysis revealed no significant relationship between indoor and outdoor culturable mould levels. A significant but weak association was, however, observed for *Botrytis* spp. *Botrytis* is a plant pathogen and is not commonly reported in indoor environments. The indoor/outdoor *Botrytis* ratio of 0.5 would suggest that, on the average, 50% of indoor total culturable mould could be explained by outdoor concentrations. This is consistent with the ratio of 0.48 that we observed for the concentrations of *Penicillium* and *Aspergillus* (considered to be primarily indoor moulds) divided by the summed concentrations of *Cladosporium, Alternaria, Epicoccum,* and Basidiomycetes (considered to be primarily outdoor moulds).

Studies reported here are notable in that they have attempted to evaluate housing characteristics and environmental variables as potential contributing factors to elevated culturable and total mould spore levels. The results of the analyses indicated that both culturable mould and total mould spore levels were independently related to house age and relative humidity. The apparent relationship between house age and airborne mould levels is not surprising. Older houses would be expected to be at greater risk for mould infestation due to the greater probability of water intrusion, general maintenance problems, and other conditions which could contribute to mould growth. Houses older than 20 years differed from newer houses in cladding and substructure types; however, these were not observed to be independent contributing factors to elevated indoor mould levels.

A variety of investigators have either suggested [18, 19] or calculated [14, 20] statistically significant relationships between culturable mould levels and indoor relative humidity. In evaluating potential interaction effects with other environmental variables and housing characteristics, this study confirmed that elevated relative humidity (RH $\geq$ 70%) was an independent contributing factor to elevated indoor mould levels. Relationships between water intrusion and mould levels appear to be somewhat problematic. Our results suggest that water intrusion through the building envelope affected total airborne mould spores but not culturable mould levels.

Elevated absolute humidity and the presence of mould odour did not appear to be associated with elevated mould levels. The absence of any apparent relationship between mould odour and mould levels is interesting in light of dose-response relationships reported for mould odour and respiratory symptoms among pre-school children in Finnish dwellings [4]. In an analysis of health and housing variables [21] in another phase of this study, we observed that mould odour was the major independent risk factor for cough and shortness of breath, two of the most prevalent symptoms reported by occupants of the 40 houses surveyed. Mould odour varied considerably, from being pervasive throughout an individual house, to being present in a single or several rooms, to being associated with mould-infested mattresses, draperies and other furnishings and materials. Because of this considerable variability, the log-linear relationship between odour and human perception of odour intensity [22], significant temporal variations [23] in mould levels which may occur in a building, and outdoor sources of mould spores, the likelihood of observing a significant relationship between mould odour and airborne mould levels would have been small.

Mould levels reported here were based on one-time sampling results. As such they do not take into account the considerable temporal variability in airborne levels in both the indoor and outdoor environment [23]. Such variability is of particular importance in health-based studies because it diminishes the statistical power to detect significant relationships between exposures and health endpoints. Despite the limitations of one-time mould sampling, we were able to show a number of statistically significant relationships between airborne mould levels and a variety of environmental and housing factors evaluated.

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