

STUDY OF AIRBORNE CULTURABLE MOLD IN SEVENTEEN EAST-CENTRAL INDIANA SCHOOL BUILDINGS

Diana R. Godish
Department of Physiology and Health Science
Ball State University
Muncie, Indiana 47306

ABSTRACT: Airborne levels of culturable mold were sampled in 167 randomly selected school rooms in 17 east-central Indiana school buildings. Culturable indoor mold levels were relatively low, with basidiomycetes, *Penicillium*, *Cladosporium*, and yeasts the most prevalent taxa identified. Indoor levels were significantly lower than those in concurrently collected outdoor samples. Based on univariate analysis of variance, significantly higher mold levels were observed in school rooms (1) which were climate-controlled by unit ventilation systems (univents) as compared to central systems, (2) which did not have operating ventilation systems (fan off), and (3) which were sampled during school hours. Except in a limited number of school rooms, airborne mold levels were not considered to be a major indoor air quality and occupant health concern in these 17 school buildings.

KEYWORDS: Air quality, fungal contamination, fungi, indoor air, indoor environment, mold, schools, ventilation.

INTRODUCTION

In the past decade, increasing attention has been given to health problems associated with human exposure to indoor air contaminants. This attention is the result of a variety of field investigations of health complaints among occupants of both residential (Gary, *et al.*, 1980; Dally, *et al.*, 1981; Godish, *et al.*, 1990) and non-residential buildings (Seitz, 1988).

Mold exposures are of particular concern because of the ubiquitous presence of mold spores in indoor air and their causal association with chronic allergic rhinitis (Lowenstein, 1979; Schata, *et al.*, 1989), asthma (Reed and Tocouley, 1983), and hypersensitivity pneumonitis (Fink, 1983; Morey, *et al.*, 1984). Because mold is frequently identified by physicians as contributing to a patient's asthma or allergy, a significant amount of research effort has focused on studies of airborne-mold levels and taxa in residential buildings (Hirsch and Sosman, 1976; Soloman, 1976; Kozak, *et al.*, 1979; Strachan, *et al.*, 1990; Reponen, *et al.*, 1992; Su, *et al.*, 1992; Dekoster and Thorne, 1995). Studies of airborne-mold levels in office and institutional buildings have been more limited in scope.

Chronic allergic rhinitis and asthma are major pediatric health concerns. To better manage these conditions, the nature of exposures in major exposure environments such as homes and school buildings must be understood. Little is now known about the potential for exposure to airborne mold and the factors

which may contribute to it in schools in the United States. As a consequence, this study was designed to expand our understanding of potential indoor air quality problems in schools, with a focus on the quantitative and qualitative assessment of airborne mold and an evaluation of school/room characteristics which might be related to mold concentrations.

MATERIALS AND METHODS

Seventeen school buildings (including elementary, middle, and high school buildings) in a single east-central Indiana school corporation were selected as the study population. Culturable mold sampling was conducted in 148 randomly-selected classrooms and student use areas such as gyms, auditoriums, and locker rooms. In addition, 16 libraries and 3 pool areas were evaluated. The total number of school rooms sampled (N) was 167. Samples were collected in 20%-25% of the classroom/student use areas in each of the 17 school buildings.

All sampling for airborne mold was conducted by collection on 2% malt extract agar using an Andersen N-6 single-stage viable particle sampler. Two-minute samples were collected at each assessment location; the exposed plates were incubated at room temperature (22°-24° C) under natural lighting for 4-7 days. Mold concentrations are said to be based on "culturable mold" if they are limited to those spores, mycelial fragments, and taxa which can grow on the commonly used malt extract medium. Airborne mold levels were determined from colony counts and expressed as colony-forming units/cubic meter (CFU/m³) after correction for multiple impactions (Andersen, 1958). Individual colonies were identified to genus whenever possible.

Samples were collected during normal school hours whenever feasible (83%) to assess the mold levels which were typical of actual exposure conditions for students and staff; the remaining samples (17%) were collected within three hours of dismissal. All sampling was conducted during the heating season (15 February to 25 March), when outdoor mold levels should be low, and the room's windows were less likely to be open. A minimum 16-hour room closure period was requested from school staff prior to any room being sampled to reduce the influence of outdoor mold on indoor mold levels. Airborne mold samples were also collected at two different locations immediately outside each school building for comparative purposes.

The physical characteristics of each sampled room were recorded. These characteristics included the type of floor covering, ceiling material, and ventilation system; the operating status of the ventilation system; evidence of water damage/condensation/musty odor; and the presence of plants and potential water sources. The potential relationships between airborne mold levels and these physical characteristics were evaluated using univariate analysis of variance after the mold data were log transformed (the mold data were not normally distributed). More advanced statistical analyses were not possible due to the limited sample size. Mold data below the limit of detection (18 CFU/m³) were scored as "one" to avoid eliminating them from the SPSS statistical pack-

Table 1. Airborne culturable mold levels associated with 17 east-central Indiana school buildings.

School	# Classrooms Sampled (n)	Indoor Geom. \bar{X}	Indoor Range	Outdoor Geom. \bar{X} ^a	Outdoor Range
1	11	10.5	BLD ^b - 83	311.9	254- 383
2	7	297.3	18-12977	343.0	343
3	8	207.7	199- 449	624.8	569- 686
4	5	15.8	BLD- 88	9.4	BLD- 88
5	6	30.1	BLD- 106	138.5	71- 270
6	6	229.3	71- 629	463.2	419- 512
7	15	28.1	BLD- 106	121.7	18- 823
8	19	10.2	BLD- 1191	215.3	161- 288
9	10	100.1	35- 270	627.3	362-1087
10	17	17.1	BLD- 161	14.7	BLD- 216
11	11	9.1	BLD- 216	640.2	161-2546
12	8	23.9	BLD- 495	75.0	53- 106
13	8	13.4	BLD- 143	279.6	216- 362
14	9	14.0	BLD- 53	528.8	474- 590
15	7	15.6	BLD- 88	133.7	125- 143
16	10	68.8	18- 251	4691.4	2446-8998
17	10	7.4	BLD- 106	316.5	251- 399
TOTALS:	167	25.3	BLD-12977	229.6	BLD-8998
Frequency range		% Indoors (N=167)		% Outdoors (N=34)	
BLD		22.2		5.9	
18- 100		55.7		11.8	
101- 200		12.0		14.7	
201- 500		7.8		41.2	
501-1000		0.6		14.7	
>1000		1.8		11.8	

^a n = 2/school.

^b BLD = Below limit of detection.

age database on log transformation. A probability (*P*) value of 0.05 was accepted as significant in these and other statistical analyses.

RESULTS

The culturable airborne mold levels determined from both indoor and outdoor samples associated with 17 east-central Indiana school buildings are summarized in Table 1. Geometric mean values of samples collected in individual schools varied from 7.4 to 297.3 CFU/m³, with a geometric mean value for all data of 25.3 CFU/m³. The sample range was BLD (below the limit of detection) to 12,977 CFU/m³. Outdoor airborne mold levels determined from samples collected concurrently with the indoor samples at each school were in the range of BLD to 8,998 CFU/m³, with a geometric mean value of 229.6 CFU/m³.

Table 2. Prevalence of airborne culturable mold taxa in indoor and outdoor samples associated with 17 school buildings.

Taxa	INDOOR			OUTDOOR
	% of Total Colonies	% of Rooms	% of Buildings	% of Total Colonies
Basidiomycetes	58.0	38.3	82.4	5.7
<i>Penicillium</i>	18.5	31.7	82.4	1.5
<i>Cladosporium</i>	7.3	34.1	94.1	42.9
Yeasts	7.1	32.9	94.1	10.1
<i>Paecilomyces</i>	1.5	8.4	47.1	0.1
<i>Alternaria</i>	0.4	3.6	29.4	1.8
<i>Aspergillus</i>	0.3	1.8	17.6	—
<i>Rhizopus</i>	0.1	1.2	11.8	—
<i>Fusarium</i>	0.1	0.6	5.9	2.7
<i>Epicoccum</i>	0.1	0.6	5.9	0.1
Unknowns	6.7	31.7	88.2	35.2

In general, the indoor airborne mold levels were relatively low; 22% of the collected samples were below the detection limit, and 78% were below 100 CFU/m³. Only a small percentage of mold samples (Table 1) exceeded 500 CFU/m³ (2.4%) and 1,000 CFU/m³ (1.8%). A comparison of indoor and outdoor mold levels using analysis of variance confirmed that the geometric mean for outdoor culturable mold was significantly higher ($P < 0.001$) than the geometric mean for all indoor samples. However, in two individual cases (buildings 4 and 10), the outdoor mean values were less than the indoor mean values. No significant correlation was observed between indoor and outdoor culturable mold levels, when these values were evaluated using Pearson's product-moment test on the log-transformed data.

Major mold taxa and their frequency of recovery as a function of total colonies and occurrence in individual rooms and school buildings are summarized in Table 2. The basidiomycetes had the highest frequency of occurrence as a function of total colonies recovered. They were also observed in a high percentage of the rooms and buildings sampled. Relatively high prevalence rates were also observed for *Penicillium*, *Cladosporium*, and yeasts. Their prevalence was less as a percentage of total colonies when compared to the basidiomycetes; however, they were recovered from a similarly high percentage of both school rooms and buildings. Outdoor samples were dominated by *Cladosporium*, yeasts, and basidiomycetes (Table 2).

Because of the potential for interaction effects, mold levels in the 167 school rooms were evaluated by a univariate analysis of variance to determine whether or not significant differences in airborne mold levels were associated with sampling location/time and a variety of room/building characteristics. No significant differences in total culturable mold levels were observed when sam-

Table 3. Relationships between airborne culturable mold levels and building/room variables.

	n	Mold Geom. \bar{X}	Ventilation Type ^a	Ventilation Operating Status ^a	Sampling Time ^a
Ventilation type			—	.034*	.155
Univent	83	38.0			
Central	68	15.4			
Ventilation operating status			.059	—	.037*
On	123	20.4			
Off	24	53.7			
Time sampling began			.019*	.133	—
During school	138	29.5			
After school	29	12.0			

^a *P* values determined by univariate analysis of variance using the SPSS statistical package.

* Significant at *P* = 0.05.

ples from classrooms (*n* = 133), libraries (*n* = 16), gymnasiums, locker rooms, and pool areas (*n* = 14), and miscellaneous rooms, such as auditoriums and cafeterias (*n* = 4), were compared. No significant associations were observed for mold levels and the presence of indoor plants, water sources (sinks, fountains), insulated water or steam lines, water-damaged materials or musty odor, or with the type of ceiling or floor/floor covering materials.

Significant relationships were observed between airborne mold levels and three room characteristics/sampling variables — the type of ventilation system, the operating status of the ventilation system, and the time of sampling (Table 3). Significantly higher mold levels were observed in school rooms which were climate-controlled by unit ventilation systems (univents) as compared to central systems, independent of the system's operating status but not of the time when sampling was conducted. The significantly higher mold levels measured when the ventilation systems were not operating (fan off) were observed to be independent of the time of sampling but not of the type of ventilation system. The significantly higher level of mold in samples collected during school hours was observed to be independent of the type of ventilation system but not of the operating status of the ventilation system units.

DISCUSSION

The culturable mold levels determined from air samples in 17 east-central Indiana school buildings are consistent with mold levels reported for mechanically-ventilated and fully air-conditioned office buildings in the California Healthy Building Study (Fisk, *et al.*, 1993), in office buildings in the United

Kingdom (Harrison, *et al.*, 1992), and in Danish town halls (Skov, *et al.*, 1990). The culturable mold levels are also consistent with, but somewhat higher than, geometric means reported for 10 naturally-ventilated Italian schools (means' range = 11-18; overall mean = 15; Maroni, *et al.*, 1993) but lower than results reported for Finnish day care centers (log mean = 70 CFU/m³; Nevalainen and Jatunen, 1987), Swedish school buildings (range = 150-3,000 CFU/m³; Strom, *et al.*, 1990), and school buildings in Paris (\bar{X} = 100 CFU/m³, with a few samples exceeding 1,000 CFU/m³; Mouilleseaux, *et al.*, 1993). However, culturable mold levels appeared to be significantly lower than in a spring study of 60 classrooms in ten California school buildings (\bar{X} = 1,040 CFU/m³; Dungy, *et al.*, 1986).

The most prevalent mold types identified in indoor samples included basidiomycetes, *Penicillium*, *Cladosporium*, and yeast. Few office and institutional building studies have reported the type and prevalence of the mold taxa present. In a broad range of unidentified workplaces, Yang, *et al.* (1990) reported that *Cladosporium*, *Aspergillus*, *Penicillium*, basidiomycetes, and *Alternaria* were most prevalent. In the studies of Morey and Jenkins (1989), *Aspergillus*, *Penicillium*, *Cladosporium*, *Aureobasidium*, and *Chaetomium* were reported to be the most prevalent indoor taxa. For the most part, these results are consistent with those of the study reported here.

The higher culturable mold levels observed in the outdoor samples taken during this study are consistent with the levels reported in mechanically ventilated buildings (Morey and Jenkins, 1989; Holt, 1990; Fisk, *et al.*, 1993). The mold taxa reported here, including basidiomycetes, *Cladosporium*, *Alternaria*, *Epicoccum*, and *Fusarium*, are considered to be associated with outdoor sources (Su, *et al.*, 1992). Despite the fact that basidiomycetes are usually associated with outdoor sources, the very high basidiomycete levels observed in two samples from building 2 were probably associated with their infestation of wooden materials. Basidiomycete colonies in these two samples accounted for approximately 47.4% of the total mold colonies recovered from the 167 indoor samples collected in this study.

Two-by-two univariate analysis of variance revealed that the elevated mold levels associated with unit ventilators were independent of the operating status of the ventilation systems. However, higher airborne mold levels were associated with all types of ventilation systems which were not operating at the time of sampling, independent of the time the samples were collected. In addition, higher mold levels were found in air samples collected during school hours, independent of the type of ventilation system.

The higher mold levels associated with unit ventilation systems may have been due to a variety of factors. These systems are typically located in individual classrooms on an outside wall having openable windows. Though sampling was conducted during the heating season and teachers were requested to keep their windows closed during the day, the actual compliance rate prior to sampling was not known. Teachers often open such windows during the heat-

ing season, when the air is perceived to be stuffy or thermally uncomfortable. The buildings with central ventilation systems did not have openable windows, and the sampled rooms were not subject to occupant-induced ventilation changes. In addition, central ventilation systems are better designed and equipped to filter airborne dusts (which include mold spores) than univents are.

The lower mold levels associated with operating ventilation systems may have been due in part to the removal of mold spores and other particles from room air recirculated through the ventilation system's filters. This effect was independent of the time when sampling occurred and is consistent with the observation that a similar percentage of sampled rooms had ventilation systems which were operating during (84%) and after (81%) school hours. On the other hand, the higher mold levels that were observed for samples collected during school hours (but not independent of ventilation system operating status) are consistent with the Norwegian studies of Hanssen (1993), who reported elevated airborne dust levels during school hours, presumably associated with the disturbance of settled dust by the students.

The results of these studies indicate that the levels of culturable airborne mold in randomly selected classrooms in 17 school buildings under presumably closure conditions were very low and, as a consequence, were not likely to be a significant risk factor for building-related health complaints. However, a few rooms, representing approximately 2% of the total number of rooms sampled, were observed to have relatively elevated mold levels. Included were two classrooms in a single wing of an elementary school building having wooden floors and significant mold infestation on window sills and wooden materials associated with a heavy condensation problem on windows. Sample concentrations in these two rooms were dominated by the basidiomycetes, common wood-rotting fungi. In a second case, high *Penicillium* levels were found in a pottery classroom in which wet clay and other periodically wet pottery-making materials were present. Mold-infestation problems and the resultant elevated levels of airborne culturable mold are a relatively limited air quality and health concern in school buildings with a similar design and maintenance practices as the school buildings examined in this study.

LITERATURE CITED

- Andersen, A.A. 1958. New sampler for the collection, sizing and enumeration of viable airborne particles. *J. Bacteriol.* 76: 471-484.
- Dally, K.A., L.D. Hanrahan, M.A. Woodbury, and M. Kanarek. 1981. Formaldehyde exposure in non-occupational environments. *Arch. Environ. Health* 36: 277-284.
- Dekoster, J.A. and P.S. Thorne. 1995. Bioaerosol concentration in non-complaint, complaint, and intervention homes in the Midwest. *Amer. Ind. Hyg. Assoc. J.* 56: 573-580.
- Dungy, C.I., P.P. Kozak, J. Gallup, and S.P. Galant. 1986. Aeroallergen exposure in the elementary school setting. *Ann. Allergy* 56: 218-221.
- Fink, J. 1983. Hypersensitivity pneumonitis. In: E. Middleton (Ed.), *Allergy: Principles and Practice*, pp. 1085-1099. C.V. Mosby, St. Louis, 1440 pp.
- Fisk, W.J., M.J. Mendell, J.M. Daisey, D. Faulkner, A.T. Hodgson, and J.M. Macher. 1993. The California healthy building study, phase 1: A summary. *Proc. Sixth Internat. Conf. Indoor Air Qual. Climate — Helsinki* 1: 279-284.

- Gary, V.F., L.A. Oatman, R. Pleus, and D. Gray. 1980. Formaldehyde in the home. Some environmental disease perspectives. *Minnesota Med.* 63: 107-111.
- Godish, T., T.W. Zollinger, and V. Konopinski. 1990. Residential formaldehyde — Increased exposure levels aggravate adverse health effects. *J. Environ. Health* 53: 34-37.
- Hanssen, S.O. 1993. Increased ventilation reduces general symptoms but not sensory reactions. *Proc. Sixth Internat. Conf. Indoor Air Qual. Climate — Helsinki* 5: 33-38.
- Harrison, J., C.A.C. Pickering, E.B. Faragher, P.K.C. Austwick, S.A. Little, and L. Lawton. 1992. An investigation of the relationship between microbial and particulate indoor air pollution and sick building syndrome. *Respir. Med.* 86: 225-235.
- Hirsch, S.R. and J.A. Sosman. 1976. A one-year survey of mold growth inside twelve homes. *Ann. Allergy* 36: 30-38.
- Holt, G.L. 1990. Seasonal indoor/outdoor fungi ratios and indoor bacterial levels in non-complaint office buildings. *Proc. Fifth Internat. Conf. Indoor Air Qual. Climate — Toronto* 4: 33-38.
- Kozak, P., J. Gallup, L.H. Cummins, and S.A. Gillman. 1979. Factors of importance in determining the prevalence of indoor molds. *Ann. Allergy* 43: 88-94.
- Lowenstein, H. 1979. Airborne allergies — Identification of problems and the influence of temperature, humidity, and ventilation. *Proc. First Internat. Indoor Climate Symp. — Copenhagen*, pp. 111-125.
- Maroni, M., M. Bersani, D. Cavallo, A. Anversa, and D. Alcini. 1993. Microbial contamination in buildings: Comparison between seasons and ventilation systems. *Proc. Sixth Internat. Conf. Indoor Air Qual. Climate — Helsinki* 4: 137-142.
- Morey, P.R., M.J. Hodgson, W.G. Sorenson, G.J. Kullman, W.W. Rhodes, and G.S. Viservara. 1984. Environmental studies in moldy office buildings: Biological agents, sources, preventative measures. *Ann. Amer. Conf. Govt. Ind. Hyg.: Evaluating Office Environmental Problems* 10: 21-36.
- _____ and B.A. Jenkins. 1989. What are typical concentrations of fungi, total volatile organic compounds, and nitrogen dioxide in an office environment? *Proc. IAQ '89: The Human Equation: Health and Comfort*, Amer. Soc. Heating, Refrigerating, and Air-Conditioning Eng., pp. 67-71.
- Mouilleseaux, A., F. Squinazi, and B. Festy. 1993. Microbial characterization of air quality in classrooms. *Proc. Sixth Internat. Conf. Indoor Air Qual. Climate — Helsinki* 4: 195-200.
- Nevalainen, A. and M. Jantunen. 1987. Airborne bacteria, fungal spores, and ventilation in Finnish day-care centers. *Proc. Fourth Internat. Conf. Indoor Air Qual. Climate — West Berlin* 1: 678-680.
- Reed, C.E. and R.G. Tocouley. 1983. Asthma — Classification and pathogenesis. *In: E. Middleton (Ed.), Allergy: Principles and Practice*, pp. 811-831, C.V. Mosby, St. Louis, 1440 pp.
- Reponen, T., A. Nevalainen, M. Jantunen, M. Pellikka, and P. Kalliokoski. 1992. Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. *Indoor Air* 2: 26-31.
- Schata, M., J.H. Elixmann, H.F. Linskens, and W. Jorde. 1989. Allergies to molds caused by fungal spores in air-conditioning equipment. *Environ. Internat.* 15: 177-179.
- Seitz, T. 1988. NIOSH indoor air quality investigations: 1971-1988. *In: D.M. Weekes and R.B. Gammage (Eds.), The Practitioner's Approach to Indoor Air Quality Investigations*, pp. 163-171, *Proc. Indoor Air Qual. Internat. Symp. Amer. Ind. Hyg. Assoc.*, 171 pp.
- Skov, P., O. Valbjorn, and B.V. Pedersen. 1990. Influence of indoor climate on the sick building syndrome in an office environment. *Scand. J. Work Environ. Health* 16: 367-371.
- Soloman, W.R. 1976. A volumetric study of winter fungus prevalence in the air of Midwestern homes. *J. Allergy Clin. Immunol.* 57: 46-55.
- Strachan, D.P., B. Flannigan, E.M. McCabe, and F. McGarry. 1990. Quantification of airborne moulds in the homes of children with and without wheeze. *Thorax* 45: 382-387.
- Strom, G., B. Hellstrom, and A. Kumlin. 1990. The sick building syndrome. An effect of microbial growth in building constructions? *Proc. Fifth Internat. Conf. Indoor Air Qual. Climate — Toronto* 1: 173-178.
- Su, H.J., A. Protnizky, H. Burge, and J. Spengler. 1992. Examination of fungi in domestic interiors using factor analysis: Correlation and association with home factors. *Appl. Environ. Microbiol.* 58: 181-186.
- Yang, C.S., L.L. Hung, F.A. Lewis, and F. Zampello. 1990. Airborne fungal populations in non-residential buildings in the United States. *Proc. Fifth Internat. Conf. Indoor Air Qual. Climate — Toronto* 4: 218-224.