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Air Sampling Results in Relation to Extent of Fungal Colonization of Building Materials in Some Water-Damaged Buildings

J. DAVID MILLER[1]*, PHILIP D. HAISLEY[2] AND JAMES H. REINHARDT[2]

Abstract We studied the extent and nature of fungal colonization of building materials in 58 naturally ventilated apartments that had suffered various kinds of water damage in relation to air sampling done before the physical inspections. The results of air samples from each apartment were compared by rank order of species with pooled data from outdoor air. Approximately 90% of the apartments that had significant amounts of fungi in wall cavities were identified by air sampling. There was no difference in the average fungal colony forming unit values per m^3 between the 15 apartments with the most fungal contamination and the 15 with the least. In contrast, the prevalence of samples with fungal species significantly different than the pooled outdoor air between the more contaminated versus the less contaminated apartments was approximately 10-fold. We provide information on methods to document fungal contamination in buildings.

Key words Fungi; Indoor air; Water damage; Air sampling; Fungal sampling.

Practical Implications
It is well recognized that the area of fungal contamination in buildings is associated with respiratory distress in occupants of these buildings. Air samples have, in the past, been used to establish the presence of fungal contamination in buildings. This study examines the association between air samples and the extent of a building's fungal contamination. The authors demonstrate a useful correlation between the results of air sampling (comparing species to an outdoor reference sample) and the extent of fungal damage.

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Introduction

Material fungal growth on building materials in houses, apartment buildings, office buildings and schools results in increased asthma and upper respiratory disease (Anon., 2000a, b; Macher et al., 1999). The principle that area of fungal contamination is associated with occupant risk was articulated by the so-called New York guidelines (Anon., 2000a), the Canadian Federal-Provincial Committee on Occupational Environmental Heath (Health Canada, 1995), the International Society on Indoor Air Quality & Climate (Flannigan & Morey, 1996) and the ACGIH Bioaerosols Committee (1999). Thus, evaluating the area of visibly moldy building materials is useful for assessing occupant risk and determining clean-up strategies. Air samples can be used to evaluate fungal contamination of air and methods have been published to standardize their collection and analysis (Dillon et al., 1996). While properly conducted air samples can identify whether a building has fungal growth there are few, if any, data on their quantitative value in relation of the extent to fungal contamination.

Fungal contamination can arise from several conditions including condensation, floods and various types of leaks. In subtropical conditions where indoor air is cooled, improper installation of the air-retarder, thermal bridging and use of non-porous wallcoverings that can become air-retarders can lead to the growth of moderately xerophilic fungi such as *Aspergillus versicolor* on wallboard or on the inner surface of the wallcovering (Dillon et al. 1999). Fungal growth and the consequent damage that can arise can be more common in expensive buildings with complex construction requirements than in more basic construction. Common problems were the result of uncontrolled air flow caused by duct leaks and supply/exhaust imbalances bringing in warm wet air to cold surfaces (Odom & Du Bose, 1996; Trechsel, 1994).

Failures of calking, joints, and other construction details, especially around windows, can lead to water

[1]*Ottawa-Carleton Institute of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6, Canada, E-mail: davidmiller@pigeon.carleton.ca, Fax: +1 613 520 2710,* [2]*Architectural Diagnostics Ltd., Honolulu, Hawaii, USA, *Author to whom correspondence should be addressed*

leaks into wall cavities. A study of water damaged buildings in the Pacific northwest revealed that construction detail failures were important causes. Poor installation of windows was the top cause of water leaks and fungal growth (Anon., 1996). Chronic water leaks lead to contamination by *Chaetomium* and *Stachybotrys* (Dillon et al., 1999; Morey, 1997)

A study of moisture and fungal problems in 59 single-family dwellings in southern Ontario showed that the amount of visible fungal growth on building materials to explain objective measures of fungi in settled dust and air. This demonstrated that some the fungal growth was hidden in wall cavities. On analysis, the largest source of fungal growth was explained by condensation on the envelope, closely followed by a variety of sources, including fungi on the interior walls and ceilings of bathrooms, refrigerator drain pans and sumps. Wicking of water into the basement resulted in 30% of basements demonstrating fungal growth (Lawton et al., 1998). Air flows in and out of buildings account for an appreciable percentage of water movement in residential housing. Evidence of air flows from patterns of dirt on the bottoms of closed interior doors and other clues can shed light on the movement of air (see Lstiburek & Carmody, 1994). A final category of water damage is unrepaired fungal and water damage from the water used to put out fires and from hurricanes, earthquakes, floods or other natural disasters (Morey, 1993). These formal studies support the inferences drawn in numerous case studies by investigators such as Morey (1997).

The purpose of this report is to describe the methods used to document a study of 58 apartments for fungal contamination with a view to determining the power of air sampling done according to the American Industrial Hygiene Association protocols to detect hidden fungal growth in naturally ventilated buildings.

Material and Methods

Air Samples

The study was done in a number of naturally ventilated buildings comprising 1-, 2- and 4-bedroom apartments. Fungal contamination was suspected from a variety of sources including window leaks, plumbing leaks and storm damage. Air samples (4 min) were taken with a Reuter Centrifugal Sampler using rose bengal agar strips supplied by Biotest (Denville, NJ, USA). The sampler was at ca. 1.5 m in the center of the room. The windows were closed for at least 1 h prior to sampling. This was done to minimize the impact of dilution by outdoor air of indoor-sourced molds. Samples were taken in the morning and the afternoon. This was done to test while the building was subjected to a range of solar and wind burdens. Air samples were collected the largest room and in each bedroom while the apartment was not occupied. Each apartment was sampled 4, 6 or 8 times depending on size (total 268 samples). Outdoor air samples (150) were taken in the morning and afternoon on third-storey balconies (ca. 10 m above ground) over the three weeks the sampling was done. Samples were shipped to the laboratory within 24 h under cool conditions and incubated at 25°C (Dillon et al., 1996). The colonies were counted and representative cultures were transferred to malt extract agar (20 g/l^{-1}) and Czapek Dox agar amended with 1 g/l^{-1} yeast extract (Oxoid, Sigma). After 7–10 days, all cultures were identified to species where possible. Outdoor air data were pooled to create a reference comparison. Individual air samples were compared to this outdoor air reference using Spearman correlation and scored as pass or fail (Dillon et al., 1996). Data were tabulated as the ratio of samples in an apartment that failed by this measure (i.e. 4/4=1.0).

Documentation of Area of Fungal Growth

Apartments were vacated for the entire time necessary to perform the inspection and repair the damage caused by the destructive testing. All operations were conducted with windows and balcony doors open which provided substantial cross ventilation. Contents were covered by polyethylene sheets and after the repairs were completed, all surfaces were HEPA vacuumed before occupancy. Personal protection consisting of disposable coveralls and N-95 half-face respirators were used where necessary (Macher et al., 1999).

Visible fungi behind movable items such as refrigerators was documented as described below. Preliminary attempts were made to determine extent of visible fungi in wall cavities by drilling small (10-cm) holes and using a boroscope. The device used was an Olympus G080–034–090–55 with ALS-150U light source (Olympus, Lake Success, NY, USA). This has a rigid 8-mm diameter shaft, 90-degree lateral optics and a 55-degree conical field-of-view that can be rotated 370 degrees around the axis of the shaft. This approach proved to be of limited value for our purposes. An alternative strategy was devised. The bottom 0.3-m section of wallboard around the entire perimeter of the apartment was cut using a reciprocating saw and the wallboard was flipped down onto the floor in place (on top of the polyethylene sheet). Powerful lights were used to look up into the cavity. Modest exceptions were made to this strategy where there were

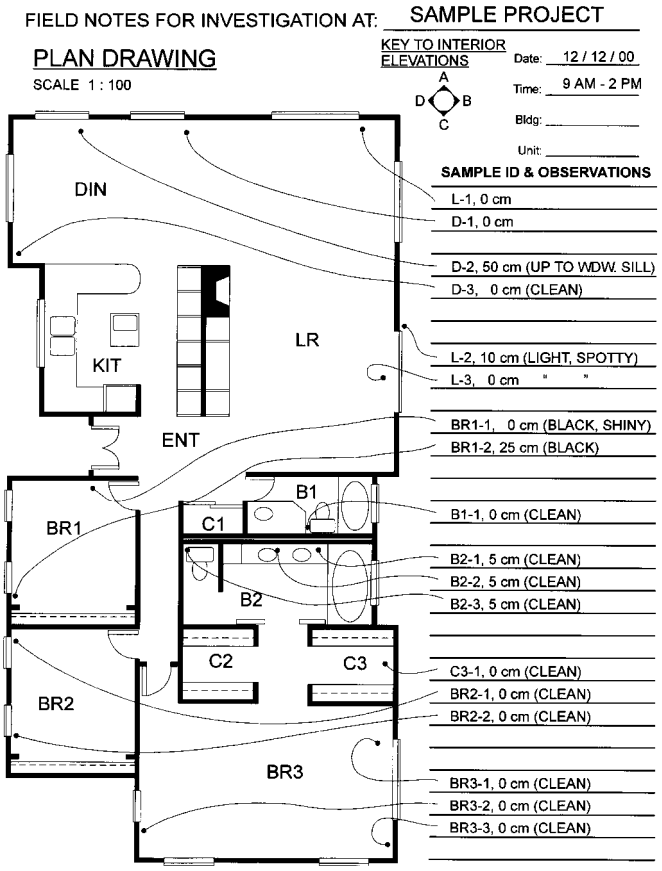

Fig. 1 Plan view of typical area under investigation with notes added

major installed items such as in the bathrooms. In these cases, the boroscope was used to determine the fungi in the adjacent wall cavity.

Survey sheets drawn to scale were prepared before the inspection (Figures 1 and 2) to record observations. Hash marks were used to indicate the observed fungal locations, as well as the side of the wall cavity where fungi were observed. Relative density of the marks approximated the density of growth. Notation on interior elevation drawings were made to identify the near side or far side of the wall cavity, or separate sheets were labelled for each side of the wall cavity. Camera locations were marked on the plan view with photo numbers for each shot. Overall photos were taken that could be paired with close-up photos, to clarify location and the surrounding context of each close-up shot.

Total observed visible fungal growth on the building materials was determined as carefully as possible from the field notes with reference to the photographs and mycological analysis. Samples of wallboard were taken everywhere there was visible fungi plus a roughly equivalent number taken nearby (within 0.25–0.5 m) that did not appear under field conditions to have visible fungal contamination. These were taken with a knife and were typically 100 cm^2 in size. Samples were placed in plastic bags and stored under air dry conditions until sent to the laboratory.

Upon reaching the laboratory, samples were examined under good lighting conditions for the presence of visible fungi. Lacotphenol cotton blue mounts were made of samples with visible fungal damage and examined under a compound microscope. Small pieces (ca. 0.5 g) were plated on 2% malt extract agar and incubated at 25°C. Colonies that arose were counted before the growth became confluent, isolated into pure culture, and plated on 2% malt extract and Czapek-Dox agar amended with 1 g/L yeast extract and identified to species where possible. The primary variables between fungal culture media are Aw and the availability of nutrients (Booth, 1971). These are similar between rose bengal and 2% malt extract agars and, appropriately handled, the use of rose bengal is not harmful to the recovery of propagules of the type of fungi considered here (Morring et al., 1983).

Statistical Analyses

Statistical analyses were performed using SYSTAT v. 8 (Systat Evanston, IL, USA).

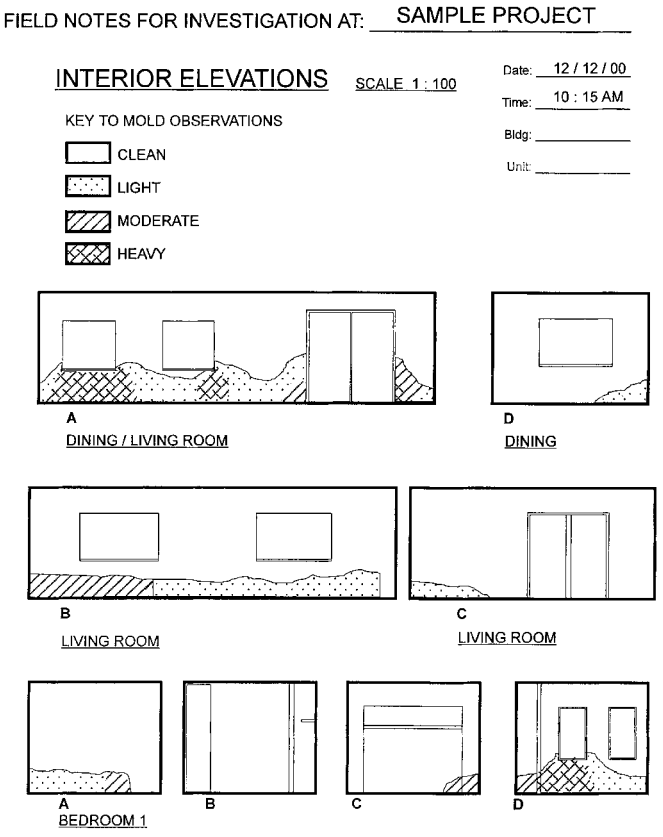

Fig. 2 Perspective views of typical area under investigation with notes added

Table 1 Fungi recovered from wallboard samples

Samples	Visible growth		Wet wallboard fungi recovered[1]		*Stachybotrys chartarum*	
	Field	Lab	Visible[2]	Not visible[2]	Culturable[3]	Not culturable[3]
978	574 59%	596 61%	596 61%	789 81%	52 9%	263 44%

[1] "Wet wallboard" fungi in this location were *Penicillium aurantiogriseum*, *P. viridicatum*, *Paecilomyces varioti*, *Chaetomium globosum*, *Memnoniella echinata* and *Stachybotrys chartarum*
[2] Percentage of total number of samples
[3] Percentage of samples with visible fungal damage

Results

Air Sampling versus Visible Fungal Growth

The 58 apartments were stratified based on physical measurements into the 15 lowest area of visible fungal growth on the building materials and the 15 highest apartments. The airborne CFU m^{-3} values between the two data sets were not significantly different using descriptive statistics (214±201 CFU m^{-3} versus 329±389). However, the proportion of samples that were significantly different from the outdoor air pooled data was significantly different (0.08±0.12 versus 0.79±0.19; $P<0.005$; t-test). Non-phylloplane species recovered in the air samples are listed in Table 1. The dominant non-phylloplane species recovered in the 264 indoor air samples comprised 8,248 colonies, 49.8% of which were *Cladosporium herbarum*, *C. cladosporioides*, non sporulating isolates and *Alternaria alternata.* The remaining colonies were *Penicillium viridicatum* (26%), *P. aurantiogriseum* (16%) and 7% were comprised of *Aspergillus versicolor, Aspergillus sydowii, P. variable, P. brevicompactum, P. crustosum, P. chrysogenum.* In addition *Paecilomyces varioti, Talaromyces flavus, Eurotium herbariorum* and 10 other taxa were recorded. The pooled outdoor data comprised 15,682 colonies of *Cladosporium herbarum*, *C. cladosporioides*, non-sporulating isolates, *Alternaria alternata* and *Eppicoccom nigrum* and 257 colonies of 12 species of *Penicillium* and 3 species of *Aspergillus* from 150 air samples.

The mean area of visible fungal growth in the apartments was 5.06±4.3 m^2 (range 0.28 to 19.1 m^2). The data were as follows: <1 m^2, n=2; 1–4.3 m^2, n=30; 4.3−8.6 m^2, n=17; 8.6−12.9 m^2, n=5; 12.9−17.2 m^2, n=3 and >17.2 m^2, n=1. Comparing the area of fungal growth to the percentage of air samples that were significantly different from outdoor air, the relationship was highly significant using the Kruskal-Wallis test for non-parametric data, $P=0.033$. When the data were transformed and subjected to ANOVA, the P-value was 0.10.

The results of wallboard sampling (Table 2) revealed a close agreement between the field and the laboratory observations of visible fungal contamination (59% versus 61% of 978 samples collected). The field investigators collected samples that did not appear visibly damaged by fungi amounting to ca. 40% of the total. Of these, however, a further 37% had fungal taxa associated with wet wallboard or 81% of the samples. This was based on multiple colonies arising from the plated material of *Penicillium aurantiogriseum*, *P. viridicatum*, *Paecilomyces varioti*, *Chaetomium globosum*, *Memnonilla echinata* and *Stachybotrys chartarum.* The adventitious occurrence of phylloplane and other species not known from wallboard in the USA were not considered (Flannigan & Miller, 2000). Culturable *S. chartarum* comprised 9% of the visibly damaged samples however, spores of this species were seen in the lactophenol cotton blue mounts of the material in 44% of such samples. The 37% of samples that had clear fungal growth by culturing but below the level required for visible growth were typically 0.25 to 0.5 m away from the line of visible fungal damage.

Discussion

Inspecting building structures for fungal damage and communicating the inspection observations to others requires an understanding of local building materials and how buildings are constructed, some ability to graphically depict observations, and knowledge of the purpose of inspection and the intended use for the results. The level and degree of visual inspection varies, but might be broadly categorized to three levels, depending on the inspection goal:

1) A visual, non-destructive inspection might be performed to identify if there is reason to suspect fungal contamination, or a need to perform a more thorough inspection. A careful walk-through inspection requires close observation of all exposed building surfaces using common inspection tools, notes and photographs. This would include determining any visible evidence of fungal growth, and any blisters, stains or discolouration which might indicate water intrusion as well as a check of around seams and crevices along the base of walls including under car-

pets, around the base of all window and door jambs, and along the tops of walls and at joints in ceiling materials.

2) A slightly destructive inspection can be performed to confirm that concealed fungal contamination exists, and to roughly gauge the severity and location. The inspection can be performed with a borescope with light source which would minimize damage while allowing reasonable observation of concealed conditions.

3) A complete and systematic inspection requires the opening of wall cavities so that the extent, location, and nature of concealed fungal contamination can be observed and noted with reasonable accuracy. This type of inspection is described here.

Our approach was guided by the air samples that failed the AIHA guidelines (Dillon et al., 1996). Some of the apartments had visible fungal growth in the bathrooms and behind the refrigerators but this did not explain the air sample data. It was only when destructive testing was done that an accurate determination of the extent of fungal growth in the apartments could be obtained. The nature of the fungi on the wallboard was determined by plating a large number of bulk samples. These tests revealed that the visual observations in the field, generally by the investigator wearing a mask, of samples that looked moldy were similar to the laboratory results (Table 1). However, 37% of the samples that looked clean taken near visibly damaged material had incipient growth of the same fungi.

Fungal determinations from wallboard were made by direct plating. This provides information on the fungi active in the building material (Miller, 2000). The technique of making a microscopic mount in addition to the plating allowed the detection of the proportion of culturable versus non-culturable *S. chartarum* (or other species).

These tests have supported the contention that properly done air sampling with analysis to species is a powerful tool for the investigation on fungal-damaged buildings (Dillon et al. 1996; 1999). The use of CFU m^{-3} alone had no value in assessing ''good''; versus ''bad''; apartments whereas the comparison to the outdoor reference value was evidently a powerful tool. The most important finding from this large study was that there was a useful correlation between the results of the air sampling and the extent of fungal damage. Comparing the area of contamination to the percentage of air samples that were significantly different from outdoor air per apartment, the correlation was significant with P-values of 0.033 and 0.10 depending on the test used. This means that there was between a 90 and 96% chance that the air samples taken in the apartments predicted eventual fungal damage. Applying that test in the other way, 6 of 58 gave a weak or no signal when there was significant hidden fungal contamination or about 10%. This was surprisingly good since there are many factors that mitigate against the predictive value of air tests including variation in the surface cleanliness of the rooms, short sampling window (4 min) and the use of a single agar medium.

Excepting *Stachybotrys chartarum*, the dominant mold species found in the air samples were similar to those recovered from the mold-damaged wallboard (see results, Table 1). In this case, the majority of the *S. chartarum* found was not viable and hence could not be detected on the air samples if present.

In naturally ventilated buildings, we have shown that properly conducted air sampling is a very useful tool for determining the extent of destructive testing that might be required. The method is not infallible and requires the exercise of good judgement. Even highly contaminated areas can ''pass'' air sampling tests by for several reasons including, for example, a deep HEPA vacuum cleaning of the space immediately before the tests, poor attention to detail in doing the tests, failure to identify the organisms present to species or doing only a few samples. We have provided examples of the methods and documentation needed to record the nature and extent of fungal contamination.

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