How To Interpret Laboratory Results for Airborne Fungal (Mould) Samples

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Introduction

Laboratory results for airborne mould and bacteria concentrations can be difficult to interpret for two major reasons. First, there are no set maximum exposure limits (MELs) or threshold level values for airborne indoor mould and bacteria concentrations. Setting MELs would be difficult due to limitations in air sampling techniques (Kung'u, 2004), variability in sensitivity to microbial exposure among the human population, occurrence of a large number of different types of biological and chemical pollutants in indoor environment (Morey, 2001) and limited data on exposure-response relationship.

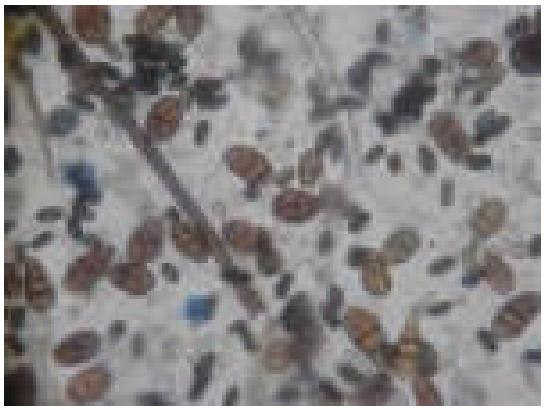

Figure 1. Spores as seen under a microscope: Non-viable Analysis

The second reason is failure by investigators to clearly define the objectives for taking air samples for microbial analysis. A well defined sampling objective helps the investigator to design an appropriate sampling strategy which includes the data required and hence the type of samples to take i.e., non-viable (figure 1) or viable (figure 2 and 3), the minimum number of samples to take, when to take them and how to interpret the resulting data. An initial walkthrough in the building under investigation is recommended prior to designing the sampling strategy.

What Information Do You Need To Interpret Results Effectively?

In absence of set MELs, concentrations and composition of indoor airborne mould and bacteria are compared with those of outdoor (Miller, 2001, Health Canada, 2004). The investigator, therefore, should have some background information on:

- typical airborne fungal or bacteria concentrations, diversity and their seasonal, diurnal and temporal variation in outdoor and indoor environments,
- factors affecting airborne microbial concentrations
- mycological or bacteriological terms used in the reports.

Typical airborne fungal concentrations for outdoor and indoor environment

There are few comprehensive data on typical airborne concentrations for fungal spores for either outdoor or indoor environment. Some useful data, however, may be found scattered in the

literature though it may be specific for the regions where the studies were conducted. From the author's experience after analysing thousands of spore traps from across Canada and reviewing published literature (Flannigan, Samson and Miller, 2001; Kendrick, 2002), outdoor fungal spore concentrations vary not only with the season but also with the day and time of the day. The spores of mushrooms (basidiospores) are numerous in early fall while rust aeciospores, urediniospores, and teliospores are most numerous in spring, summer, and fall respectively. Li and Kendrick (1995) reported diurnal periodicity for different groups of fungi. Ascospores and basidiospores concentrations showed an increased prevalence during the morning hours while concentrations of *Cladosporium* and other imperfect fungi which are released passively tended to increase during the afternoon. The highest concentration of *Cladosporium* species was at around 4:00 pm, while those of *Alternaria* and *Sporobolomyces* species were highest at around 6:00 pm and at night respectively (Kendrick, 2002).

Figure 2. RCS Samples: Viable Analysis

Indoor levels of microbial propagules broadly parallel those of outdoors during summer but counts are usually lower than outdoors (Miller, 2001). From May to October the relationships between numbers of airborne spores and hyphal fragments and the diversity of fungi in indoor and outdoor environment are very strong. *Alternaria* species, *Leptosphaeria* species, unidentified ascospores, *Coprinus* species, and *Ganoderma* species come mainly from outdoor sources. The major exception to the tendency for indoor counts in summer to be lower than the corresponding outdoor counts are for *Aspergillus* and *Penicillium* species. Spores of these two groups are primarily of indoor origin. Spores of *Cladosporium*, *Epicoccum*, unidentified basidiospores and other unidentified spores may have both an outdoor and indoor sources.

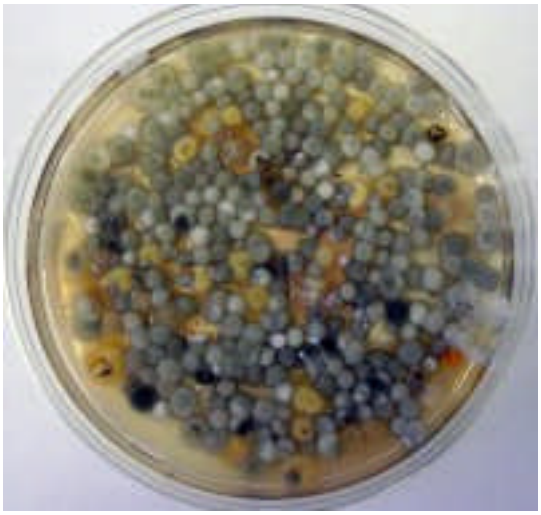

Figure 3. Andersen Sample: Viable Analysis

Hyvärinen (2002), compared microbial concentrations and composition in problem houses with "non-problem" houses (reference). In wintertime the concentrations of total viable fungi and concentrations of *Penicillium*, *Aspergillus*, and yeasts in the moisture damaged buildings were higher than in the reference buildings. Also, fungal diversity was higher in the moisture damaged buildings than in reference buildings. Certain fungal genera, such as *Stachybotrys*, *Ulocladium*, *Tritirachium*

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and *Exophiala*, were detected only in the air of the moisture damaged buildings.

The type of ventilation in a building greatly affects the indoor-outdoor airborne microbial ratios (Flannigan, 2001). Central air conditioning, for example, was found to reduce mould spores in homes by 50% and closing of windows and doors in naturally ventilated buildings excluded 98% of outdoor spores.

Factors affecting airborne microbial concentrations

Prevailing environmental factors affect airborne microbial concentrations. Wind for instance increases outdoor mould spore counts by aiding release and dissemination (Kendrick, 2002). Rain has three distinct effects. Immediately after the rain begins, release of ascospores increases. However, heavy or prolonged rain washes out most spores from the air thus reducing the concentration. Humidity, temperature and light intensity also affect release of spores into the air.

Understanding mycological terms used in laboratory reports

Understanding mycological terms used in laboratory reports, also helps in interpretation.

Terms used in non-viable analysis reports

Amerospores: refer to single celled spores (width to length ratio <15:1). Since spores from many groups of fungi can be referred to as amerospores, this term is not useful in reports.

Basidiospores: spores produced by a group of fungi called Basidiomycetes, for example mushrooms and some wood-rotting fungi.

Ascospores: refer to spores produced by a group of fungi called ascomycetes, for example *Chaetomium* spp.

Smut: refer to spores produced by smut fungi. Smut fungi are plant pathogens and are unlikely to have an indoor source.

Rust: refer to spores produced by rust fungi. Rust fungi are also plant pathogens and are unlikely to have an indoor source.

Myxomycetes: these are the slime moulds. Not true moulds.

Hyphal fragments: refer to fragments of the filamentous structures (hyphae) that make up the body of moulds by branching extensively to form a complex network called mycelium.

Terms used in viable analysis reports

Colony forming units (CFU): for moulds, a colony refers to a group of hyphae (see figures 2 and 3). The term "unit" refers to the mould component(s) from which a colony developed. The unit could be a single spore or a single hyphal fragment, a group of spores or hyphal fragments or a mixture of both spores and hyphal fragments.

Non-sporulating (non-sporing) isolates: refer to moulds that fail to produce spores either because they have lost this ability, conditions were not suitable or required very long periods to produce spores. Fungi that are strictly non-sporulating are called Mycelia sterilia (sterile mycelia).

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Sp (plural written as spp).: short form for species, e.g., *Penicillium* sp or *Penicillium* spp referring to a single species or more than one different species of *Penicillium* respectively.

Data interpretation procedures
As indicated above interpretation of airborne concentrations of indoor moulds and bacteria is primarily based on experience and professional judgement. Basic knowledge of typical outdoor and indoor moulds is important. For example, if lab results for a sample that was supposed to be an indoor sample show that dominant spores were mainly smut and rust spores while results for outdoor sample showed Aspergillus/Penicillium group, then the investigator would suspect the samples were swapped and may want to resample again. To be able to interpret the results there are key steps to follow:

1. **Review the air sampling objective.** Let us say the primary objective of the investigation was to determine if there were elevated airborne mould spore concentrations and/or indoor amplification sources in problem rooms.

2. **Bearing in mind the sampling objective, compare total airborne spore concentrations from problem rooms with those from outdoors and control (non-problem) rooms.** This would answer the question whether levels of mould spore concentrations were elevated in problem rooms compared to outdoor and non-problem rooms.

Our objective was also to know whether there were amplification sources in the problem rooms. We can deduce this from:

- dominant moulds present in problem rooms but not in the outdoor and control rooms. For examples if *Cladosporium* is dominating in problem rooms but is insignificant in outdoor and control rooms, then we can conclude the source is in problem rooms, even though *Cladosporium* can originate from outdoor,
- presence of indicator moulds. These are moulds frequently found in water damaged buildings. These include *Ulocladium* spp., *Stachybotrys* spp., *Chaetomium* spp., *Acremonium* spp., *Sporobolomyces* sp., *Tritirachium* sp., *Cladosporium* spp., and *Aspergillus* and *Penicillium* species.

3. **The third step is therefore to compare the dominant spore types (and their concentrations) from problem rooms with those from control rooms.**

Some species of *Aspergillus*, *Penicillium* and *Cladosporium* may also originate from outdoor environment. Therefore, the type of ventilation and the building history i.e., whether there has been previous water problem would provide additional information on the possible source of these moulds.

NB:

- Always be aware of the limitations in sampling and analytical methods and how these could affect the results and hence the data interpretation.

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- The idea of comparing outdoor with indoor samples is controversial. However, this comparison gives an indication as to whether the spores recovered from indoor environment originated from outdoors or were from indoor sources. During winter, this comparison is not possible since outdoor microbial concentrations, in most cases are below the detection limits.

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