FUNGI IN THE AIR: WHAT DO RESULTS OF FUNGAL AIR SAMPLES MEAN?

By Chin S. Yang, Ph.D.

Introduction

Sampling and testing for airborne fungi is a common practice during an indoor air quality (IAQ) investigation, routine IAQ survey, and monitoring during mold remediation. There are currently no standards and guidelines regarding results of fungal air samples. It is not very likely to have such standards and guidelines in the near future. Airborne fungi may change according to spatial and temporal variations. Without standards and guidelines, current approach to the interpretation of results of fungal air samples relies on comparisons of indoor vs. outdoor results and complaint vs. non-complaint area results.

This technical fact-sheet discusses how to interpreting results of fungal air samples collected during an IAQ evaluation and investigation.

What to consider when comparing results of fungal air samples

Different air samplers use different flow rates and have different collection efficiency. Therefore, never compare results derived from different air sampling equipment. In addition, different sampling time (duration) and different sample media may also yield different results. Different incubation (such as 25°C v. 35°C) and analysis (total spore count v. culturable fungi) may also result in varying results.

There are currently two widely used sampling and testing of airborne fungi, culturable method and spore traps. Culturable methods include the gravity/settling method, Andersen samplers, SAS samplers, Biotest samplers, filter cassettes, or other bioaerosols samplers. Airborne spores are collected and cultured on fungal nutrient media. Viable spores germinate and grow into colonies after incubation. Fungal colonies are then enumerated and identified. Spore traps use Air-O-Cell cassettes, MCE filter cassettes, Allergenco sampler, or Burkard samplers to collect spores onto a greased-coated receiver or a membrane filter. All fungal structures (including spores, conidiophores, and hyphal fragments). are counted and presumptively identified. Interpretation of results of both total spore count and culturable fungi are similar. Culturable methods usually yield lower fungal concentrations than spore trap methods because many spores may be non-viable, dormant, or unable to grow on the media used. But culturable methods give proper identification of fungal colonies. Spore trap methods generally yield higher levels of fungal structures but identifications are presumptive at best.

Interpretation of Airborne Fungal Bioaerosol Results

A detailed process on result interpretation is discussed in this fact-sheet. You may use results of both culturable and spore trap in a similar fashion. Please remember that background information, on-site observations, and history of the case and building (such as water damage history and humidity problems) are very important in the final interpretation and conclusion. Make sure that you incorporate and use the information that you collect during your field study and sampling. Make use of floor plans or blue prints to map out your data. This may allow you to correlate the results with locations better.

The process is divided into several steps. You may or may not use all steps. If you feel any step is not applicable in your case, you may skip and go on to the next step. If you have any question in the process, you may contact us by phone, fax, or e-mail.

1. If you have a large collection of airborne fungal data, you may be able to define what is considered low, moderate, or high to ***screen*** your results. However, use such data with caution and for performance evaluation only. The data should not be used for health evaluation criteria. For most culturable air samples, concentrations over 200 $\pm$ 50 CFU/m^3 may require further evaluation. Concentrations over 1000 CFU/m^3 may suggest possible indoor sources of fungi or poor filtration in the HVAC system. It is important to note that concentrations lower than 200 CFU/m^3 do not indicate a "healthy environment."

2. Compare total concentrations from indoors, outdoors, complaint, and non-complaint areas. In general, indoor concentrations should be lower than that of outdoors. However, this may not be always consistent. Residential

buildings, warehouses, schools and buildings with many entrances and openable windows, and buildings with HVAC systems with no filtration may have airborne fungal levels higher than or as high as that of outdoors. Results of non-complaint areas should consistently be lower than that of complaint areas.

3. Compare fungal types and species, indoors v. outdoors and complaint v. non-complaint areas. Fungal types and species from indoors and outdoors and complaint and non-complaint areas should be generally similar. However, in a large building, such as a convention center or a 30-story office building, indoor fungal types and species may not always reflect what are outdoors because of air dilution due to large air spaces in these buildings. In an airtight and mechanically ventilated building, indoor fungal types and species may be a collection of outdoor fungi over several days.

4. Compare the data set of complaint-area samples to determine what are the fungi consistently detected. Evaluate the entire data set of complaint area samples to determine whether the complaint area has consistent presence of certain fungi or not. For example, ten complaint area samples are collected and all samples have *Aspergillus versicolor* at low levels. This suggests that the fungus may be near or at the location.

5. Look for marker or signature fungi. Some fungi, if detected indoors, are very likely associated with water damage. They are: most *Aspergillus* and *Penicillium* species, *Acremonium* spp., *Sporobolomyces* spp., *Stachybotrys chartarum*, *Memnoniella echinata*, *Tritirachium oryzae, Ulocladium botrytis, U. chartarum, Chromelosporium spp.,* and *Chaetomium spp*. Keep in mind that these fungi may also come from outdoors. *Aspergillus fumigatus* and *A. niger* are likely to come from outdoors. *Penicillium oxalicum, P. thomii,* and a few other penicillia are common outdoors. *Cladosporium* species are common outdoors, however, they grow well indoors in fiberglass insulation or on surfaces in high relative humidity conditions with occasional condensation.

   The consistent detection of *Eurotium* species and *Wallemia sebi* is usually indicative of persistent high relative humidity or carpets on concrete slab.

6. Consider seasonal effects of airborne fungi. Indoor fungal growth may become dormant during winter heating season unless there is persistent leaks or water sources to sustain the growth. Therefore, low airborne fungal levels in winter do not suggest a "clean or healthy" environment.

7. If *Stachybotrys chartarum* (synonym *S. atra*) is detected and the condition suggest growth of the fungus, consult the "Guidelines on Assessment and Remediation of *Stachybotrys atra* in Indoor Environments" published by the New York City Department of Health. This document is still available from EMLab P&K. The guidelines are being updated and the revised one should be available in 2000.

8. Identify problem fungi, such as *Aspergillus fumigatus, A. flavus, A. niger*, or *Fusarium moniliforme* (syn. *F. sporotrichoides*). These fungi are common indoors and outdoors, and can cause opportunistic infections in immune-deficient people. Their presence is a major problem in hospitals and health care facilities.

9. Relate and correlate complaints, field observations, and laboratory results to determine fungal contamination and growth occurs in the building or complaint area or not. Remember moisture and water are the critical factor in indoor fungal growth. There is fungal growth, there must be moisture or water problem nearby.

10. Understand the ecology and background of the fungi identified. Some fungi grow at high water activity conditions. Species of *Acremonium, Chaetomium, Chromelosporium*, *Fusarium, Rhodotorula, Sporobolomyces,* and *Trichoderma*, and *Stachybotrys chartarum* require high water activity. Their detection suggests wet conditions. Another group of fungi are xerophilic and grow at low water activity. Some common xerophilic fungi found indoors are species of *Eurotium* and *Wallemia sebi*. Their detection and growth suggest persistent high humidity conditions but not wet. There is the third group in between. They may be called xerotolerant fungi. Included in this group are *Aspergillus sydowii, A. versicolor*, and *Penicillium* species. They are common on water-damaged materials.

11. Some fungi, such as species of *Acremonium, Fusarium, Sporobolomyces, Trichoderma,* and *Stachybotrys chartarum,* produce slimy spores. Slimy spores are mostly dispersed by running water, insects, and small animals. Airborne transmission of the spores is not the primary route. If these fungi, even at only one colony count, are identified in indoor air samples, it should be considered significant. Look for possible indoor source.

12. Basidiomycetes, when identified in air samples, are likely to come from outdoors. However, basidiomycetes may grow on indoor wood products and causing wood decay if there is a prolonged water and moisture-related problem. Look into the possibility if the samples are collected from an old, wood-structured building with a known water damage history.

Protocol for Collecting Airborne Fungal Bioaerosols (culturable and total spore count)

1. If you use sampling equipment, such as Andersen, SAS, or Biotest samplers, follow the manufacturer's suggestions. These samplers usually collect for one to eight minutes at a set flow rate. However, under unusual conditions (such as a clean room or a hospital isolation area), longer sampling time may be used.

2. Disinfect the sampler with 70% rubbing alcohol, and allow alcohol to dry before loading your agar collection plate.

3. Load the agar collection plate and sample.

4. Remove and cover the agar plate. Label and seal the agar plate with 3/4" masking tape. Parafilm seal is fine. However, parafilm is likely to tape moisture and cause condensation in the plate. Parafilm tends to break when temperature higher than 80°F

5. Place agar plates in paper bags, and complete your chain-of-custody sheet. Indicate special instructions (such as incubation at 35°C) on the C-O-C sheet when necessary.

6. If your samples require special incubation (such as 35 or 55°C), ship them on ice in a cooler.

7. If you sample with MCE filter cassettes for culturable fungi, we suggest that you use 37mm, three-piece cassettes. You may sample, with open face, for 100 to 200 liters (L) total air volume. Flow rates may vary between (2)-6-10 liters per minute. Varying flow rate allows you to determine whether you want a short or long sample time. Cap and seal the cassette after sampling.

8. When taking spore trap samples, total air volume between 50-100 L is suggested. Dirty and dust air, such as in a remediation containment area or in a composting facility, lower air volume (50-75 L) is recommended. If you use MCE 25 mm cassettes (37 mm is fine), collect at least 3000 L to reduce the detection limit. Lower air volume is fine if you expect high levels of spores

9. Place your grease-coated slide in a slide box. If the slide box is re-useable, wash it to avoid contamination. Cassette samples should be securely sealed and taped for shipping. Do not ship your samples in an envelope, whether padded or not. Ship your samples in a box. Have them delivered to our laboratory as soon as possible if you would like to have a quick turnaround for the results.