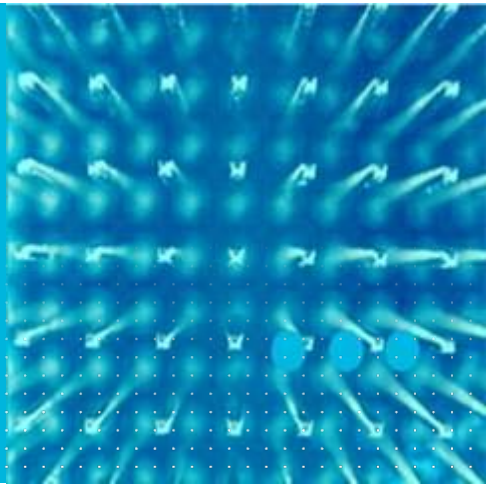
Basic Industrial Hygiene Sampling
Overview of Indoor Air Quality



Bryan K. Stolte, Ph.D., C.I.H.
Alcatel-Lucent – Corporate EHS Office

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Agenda

1. Introduction – Requirements for Mold Growth
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Requirements for Mold Growth

Source (spores, hyphal fragments, etc.) mold spores are found everywhere, but generally originate outdoors

Water Activity (condensation, high humidity, leaks from pipes) Indoor mold growth is always associated with excess moisture

Nutrients – soil, dirt, cellulose in paper, carpet backing, pipe wrap, etc.

Suitable Temperature (generally around 25 C for indoor molds)

Health Effects Associated with Indoor Mold

1. Who is at Risk of Mold Exposure?

Virtually anyone exposed to airborne mold may react to toxic and/or allergenic properties of mold spores;

Individuals with Asthma or other allergy problems

People working in heavily contaminated environments: farmers, renovation and demolition workers, and abatement workers

Infants and susceptible individuals (i.e., persons with compromised immune systems and the elderly)

Health Effects Associated with Indoor Mold

2. How Do Molds Affect Our Health?

   Infection – *Aspergillus* species (Aspergillosis)

   Toxicity – By ingestion (best known are aflatoxins produced by various species of *Aspergillus* and *Penicillium);* By inhalation (toxic black mold from *Stachybotrys*)

   Immune Response - Allergic sensitivity, Hypersensitivity pneumonitis (HP)

Common Outdoor Molds

Saprophytes (decomposers of organic material)

Plant pathogens (molds causing plant diseases)

Daytime dominated by “Dry Air Spora” (*Cladosporium*, *Alternaria*, *Curvalaria*, and *Pithomyces*

Common Indoor Molds

Indoor spores generally reflect outdoor species unless there is a source of contamination

Many different types of fungi have been reported indoors (about 150 different species) but only a few are frequently found indoors

Penicillium and *Aspergillus* often exist at higher concentrations indoors compared to outdoors

Common Indoor Molds

Most common molds in water damaged buildings and their frequency are:

Penicillium (68%);

Aspergillus (56%);

Chaetomium (22%);

Stachybotrys (19%);

Cladosporium (15%);

Mucor (14%);

Alternaria (8%).

Common Indoor Molds

Alternaria found worldwide in a wide range of plants/soils.

Fairly common indoors. Common in floor, carpet and mattress dust, occasionally found on gypsum board and wallpaper

Alternaria alternata is regarded as the main cause of allergy and asthma in children aged 6-11 years.

Aspergillus very important species concerning indoor air quality as various species are allergenic in nature

Found worldwide in in soil and plant matter, and common in floor, carpet, and mattress dust. Also gypsum board and damp walls

Common Indoor Molds

Cladosporium species found more often in the outdoor air than indoors.

Often found in various materials by laboratories engaged in environmental mycology

Allergenic to humans (42% positive reaction to skin patch tests in children up to age four)…with increasing age positive reactions showed decreasing tendencies

Common Indoor Molds

Penicillium species found in a wide range of habitats.

Extremely common on damp building materials, walls and wallpaper; floor, carpet mattress and upholstered furniture dust.

Produces a number of toxins of moderate toxicity.

Allergenic to humans.

Common Indoor Molds

Stachybotrys chartarum grows well on materials rich in cellulose, including wet ceiling tiles and cardboard.

Extremely common on damp building materials, walls and wallpaper; floor, carpet mattress and upholstered furniture dust.

Produces a number of toxins of moderate toxicity.

Allergenic to humans.

It is now popularly believed and promoted that *Stachybotrys* is a "toxic mold," though there is no scientific consensus that this is true

Investigating and Recognizing Mold Growth

1. Determine if conditions favorable for mold growth are present or existed in the past (water leaks, high humidity, previous flooding, and poor ventilation).

2. Look for visible signs of mold growth (mycelia or mold growth readily visible as blue, green, bluish, yellowish, grey or black shades)

- Water stains on ceiling tiles following a roof leak
- Discoloration or spotting on surfaces
- Damage to surfaces

Investigating and Recognizing Mold Growth

3. Odors or descriptions of “mildew,” “musty,” or “cellar-like” odors in a space are usually good indicators of a persistent moisture problem and associated mold growth

4. Actively growing mold has a hairy appearance and may be soft, slimy, or damp to the touch

5. Inactive or dead mold is dry, appears powdery and rubs off the surface easily, subsequently becoming airborne

Developing an Effective Sampling Strategy

1. Before sampling evaluate problem background
   - What is the nature of the problem (visible mold, odors, mold related symptoms)
   - Where and when did the problem occur, including building conditions/history
   - What symptoms, if any, were reported, and by whom (timing, location within building, frequency, severity, and duration)

Developing an Effective Sampling Strategy

2. Perform a careful walkthrough of the suspect areas
   - Check for mold growth, and signs of excessive moisture or water damage
   - Use a moisture meter to determine locations of elevated moisture within materials or at surfaces in problem areas.
   - Use the sense of smell to locate sources of odor
   - Inspect areas behind cabinets, above ceiling tiles, under carpet and pad, behind wall coverings, within duct work, within wall cavities; and inside mechanical systems (but limit destructive testing)

Developing an Effective Sampling Strategy

3. Is sampling necessary?

Possible objectives/reasons for sampling

- To confirm mold growth
- To determine if spores from visible growth sources have become airborne
- To evaluate whether the HVAC system is a possible source of mold
- To evaluate whether a specific organism is present in the indoor environment (i.e., cause of observed allergic reactions among building occupants)

Developing an Effective Sampling Strategy

4. Clearly identify what are the specific objectives for sampling

- To determine if there is an indoor mold source (but, remember mold spores are everywhere, so the challenge is to determine whether there is a difference between the types and concentrations of mold spores indoors compared to the outdoors, indicating an indoor source)
- Determine the type of data required to meet the objective (Qualitative, Quantitative, ID of genus/species, turnaround time required, cost of sample analyses)

Developing an Effective Sampling Strategy

5. An effective sampling strategy should include:
   - A description of the types of samples to be taken, how many, where and when to take them
   - A description of how the data are to be interpreted and used to meet the objectives
   - A description of how the sampling protocol will achieve the objectives

Various Air Sample/Surface Sample Techniques

Air Samples

- Viable counts (also referred to as culturable)
- Total fungal spore counts (non-viable)

Surface (source) Samples

- Tape-Lift samples
- Swab samples
- Bulk samples
- Contact plates
- Contact slides

Various Air Sample/Surface Sample Techniques

Air Samples

Describe environmental and other conditions during and prior to sampling. Such as:

- Outdoor weather conditions
- Temperature and RH in the sampled space
- Occupation of space, type of activity
- Windows open or closed
- HVAC isolated or not

Various Air Sample/Surface Sample Techniques

Air Samples

Close windows and doors before sampling

Types of samples:

- Quiet samples: with no activities
- Normal samples: with normal activities
- Aggressive samples: deliberately disturbing source

Take Outdoor reference samples for comparison (may not be necessary in winter due to low average counts)

Various Air Sample/Surface Sample Techniques

Advantages of Air Samples

Useful in ability to quickly sample an area of concern (usually sampling is extremely short in duration)

May detect hidden mold in HVAC duct or cavities (none visible)

Useful in hazard assessment

Useful tool for pre and post-remediation conditions

Disadvantages of Air Samples

All samples are of short duration. A short sampling time only gives a snapshot of what the airborne mold concentration is in the building.

It is not uncommon for concentrations of airborne mold to vary by a factor of 2-3 or more over the course of a single day when sampling is conducted in the same location

Various Air Sample/Surface Sample Techniques

Volumetric Air Samplers (Viable)

Reuter Centrifugal Sampler (RCS)

Hand-held portable sampler

Powered by battery

Selection of volume and air sampling time

Uses agar-filled wells in flexible plastic strips

Flow rate of 40 liters/min

Sample usually taken for 4 minutes

Various Air Sample/Surface Sample Techniques

Volumetric Air Samplers (Viable)

Andersen N6 Single Stage Sampler

Requires separate vacuum pump and electrical sources

Flow Rate: 28.3 liters/min, sampling time 2-5 minutes

Collection (via impaction) onto agar-filled 100 mm Petri dish

More efficient than RCS Sampler

Various Air Sample/Surface Sample Techniques

Advantages of Viable Air Samples

Allows identification of mold to species level. Species are identified to :

Determine presence or absence of indicator species

Easily determine if the composition of indoor airborne mold is different from that of outdoors

Disadvantages of Viable Air Samples

Only the concentration of mold spores/fragments that are alive (viable) and can grow (culturable) on the culture medium can be detected (as such, they represent a fraction of the total airborne mold count)

Cultures take 7-10 days to be grown and identified (not realistic for needing “overnight results”)

Various Air Sample/Surface Sample Techniques

Air Samples :Total Spore Counts (Non-Viable)

Slit Samplers

- Air-O-Cell, Bio-O-Cell
- Versa Trap
- Allergenco
- Burkhard

Various Air Sample/Surface Sample Techniques

Air Samples :Total Spore Counts (Non-Viable)

Advantages of Using Slit Samplers

- Collects all airborne spores, pollen, and other particulate on a sticky surface
- Collected material analyzed by direct microscopy
- Mold spores identified to genus level
- Results can be obtained the same day
- Samples usually taken from 1-10 minutes, depending on sampler and environment being sampled (typically indoors a 5 minute sample at 15 liters/min)

Various Air Sample/Surface Sample Techniques

Air Samples :Total Spore Counts (Non-Viable)

Disadvantages of Using Slit Samplers

- Many spores are morphologically similar and cannot be identified to genus level (*Penicillium* and *Aspergillus*)
- Many spores are small and colorless and can be extremely difficult to see, thus concentrations may be underestimated
- The trapping efficiency of volumetric samplers decreases once the spore diameter falls below 5 microns, thus once again possibly underestimating actual spore counts

Various Air Sample/Surface Sample Techniques

Surface (source) Samples

- Tape-Lift samples
- Swab samples
- Bulk samples
- Contact plates
- Contact slides

Various Air Sample/Surface Sample Techniques

Surface (source) Samples

- Tape-Lift samples – Clear tape used on flat, dry surfaces with visible or suspected mold growth. Analyzed by direct microscopy. Useful for mold confirmation and ID
- Swab samples – Uses sterile cotton swabs on flat or irregular surfaces (dry or wet). Analyzed by direct microscopy or culturing. Can be used for quantification.
- Bulk samples – Pieces of building material (drywall, carpet, insulation, wallpaper). Analyzed by direct microscopy or culturing.
- Contact plates - Filled with agar media, used on flat surfaces to test for confirmation of viable fungi
- Contact slides – Microscope slide design, similar to plates, except no agar media

Guidelines for Interpreting Lab Results

Data for Sample Analyses

Tape/Bulk Sample – Qualitative (a listing in rank order of fungal genera present on the sample)

Viable Samples (RCS, Andersen Impactors, Swab, Contact plates/slides) – Qualitative and Quantitative: A listing of fungal Genera/Species and their counts reported as colony forming unit (CFU) per unit (volume, area, weight)

Spore Traps (Air-O-Cell, Allergenco, etc.) – Qualitative and Quantitative : A listing of fungal Genera/species identified and their counts reported as total counts per cubic meter of air

Guidelines for Interpreting Lab Results

What can we say about certain types of molds (air and surface samples) ?

If correctly identified we can draw the following conclusions:

1. Whether the molds are toxigenic and/or allergenic (i.e., *Aspergillus sp., Penicillium sp., Stachybotrys sp.*)

2. Conditions existing within a building (i.e., moisture damage and various molds that thrive in that type of environment), with molds such as *Alternaria, Chaetomium sp, Fusarium sp*, etc. that warrant remediation/renovation

Guidelines for Interpreting Lab Results

What can we say about Total Spore Counts and Colony Forming Units ?

1. Counts can help determine if there was hidden mold growth in the building

2. The basic analyses of spores and CFU counts involves calculating the Indoor/Outdoor ratios (the I/O ratio should be ≤ 1.0). Agreement ratio of 1 ? Of 0 ? of 0.8 ?

3. Compare the level of contamination:

- Over time in the same building
- Between rooms in the same building
- Between different buildings in the same location

Guidelines for Interpreting Lab Results

There are no internationally accepted guidelines

1. The collection of the right samples, at the right time, and in the right places is key to being able to properly interpret laboratory data from an indoor environment
2. Guidelines not meant to determine exposure, but to determine if a mold problem exists
3. Professional judgment is very important, as there is no exposure limit or standard to reference
4. Understand that data from airborne mold sampling is truly a snapshot in time, and usually non-representative of long-term conditions
5. Statistical methods available to analyze mold air sampling data are of very limited use due to the lack of numeric standards and the imprecision of the data set

Reference Materials

1. Canadian Guidelines
2. AIHA “Green Book” (2008)
3. University of Minnesota
4. Guidelines on Assessment and Remediation of Fungi in Indoor Environments (2008), NYC Dept. of Health
5. Mold Remediation in Schools and Commercial Buildings (2001) by the US EPA