Initial Information – Copper Hill Elementary School
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Discussion of Sampling Interpretation and Procedures

Wei Tang, Ph.D. completed analysis of our samples at Q Lab in Metuchen, NJ. They are successful participants in the Environmental Microbiology Proficiency Analytical Testing (EMPAT) program administered by the American Industrial Hygiene Association (AIHA). Copies of the laboratory reports and sample logs showing sample locations and analyses performed are appended at the end of this report.

1.1 Interpretation of Sampling Results

When reviewing the results of any air or surface tests for fungal contamination, it is important to note that there are no specific standards or accepted exposure levels to differentiate between "safe" or "unsafe" exposure levels and no Permissible Exposure Levels (PEL's) or Threshold Limit Values (TLV's) exist for exposure to bioaerosols, There are also no "safe" or "unsafe" levels of surface contamination or "safe" or "unsafe" levels of occupant exposure. There are too many variables to even begin to develop appropriate levels of exposure. Additionally, no one sampling method is able to capture all the biological and microbiological agents of interest. All sampling methods have inherent limitations that impact the usefulness, accuracy, and precision of the results.

Given these limitations, we advise our clients to keep their buildings clean and, most importantly, dry. All living organisms need water to survive and fungi and bacteria are no exception. Keeping your building dry minimizes the ability of these unwanted biological organisms to reproduce and produce bioeffluents or particles that may impact occupant health.

Of more importance than quantified levels of fungi in the interpretation of sampling results is the assessment of the following:

1. What specific organisms are present in the areas of concern
2. The biodiversity present in the samples
3. The rank order of fungal genera and species in the sample
4. How the results compare with the reference sampling data
5. The possible presence of uncontrolled moisture sources
6. The dominant presence of species of fungi known to be present in damp or water damaged buildings (WDB's).

There are no standards for dose-response, there are no dose-response curves available regarding fungal exposure and individual responses vary greatly. Also, there are more than 100,000 species of fungi to consider and most exposures are mixtures of many fungal genera and species. In other words, there is no established threshold of exposure to cause pathological responses in humans. Other variables related to individual exposure to fungi and other microbiological agents involved in determining health effects are referred to as host factors that an individual "brings to the table."

This is true for many chemical, physical, or biological environmental stressors and not simply toxigenic or allergenic fungi. In general, fungi are considered allergens and can aggravate asthma and other hypersensitivity conditions/

In the absence of applicable exposure standards, it is generally accepted that, in mechanically ventilated facilities, the indoor levels of fungi in the air (fungal bioaerosols) should be less than outdoor levels. Also, the biodiversity (the various fungal genera and species present in a given sample) of fungi identified indoors and outdoors should be similar. In naturally ventilated facilities, the observed indoor and outdoor bioaerosol levels should be similar in quantity and types of microorganisms. Since there should be no significant microbial sources present in an occupied building that are not associated with the presence of humans, the types of organisms found in the indoor environment should mirror those in the outdoor environment. Therefore, the indoor microbial spectrum identified should be reflective of the outdoor spectra.

Results which fall outside these guidelines with respect to comparison with reference samples, the species present in samples, the overall counts, and/or the significant presence of so-called "indicator" organisms or "toxigenic" species of fungi or bacteria seen in WDB's are indicative of unusual fungal conditions or populations, and are considered unacceptable from two perspectives. The first is the from an occupant health perspective due to the potential for adverse health effects in occupants exposed to these unusual fungal populations. The second perspective involves building performance and the potential for deterioration of building materials amplifying fungal growth. Obviously, indoor amplification or growth of fungi is considered unacceptable and should be immediately remediated. Clean and dry buildings are healthy while damp and dirty buildings can impair human health and will not last long.

"Unusual fungal conditions or populations" or "unusual fungal activity" or "atypical biodiversity" are terms used in this report to indicate that the fungal activity identified in sample results is not consistent with the types/species of fungi seen in indoor environments that have not been impacted by uncontrolled water or moisture. Fungal species in indoor locations that have not been impacted by water (resulting in the presence of wet or damp building materials), and the outdoor fungal species represent "typical" or "usual" conditions when the observed dominant indoor fungal species is similar in types and numbers to the observed outdoor fungal species.

Different species of fungi have different growth requirements or ecological niches, and are predictable with respect to the types of environments where they will grow. Indoor ecologies are affected by the amount of water available for growth, the type of substrates on which the fungi may grow, and ambient temperatures. Certain species of fungi are referred to as "moisture loving species" or "indicator species" that tend to grow on damp building materials impacted by moisture. Their significant indoor presence indicates that building materials are water damaged, since, if these building materials were not wet, these fungi would not be present in the indoor environment at significant or dominant levels.

In order for these "indicator" species to grow, significant amounts of water must be present in or on the surfaces of building materials where growth is noted. These same species should not be seen at excessive levels in an environment that has been remediated since this indicates that

moisture and/or wet building materials are still present or cleaning by the remedial contractor was not effective.

Species that are considered to be “toxigenic species” associated with damp indoor environments include some *Penicillium* species, many *Aspergillus* species including *A. versicolor*, *A. niger* and *A. fumigatus, Eurotium, Trichoderma, Memnoniella, Wallemia, Chaetomium, Scopulariopsis, Paecilomyces* and *Stachybotrys chartarum* among others. *Aspergillus/Penicillium-like* fungal genera seen at dominant or sub-dominant levels or at higher percentages of the total than in outdoor or indoor reference samples also indicate the presence of unusual or atypical fungal populations.in samples for countable fungi.

Non-toxigenic moisture indicator species include *Rhodotorula, Acremonium, Ulocladium, Aureobasidium* and yeasts.

When the significant presence of these “indicator species” occurs in the indoor environment and samples, it requires that the indoor ecology be such that it will support damp conditions and result in the presence of these “unusual fungal populations.”

Our assessment protocols include looking at all the sampling results from the different methods used as a group rather than at each distinct sample set as individual results. This is done because all the sampling methods we use have advantages and disadvantages, and therefore, it makes sense to look at the results in their entirety as opposed to discreet sample sets.

Countable methods using spore traps utilize microscopic and staining techniques that allow for quick turnaround times at the laboratory. This methodology, however, cannot differentiate between certain species of fungi, e.g., *Aspergillus* and *Penicillium* spores that look the same under the microscope, and thus, the results are reported as *“Aspergillus/Penicillium*-like” (*Asp/Pen*-like) spores. This is sometimes problematic since *Aspergillus* species are not seen at significant levels outdoors or in clean, dry indoor environments while some *Penicillium* species are much more cosmopolitan, and therefore, present in both outdoor and clean, dry indoor environments. Typically, the elevated presence of *Asp/Pen*-like spores in the indoor environment when compared to outdoor reference samples indicates impacts from uncontrolled moisture and likely fungi growth in some building materials or furnishings, particularly when no *Asp/Pen*-like spores are seen in reference samples collected outdoors.

Culturable methods involve inoculating samples onto nutrient agar and then incubating the agar plates prior to plate counting. They have the advantage of being able to identify the genera and species of fungi seen in samples. *Aspergillus* species seen on a countable sample can be identified to species level as “*Aspergillus versicolor”* or “*Aspergillus niger”* for example, by culturable methods. This method is able to differentiate between *Penicillium* and *Aspergillus* making interpretation of the results easier and species-specific. The disadvantage of culturable sampling is that only living or viable materials will be detected. Additionally, results take about two weeks since the samples must be incubated for a period of time before they can be analyzed.

Finally, it must be recognized that any air or surface sampling results provide only a "snapshot" of the fungal or microbial activity at the specific time and locale of the test. Fungi commonly found

in the indoor environment release spores under different conditions, time of day, season, etc. Therefore, sampling results should be viewed as one investigative tool of several from which favorable or unfavorable trends may be apparent.

As noted previously, *Aspergillus* and *Penicillium* are two completely difference fungal genera just like oak trees and sycamore trees are two completely different tree genera and species. The reason they are reported as "*Aspergillus/Penicillium*-like" spores is that both *Aspergillus* and *Penicillium* spores are identical under the microscope and thus cannot be differentiated microscopically. If oak and sycamore leaves looked identical, when a tree expert looked and the leaves from either tree, the best he could say was that the leaf was an "oak/sycamore-like" leaf even though it could have come from two different types of trees.

Asp/Pen-like spores are considered indicators of wet or damp conditions when seen at significant levels indoors. At the same time, some *Penicillium* species and most *Aspergillus* species are considered potentially toxigenic or able to produce toxins under certain environmental conditions. The mechanism for toxin production is not well understood and the mere presence of toxigenic species of fungi does not necessarily indicate the presence of toxins associated with these species. Thus, Asp/Pen-like spores are considered indicators of dampness and also may be toxigenic.

We collected air samples using Allergenco-D cassettes and a high volume air sampling pump operating at a flow rate of 15 liters per minute for 5 minutes yielding a collection volume of 75 liters. Composite samples are taken in various locations in a given room rather than one stationary location in an effort to approximate the actual airborne biodiversity in the room.
Air samples for countable fungal material identify both viable and non-viable fungal spores resulting in a "spore count" for all spores in the sample regardless of viability of the individual spores. Viable spores will reproduce if given sufficient moisture and nutrients whereas the non-viable spores will not. Please note that, with the exception of direct infection of individuals with significant immune system performance or other serious health conditions, the health effects associated with exposure to fungi are not tied to the viability of fungal spores.

As air enters the cassette, spores and other biogenic materials become impacted on a glass slide coated with adhesive material and airflow continues out the exit orifice. The air sample cassettes are forwarded to the laboratory where the glass slide containing the captured materials is removed and analyzed using direct microscopic methods and staining techniques.

Results are presented as fungal structures per cubic meter of air sampled (str/M^3). These results are considered presumptive identification of the noted species since the analysis is performed optically and does not assess colonies of fungi, reproductive structures, or associated staining and other morphological assessments.

In Copper Hill School we noted the presence of significant levels of *Aspergillus/Penicillium*-like spores, clusters and chains resulting in our interpretation of these results as indicating atypical or unusual fungal populations in the areas we assessed. Chains and clusters of spores, comprising 3-9 and greater than 10 spores respectively, tend to indicate indoor amplification. Clusters and chains are groups of spores rather than individual spores. Single spores would have an easier time entering

the building from outdoors due to particle aerodynamics and gravity. Once again, the presence of clusters and chains in significant quantities suggests indoor growth of fungi.

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