

Fungi and the indoor environment.

Fungi can lead to health problems ranging from hay fever to cancer. It is important to know when and how to sample for these organisms, as well as how to interpret the results.

Fungi are eukaryotic organisms and include yeasts, molds and mildews, as well as large mushrooms and puffballs. Fungi can exist as single cells, such as yeast, but are more commonly found as thread-like hyphae. Hyphae usually branch out extensively, with the collective mass of interwoven filaments being referred to as mycelium. Fungi normally reproduce by forming spores which may result from either sexual or asexual processes. The spores can range in size from less than 2 microns ([micro]meter) to more than 100 [micro]meter. Most fungal spores disperse through the air, although some are specialized for other forms of dispersion, such as via water or insects.

Allergic Reactions to Fungi

The health effects noted from fungi are typically a result of their spores. Repeated heavy exposures to fungal spores may result in two different types of allergic reactions, Type I and Type III. In Type I, or "immediate reaction," repeated exposures to the offending agent(s), over a period ranging from months to years, may eventually result in an overreaction of the immune system. Once the immune system has been triggered, even minute amounts of the specific allergen can elicit an allergic reaction. An estimated 8 percent of the adult allergic patients and 20 to 25 percent of children in the population suffer Type I allergic reactions to fungi, which implies some type of genetic predisposition. Patients with this disposition produce immunoglobulin E (IgE) antibodies in larger amounts than "normal" people. The overstimulated production of these antibodies then creates a hyperreaction which induces allergic reactions, such as rhinitis, hay fever or asthma, within a matter of minutes after exposure.

Type III, or "Arhus" reactions, are mediated by IgG and IgM antibodies, not IgE. The resultant immune complexes will initiate different inflammatory responses, which may result in asthma. This condition is typically associated with occupational diseases, including farmer's lung and wood trimmer's disease, and is frequently referred to as "hypersensitivity pneumonitis" or "extrinsic allergic alveolitis." In this case, the symptoms occur approximately 4 to 8 hours after exposure, and include general malaise, flu-like symptoms, fever and muscle and joint pains. Physical findings can often include signs of severe oxygen deficit and abnormal crackling sounds in the lungs, called "rales." Long-term exposure can lead to fibrosis of the lung tissue.

Fungi and Mycotoxins

Several mold species, including *Aspergillus*, *Fusarium*, *Penicillium* and *Stachybotrys*, can produce a wide variety of nonvolatile chemicals, commonly referred to as "mycotoxins." Even in low concentrations, these chemicals can cause adverse health effects, including skin irritation, pathogenic disease, cancer and immune disorders. Unlike allergens, mycotoxins elicit toxic responses in virtually all individuals who come in contact with them. *Aspergillus flavus*, a common indoor fungus, produces aflatoxins, notoriously potent animal carcinogens. However, *A. flavus* is capable of producing other toxins, as well. *Penicillium*, while unable to produce aflatoxin, may produce 100 or more different classes of mycotoxins.

Stachybotrys, one of the more famous mycotoxin producers, has been

implicated in a number of health-related cases, including the hospitalization of 10 infants in Cleveland in the fall of 1994 for pulmonary hemorrhaging. Various species of *Stachybotrys*, as well as *Fusarium*, can produce macrocyclic trichothecenes, which have potent adverse effects on the immune system, as well as with protein synthesis. *Stachybotrys chartarum* produces at least five different trichothecenes, which are both dermatotoxic and cytotoxic. In one study, an extract of the various *Stachybotrys*-produced trichothecenes was given to rats. It resulted in their deaths within 24 hours. Reported health symptoms of exposure to *S. chartarum* include signs of cold and flu, sore throat, diarrhea, headaches, chronic fatigue and dermatitis.

The toxic effects of ingesting moldy foodstuffs can include acute and chronic damage to the liver, kidneys, gastrointestinal tract, heart, central nervous system and the immune system. There have been numerous documented cases of fatalities due to ingestion of fungal contaminated food.

Mycotoxins also can enter the body via inhalation or contact with the skin. Inhalation of mycotoxins is a much more potent route of exposure, compared with ingestion. In most cases, the dose required can be an order of magnitude less when inhaled. Adverse health effects have been noted in individuals who came in contact with *Stachybotrys*, suggesting that the toxins were absorbed through the skin. In one study, a majority of the workers exposed to airborne dust from straw on the floor of a room developed acute symptoms indicative of mycotoxicoses.

Fungi also produce a wide range of volatile organic compounds (VOCs), consisting mainly of alcohols, ketones, hydrocarbons and aromatics, many of which have distinct odors. These VOCs, which are sometimes referred to as microbial VOCs or MVOCs, are typically what cause the characteristic musty or dank smell which people associate with mold growth. The most common compounds include 3-methyl-1-butanol, 3-octanol, 3-octanone and 1-octen-3-ol. Odor thresholds for some MVOCs are very low, as low as 1 part per trillion (1,5-octadien-3-ol). Studies are currently being conducted to try to determine the intensity of sensory irritation and potential adverse health effects caused by MVOCs.

Conditions Necessary for Growth

Without sufficient amounts of moisture, a growth substrate and some type of organic nutrient base, microbial amplification simply will not occur. Many fungi grow quite well on any cellulose-rich material, such as wallboard or ceiling tiles, particularly when there is a high level of moisture present. However, fungal growth and amplification can occur virtually anywhere, including carpets and HVAC systems, as long as the three elements are present. Molds have been observed growing on a variety of unusual substrates, including fiberglass piping, photographic paper, synthetic rubber and creosote-treated wood. Most fungi prefer warm temperatures, although some can grow at near-freezing temperatures, while others can grow at temperatures as high as 35 to 40 C.

Moisture can be introduced to the indoor environment through a variety of means. Water intrusion through the building envelope is a common problem in older buildings. Lack of building maintenance can often result in roof leaks and/or water leaking through the outer walls. Numerous cases of poor or misguided construction in the humid South has resulted in vapor barriers being installed on the wrong side of the exterior wall. This, in turn, results in condensation occurring on the interior walls, which are typically constructed of cellulose-rich wallboard. Bathrooms, which by nature are humid environments, frequently are not properly vented, resulting in moisture accumulating on the walls.

Too often, damage caused by water is discounted or treated lightly until microbial growth and its associated odors have become a problem. Microbial amplification can occur within a relatively short period of time after water infiltration. Fungi may start to grow and produce new spores within 24 hours, if sufficient air is also present. These microcolonies are not visible to the naked eye; however, if humid conditions continue, fungal growth on indoor surfaces may become rapidly visible. Once fungal growth and amplification have begun, it is often difficult to eradicate the problem without having to completely abate the infected materials.

Inspecting and Sampling for Fungi

When performing an investigation, the inspector should not arbitrarily take air samples for fungi. Results from air samples can be confounding and may inaccurately represent conditions within the indoor environment. Fungal spore levels often fluctuate widely over the course of a day, and a single air sample reflects only a momentary situation. In addition, the origin of the spores often remains unknown. Certain fungi, such as *Stachybotrys*, have sticky spores and are very rarely airborne. In these cases, air sampling methods will be prone to false negative results and should not be used to rule out contamination.

Surface sampling of building materials often provides more reliable information about contamination, since other sources of fungal spores do not confuse the results. Swab, tape or bulk samples can be collected wherever there is visible growth to determine the actual species present. When taking bulk samples of water-damaged wall board, always take a core sample, since fungi are often found growing on both the front and back sides. A boroscopic examination of the back side can be invaluable when trying to verify the presence or absence of visible growth and to assist in determining sampling locations.

Both viable and nonviable fungal particles are important in relation to health effects. Therefore, when performing air sampling, collect both types of particles. A variety of techniques have been developed for volumetric sampling of fungi in the environment. Viable sampling is usually performed using an Andersen or similar impaction sampler which pulls air across an agar plate at a flow rate of 28.3 l/min. Small particles are deposited upon this plate, which is subsequently incubated and the resultant colonies identified and enumerated. Counts are then converted to the number of colony-forming units (CFU) per cubic meter of air sampled. There are a variety of different types of agar which can be used, depending upon the type of fungi being sampled. No one agar is ideal for all types of fungi, although malt extract agar (MEA) is a good general purpose agar for screening. When sampling specifically for *Stachybotrys*, cornmeal agar (CMA) or Czapek cellulose agar (CCA) are more suitable.

Nonviable particles are typically collected using a spore trap sampler, such as the Burkhard or a slit impaction device, such as the AIR-O-CELL Bioaerosol Cassette. Air is pulled through the sampler and particles deposited on a grease-coated glass slide. The samples are then microscopically examined and the total number of spores per cubic meter determined. Tentative identification of many types of spores can also be made, though typically only the genus (i.e., *Penicillium*) can be ascertained. Sampling can also be accomplished by using membrane filters, which can then be cleared and stained with biological dyes.

When performing any type of air sampling, samples must also be collected outside the building in order to determine background levels of both total spore counts and specific species present. Outdoor samples should

be taken in proximity to the building's fresh air intakes. It is good practice to take at least one sample in a "noncompliant" area of the same building to determine if an unusual exposure situation exists in the subject area in comparison with a symptom-free environment.

Interpretation of Results

There are no "official" standards or guidelines for fungal or bacterial bioaerosols. Some researchers have expressed the opinion that 100-250 CFU/[m.sup.3] are acceptable, provided no opportunistic fungi are identified. The same range is also used by the U.S. Public Health Service, Federal Employee Occupational Service Region III. A range of concentrations proposed by the World Health Organization and Health Canada suggests that microbial concentrations below 50 CFU/[m.sup.3] for a single species (other than common outdoor fungi), 150 CFU/[m.sup.3] for a mixture of species reflective of the outdoor air spores, or 500 CFU/[m.sup.3] during the summer for common outdoor fungi (such as Cladosporium) are acceptable. There are other ranges (called background numbers or guidelines) used by organizations such as the American Conference of Governmental Industrial Hygienists (ACGIH) and OSHA, but for the most part, these numbers are arbitrary.

Since there are no generally accepted guidelines to follow regarding airborne fungi, indoor results must be interpreted with respect to the control samples. In general, mechanically ventilated buildings should have indoor fungal counts that are lower than those found outside. In addition, the species found inside should be similar to those identified outside the building. A situation can be considered unusual when fungal levels in the complaint area are an order of magnitude or greater than those found in the control areas. The presence of any slimy-spored toxigenic fungi, such as *Stachybotrys chartarum* and *Fusarium moniliforme*, should be considered unusual, and may suggest an indoor contamination source. The consistent detection of some fungi, such as *Aspergillus*, *Paecilomyces*, or various species of *Penicillium*, could indicate water damage and subsequent fungal amplification.

Bulk and wipe sample results can give a strong indication as to whether or not a material is contaminated. Contaminated samples will often result in a pure culture or a mixture of no more than two to three types of fungi. A high fungal level which is dominated by one or two fungi is indicative of fungal amplification. Materials which are found to be contaminated should then be either removed or decontaminated with some form of fungicide.

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