The Role of Microscopy in Indoor Air Quality Investigations

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Numerous studies have demonstrated that fungi are capable of colonizing a range of indoor construction and finishing materials as well as a number of air handling system components [1-6]. Many of the species found are known to produce mycotoxins and demonstration of the presence of 'toxic mould' in both the workplace and in dwellings has become a major issue. Mould issues now are the focus of litigation or are currently in contention in a number of states and hundreds of millions of dollars are at stake [7,8].

The indoor environment harbors a variety of microorganisms, including bacteria and fungi. Under normal indoor conditions most of these organisms are present as dormant forms. The fungi persist either as conidia (asexual spores), sexual spores, or hyphal fragments. The presence of water in an environment may lead to the growth and proliferation of fungal species, sometimes resulting in deterioration of the colonized substrates with possible negative effects on IAQ. Fungi growing on various substrates often produce volatile organic compounds that are released into the air. Cryptic colonization of structural materials may produce olfactory evidence of fungal growth without yielding any culture data from air samples. Humans find a number of these substances irritating and may detect them at extremely low concentrations (< 1 part per million). Detection of these offensive substances is often the first indicator of a fungal colonization problem and may lead to an investigation into the source. Methodology of these investigations may be crucial to an accurate assessment of the location and scope of the problem[9].

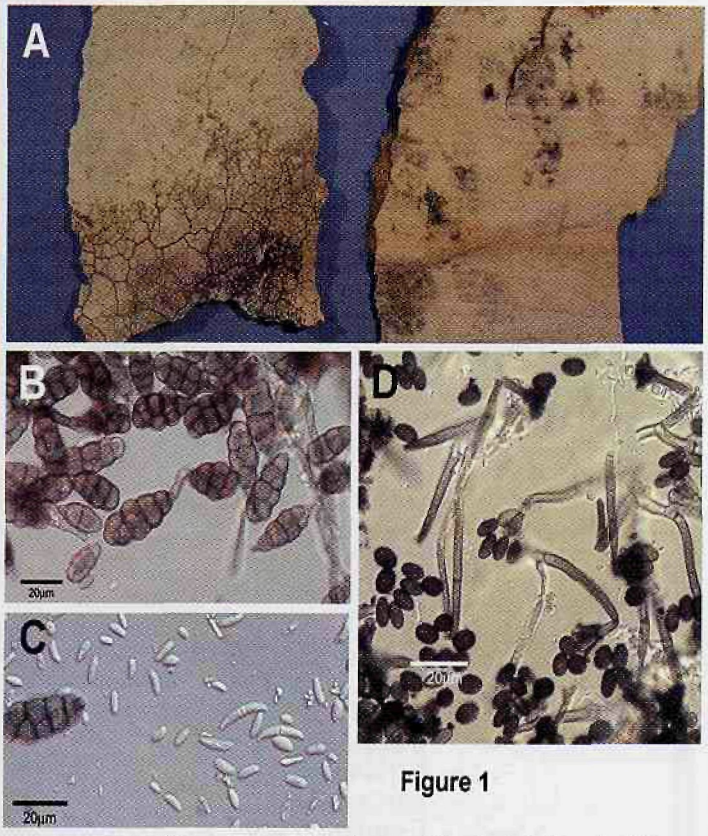

Figure 1

Figure 1. *A. Wall covering materials with apparent fungal colonization collected from 2 different sites. B. Alternaria conidia as seen by light microscopy of a tape mount from the sample on the left. Scale bar = 20 m. C. Fusarium conidia as observed from a tape mount of the sample on the left. Scale bar = 20 m. D. Stachybotrys chartarum taken from the sample in the right. Scale bar = 20 m.*

Emerging health concerns about fungi fall into four major areas. Adventitious pathogens, such as *Aspergillus fumigatus, A. flavus* and *A. niger* are airborne fungi primarily of concern to individuals with suppressed immune systems which make them susceptible to infection. These fungi are commonly found in both the indoor and outdoor environments. Overt fungal pathogens (*Blastomyces, Cryptococcus, Coccidioides* and certain dermatophytes) may occasionally be found indoors but rarely colonize indoor substrates. Potentially toxigenic fungi such as *Stachybotrys chartarum* are currently the focus of much attention. Representatives of this species complex may colonize indoor substrates, particularly cellulose coatings of gypsum wallboard and cellulose fiber ceiling tiles, under certain conditions of moisture and temperature. Review of the literature indicates that there is inadequate evidence linking indoor exposure to fungal toxins with reported symptomology of building inhabitants[10]. By far the most common fungi colonizing the indoor environment are members of the genera *Aspergillus, Cladosporium,* and *Penicillium.* At least some species of all of these genera have proven to be allergenic for certain individuals[11].

Until recently, air sampling techniques (both volumetric sampling and settling plates) have been commonly applied when fungal colonization was suspected. Growth media-based techniques provide some small insight into the nature of a suspected problem, but are inadequate for a complete investigation. Culture data demonstrate the presence of only those fungi amenable to the culture media applied, and no single culture medium is suitable or appropriate for recovering in culture all the types of fungi encountered in the indoor environment.

Many different species of fungi look similar on natural substrates to the unaided eye. Light microscopy of samples collected on clear polypropylene adhesive tape (clear packing tape will do) often gives a quick answer to the question of what groups of organisms are actually growing on a surface, or if the suspect surface features are not biological in origin. Scanning electron microscopy of materials will show surface features in great detail. Figure 1 shows examples of 'black mould' on wall coverings from 2 different sites. Visual inspection might lead to an identification of *Stachybotrys* at both sites. However, the sample on the left shows the presence of *Alternaria* and *Fusarium* (both potential, but rare pathogens) whereas the material on the right shows *Stachybotrys.* Figure 2 shows a colorful stain on a water damaged ceiling tile, which, under microscopic examination is actually a mineral deposit. Microscopic examination of a similar stain from another ceiling tile shown in Figure 3 clearly demonstrates the presence of the fungus *Stachybotrys.*

Direct sampling of surfaces and application of light or scanning electron microscopy technologies has been the method of our choice for identification of actively colonizing fungi. These observations often facilitate evaluations of the need and most appropriate remediation processes.

There appears to be adequate evidence of a correlation between asthma-like symptoms among inhabitants in damp-mouldy environments, but heightened fear of toxicities or perception of incapacity from fungal toxins have fueled the increasing frequency of litigation in cases of fungal colonization. Whether any factual basis may for the role of fungi in such litigations can often be resolved (in part) by direct microscopic examination of materials and surfaces. ■

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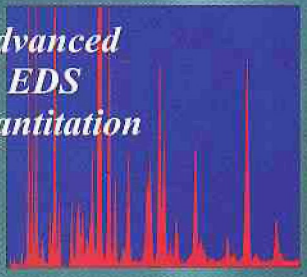

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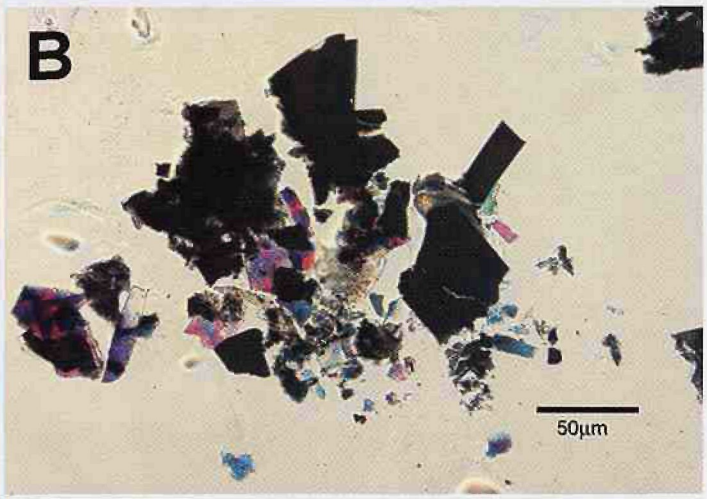

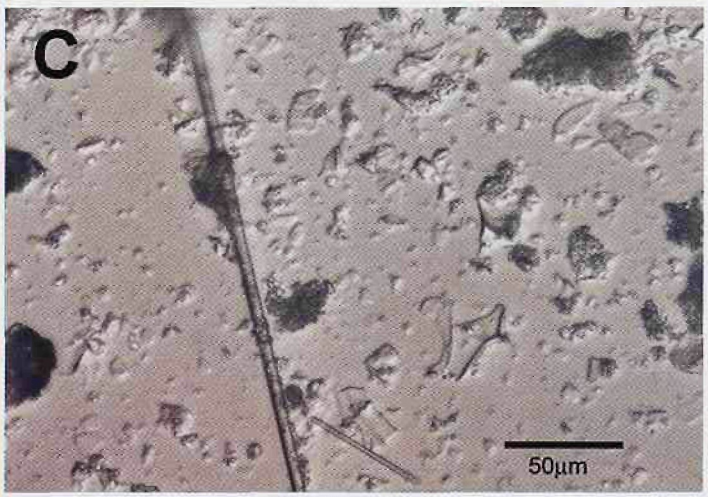

Figure 2. *A. Textured black and red stain as observed on a water damaged cellulose fiber ceiling tile. B. Mineral deposits identified by light microscopy. No fungi were detected. Scale bar = 50 m.*

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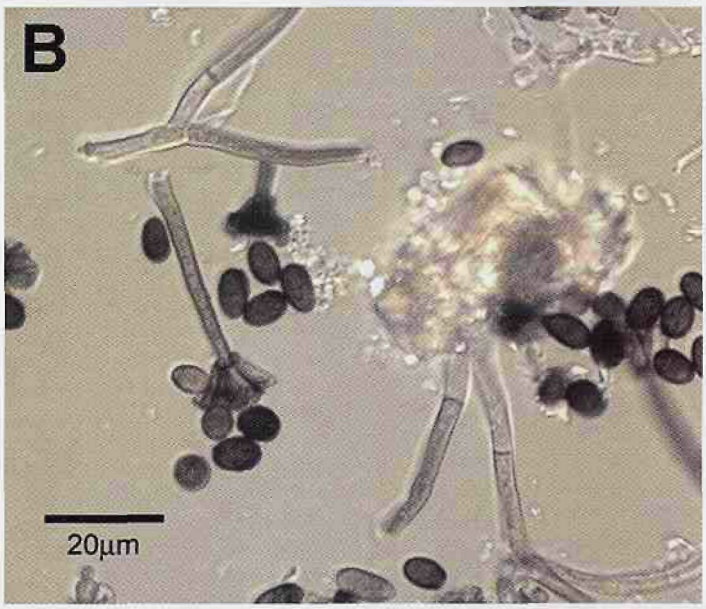

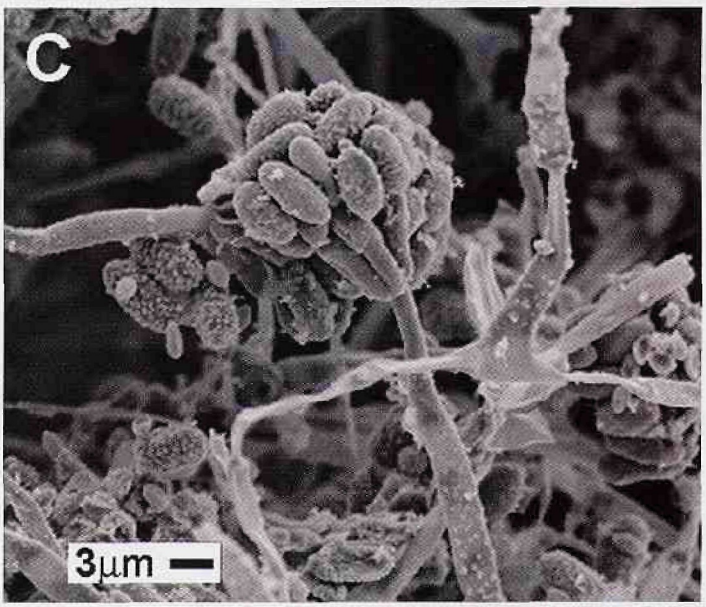

Figure 3. *A. Textured black and red stain as observed on a water damaged cellulose fiber ceiling tile. B. Light micrograph showing conidia and conidiophores of Stachybotrys chartarum. Scale bar = 20 m. C. Scanning electron micrograph of the ceiling tile surface showing mature Stachybotrys conidiophores with conidia. Scale Bar = 3 m.*