# FUNGAL COLONIZATION AND WATER ACTIVITY STUDIES OF HEATING VENTILATING AND AIR CONDITIONING SYSTEM INSULATION MATERIALS FROM A SICK BUILDING

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## ABSTRACT

Heating ventilating and air conditioning (HVAC) duct insulation in a county government facility in south Florida USA was removed because of heavy fungal infestation of the building. Samples of these materials were examined by direct microscopy and enrichment culture. Additional insulation samples were desiccated for three weeks over silica drying gel, weighed, then placed over saturated salt in water solutions at known relative humidities. At relative humidities approaching 95%, some insulation samples on an average gained 6% of their dry weight in water. The water gain at various relative humidities ranged from a low of 2 % to a high of 9.3% of the dry weight. The hygroscopic nature of duct insulation and mechanical insulation in HVAC systems is most likely one of the contributing factors in fungal colonization. The porous nature of the insulation is also a contributing factor because of its propensity to trap organic and inorganic particulates. Understanding which fungi colonize these substrates and under what moisture and nutrient conditions will help design engineers make recommendations on HVAC system operational parameters including relative humidity and moisture control.

## KEYWORDS

fiberglass duct insulation; fungal colonization; water activity; HVAC systems

## INTRODUCTION

HVAC systems have been associated with increased airborne densities of fungi of various genera particularly *Cladosporium* and *Penicillium* (Hirsch *et al.* , 1978, Mishra *et al.* , 1992). Ahearn *et al.* (1991) reported the colonization of painted metal surfaces of HVAC systems by *Cladosporium* spp. in a study in which airborne conidia appeared in low densities. The fungi were present not in the form of dormant conidia originating from the outdoor air, but as reproducing fungal colonies tightly adhered to the metal surfaces. Fiberglass duct insulations used in HVAC systems also have been found susceptible to fungal colonization (Morey *et al.*, 1991, Ahearn *et al.*, 1992, Pasanen *et al.*, 1993, Price *et al., 1994*, Ezeonu *et al.* 1994). The colonization of fiberglass duct liners and duct boards and the species involved are affected by relative humidity (Price *et al., 1994*, Ezeonu *et al., 1994*). The number of fungal species colonizing these materials was fewer than that obtained from initial direct culture of these insulations. Whereas *Acremonium obclavatum* was the predominant colonizer at

humidities above 95%, *Aspergillus versicolor* dominated from about 65% to near 90% relative humidities. No colonization was observed at relative humidities below 50% (Ezeonu *et al., 1994*). Even more important than ambient relative humidity is the fact that the fiberglass materials are hygroscopic in nature and may pick up significant amounts of moisture, particularly when laden with dust (Morey *et al.,* 1992). West and Hansen (1989) noted that fiberglass behaves hydrophobically when at low moisture content. At high moisture content, the water retained on and between the fibers attracts water with sufficient force to replace the water lost to evaporation at room conditions. This means that dry fiberglass duct lining in contact with liquid water (for example, a condensate drip pan) will remain dry above the water line, but any section of lining that becomes wet will remain wet as long as part of it remains in contact with liquid water). Thus under any given set of environmental conditions, the actual water activity within the fiberglass materials may vary significantly from the relative humidity of that environment (Flannigan *et al.,* 1992).

A government building in south Florida was vacated in late 1992 due to massive microbiological contamination that resulted from a combination of water leaks through exterior walls, condensation on cold interior surfaces (wallpaper, ceiling tiles etc.), and consistently elevated (>70%) relative humidity. Fungal contamination of the building components and interior furnishings (including carpets, textiles, gypsum wall board, and vinyl wall coverings) was extensive because of the uncontrolled moisture incursion. Herein, we examine duct liner and duct board samples from the HVAC system of this building for viable fungi as well as the ability of these materials to absorb water under different relative humidity conditions.

## MATERIALS AND METHODS

Samples of fiberglass duct liner and rigid fiberglass duct board were removed from the air handling systems, placed into clean plastic zip lock bags, and transported to our laboratories for evaluation. For comparative studies, new duct liner and duct board was purchased retail. Acetate tape mounts were taken from the surfaces of the insulation samples and examined by stereo-microscopy, direct epi-illumination microscopy and by transmitted light microscopy with Nomarski differential interference contrast optics (Ahearn *et al.*, 1992). When mature conidiophores with conidia were observed with the microscope the colonization was termed microscopic; if fungal growth was observed with the unaided eye, the colonization was termed macroscopic. Sections of the duct liner and rigid duct board facing were imprinted onto Mycological agar (Difco Laboratories, Detroit, Michigan) containing 0.05% chloramphenicol. Selected sections of fiberglass, where colonization was confirmed by direct microscopy, were picked using sterile forceps and placed onto Mycological agar plates. All plates were incubated at 30°C. for up to 14 days. Fungi developing in culture were identified by standard morphological procedures (Ahearn *et al.*, 1991). Sections of contaminated duct liner and duct board as well as new materials purchased retail were cut into 6 to 8 $cm^2$ sections. These samples were desiccated for 1 week over silica drying gel and then weighed using a Mettler® analytical balance. These sections were placed into polycarbonate chambers (Nalgene® 1 liter) in which two relative humidities, 79% and 98%, were established using saturated salt solutions: potassium sulfate, 98% relative humidity, and ammonium sulfate, 79% relative humidity (Foarde *et al.,* 1992). Subsequent weighing of the samples, as well as the humidity readings within the chambers, were conducted at 3 days and 21 days. The samples in these humidity chambers were examined also for fungi.

## RESULTS

All samples of fiberglass insulation from the building were positive for fungi upon direct microscopic examination, enrichment culture and after incubation in the moisture chamber. In contrast to other samples (gypsum wall board etc.) from the site, none of the fiberglass samples appeared to be

colonized to the unaided eye. *Aspergillus versicolor* was the most prominent species in the fiberglass with all types of sample processing. *Cladosporium spp.* were found also in most samples. *Stachybotrys atra* was observed only in the adhesive layer that joined the outer foil facing to the fiberglass matrix of duct board. Ezeonu *et al.* (1994) made similar observations in a separate laboratory on additional samples collected from this same building. While sections of the duct liner facing material that had been cleaned and coated with a paint containing a phosphated quaternary amine complex were free of fungal colonization, enrichment culture of the fiberglass liner matrix demonstrated that viable fungi were still present in the liner.

Moisture chamber studies showed no appreciable difference in moisture content between new and used duct liner and duct board. Used colonized duct liner increased in mass from 3.5% at 79% relative humidity to about 6% at 98% relative humidity. The greatest water uptake (7%) occurred with the unused duct liner at 98% percent relative humidity.

## DISCUSSION

Our studies demonstrate that fiberglass duct board and duct liner from a building with heavy fungal contamination were hygroscopic and were colonized by fungi, particularly *Aspergillus versicolor.* Both new (retail purchased) and colonized samples were hygroscopic. *Stachybotrys atra*, a toxigenic species with relatively high $a_w$ requirements and characteristically associated indoors with moisture damaged cellulose wallboard and ceiling tiles, was found in the glue-fiberglass matrix interface next to the outer foil layer of rigid duct board. This cryptic micro-habitat at the foil vapour barrier could be expected to be periodically saturated with moisture because of condensation ($a_w$ 1.0) or mean condensation ($a_w$ <1.0) conditions. When the foil was pealed back the fungal growth was visible with the unaided eye. In spite of this obvious fungal mass, the recovery of the fungus upon subculturing was difficult. *Aspergillus versicolor*, a species that can grow at relative humidities below 78% was obtained mostly from the matrices of the fiberglass. Growth in these samples was observable only with microscopic examinations or upon enrichment culture which was readily accomplished.

Although we observed no significant differences in the water uptake between used and retail purchased materials, other investigators have noted that dust laden ducts and fiberglass show greater tendencies to absorb moisture (Pasanen *et al.*,1993, Morey and Williams., 1991). Our used samples although dusty were in use for less than three years, had been professionally cleaned and relative to certain of the aforementioned samples in the referenced studies could be considered to be relatively clean. We have shown that in as little as 48 hours the used and unused HVAC fiberglass insulation can pick up significant amounts of moisture. In-situ, these amounts of moisture can provide favorable growth and reproductive conditions for a variety of fungi.

Currently, moldy gypsum board on occupied sides of the exterior walls as well as all ceiling tiles, carpet and porous finishing materials have been removed from the South Florida building. Marked decreases in densities of airborne fungal conidia were noted after interior surfaces were cleaned with vacuum machines fitted with high efficiency particulate arresting (HEPA) filters and after some surfaces were treated with chlorine dioxide. In our water uptake studies, certain samples showed increased colonization in the moisture chambers. Where the facing of the samples had been treated with an antifungal paint (applied in the stages of initial remediation), colonization was not evident on the coated facing surface, however growth from the fiberglass matrix was still observed. It is probable that the disinfection process applied to the building will only be effective if future moisture control and good maintenance procedures are employed. In humid environments it is essential to control and limit the amount of moisture in construction and finishing materials; the use of antimicrobial preservatives may be beneficial in controlling microbial growth when moisture becomes inadvertently non-limiting.

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