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Growth Evaluation of Fungi (*Penicillium* and *Aspergillus spp.*) on Ceiling Tiles

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KEY WORDS

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ABSTRACT

The potential for fungal (*Penicillium* and *Aspergillus spp.*) growth on four different types of ceiling tiles was evaluated in static chambers. It was found that even new ceiling tiles could support fungal growth when at equilibrium with a relative humidity (RH) as low as 85% and corresponding moisture content (MC) greater than 2.2%. Used ceiling tiles appeared to be more susceptible to fungal growth than new ones. In the 70% RH chamber with wetted tiles under slow-drying, non-equilibrium conditions, fungi could still proliferate as long as the moisture level in the ceiling tiles was adequate. Fungal growth could be limited if the wetted ceiling tiles were dried quickly and thoroughly.

1.0 INTRODUCTION

With individuals spending as much as 90% of their day indoors (Ott, 1988), exposure of building occupants to biological contamination of the indoor environment is a major health concern (Miller, 1990). Although some biocontaminants are transported indoors from outdoor sources, many are produced or amplified indoors. Materials that have become contaminated and sustained a population of fungi are a significant source of indoor air contamination (Morey, 1993; Hunter et al, 1988).

A variety of structural and finishing materials have been reported as contaminated with fungi. It is well established that microorganisms can colonize and amplify on these materials if sufficient nutrients and moisture are present. Building material components or accumulated soil on the surface of the material can provide nutrients for the organisms. In addition, dirt or soiling has a profound impact on the ability of materials to retain moisture. The moisture contents of some used materials can be 10% to 40% higher than for the same materials when new (West and Hansen, 1989; Yoshida et al., 1989). Moisture available to support the growth of microorganisms may originate from sources ranging from standing water to elevated relative humidity in the air. Hygroscopic, porous materials have been found to be more susceptible to contamination (Block, 1953; Coppock and Cookson, 1951).

One of the most commonly used building materials in the United States is ceiling tile. Contaminated ceiling tiles (Morey, 1988; Reynolds et al., 1990) as well as contaminated accumulated ceiling tile dust (Streifel, 1988) may pose a serious health threat. During demolition and renovation of a building, contaminated dust could pose a considerable hazard. In addition, the ceiling space above the tiles is frequently used as a return air plenum, increasing the potential health risk associated with a contaminated surface if the tile itself or the

accumulated dust is contaminated.

The key to prevention and control of indoor biocontamination becomes the elimination of potential indoor reservoirs or sources by creating and maintaining environments unfavorable to the growth of the microorganisms. Laboratory static chamber experiments have been undertaken to evaluate the impact of different environmental factors on the ability of building materials to support fungal growth and amplification. The method has been demonstrated previously in an evaluation of microbial growth on materials (Foarde et al., 1992). Previous results have demonstrated both organism and substrate differences. At high RH (97%), *Penicillium glabrum* was able to grow and amplify on both used and most new tiles, while *Aspergillus versicolor* was not. In addition, it was demonstrated that for porous materials the bulk moisture level should be taken into account as an important factor in the selection of building materials (Foarde et al., 1993).

New experiments at a range of RHs from 54% to 97% have been undertaken. In addition to *P. glabrum*, *Penicillium chrysogenum*, and *Aspergillus niger*, have been selected as test organisms. The objective of these experiments was to determine the impact of a range of RHs on the ability of the test organisms to grow on both new and used ceiling tiles including those made of fiberglass. Experiments were conducted under both equilibrium and non-equilibrium moisture conditions.

2.0 MATERIALS AND METHODS

2.1 *Static Chamber*

Static chambers (32 x 39 x 51 cm), prepared by modifying acrylic-walled desiccators (Figure 1), were used to provide controlled environments for the fungal growth tests.

Saturated salt solutions were used to maintain specific RHs (ASTM 104-85) within each chamber. The chambers were placed in a dark, temperature-controlled (21 ± 3°C), HEPA (High Efficiency Particulate Air) -filtered room.

The six RH values and saturated salt solutions used to attain them were: 54% RH - magnesium nitrate, 70% RH - potassium iodide, 85% RH - potassium chloride, 90% RH - barium chloride, 94% RH - potassium nitrate, and 97% RH - potassium sulfate (Greenspan, 1977).

Each static chamber was equipped with three shelves, a tray containing the saturated salt solution, a hygrometer, and for some experiments a small (3v), battery-powered fan. The fan was always positioned on the top shelf, and ceiling tile samples were never placed on that shelf when the fan was in use. While that fan did significantly speed drying, the air velocities on the shelves where the ceiling tile blocks were located were always less than 13 cm s^{-1} (detection limit of the TSI Velocicheck Model 8310 hot wire anemometer). Air velocities near the unfinished side of the ceiling tile have not been reported in the literature. They are expected to be very low except near the diffusers to unducted plenum returns. Natural and forced convection and flows in rooms near walls have been reported to be in the range of 0 to 30 cm s^{-1} (Nazaroff et al., 1990). Thus the air flow rate in the chambers with the fan on was of the order of those that might be expected indoors.

2.2 *Ceiling Tiles*

Four commercially available ceiling tiles, three new and one used, were evaluated. The used ceiling tile (designated "used Class C") was approximately 10 years old and had been removed from an office. The tile was specified by its manufacturer as Class C, fire-retardant, washable, standard white, textured-faced, and suspended-ceiling tile. It contained varying

proportions of mineral fiber (0-90%), gypsum (10-15%), starch (10-15%), paper fiber (10-15%), clay (0-25%), perlite (0-30%), silica (0-12%), styrene acrylic polymer (0-12%), and phenolic resin (0-8%).

The three different types of new ceiling tiles tested were: a Class C (designated "new Class C"), a Class A (designated "new Class A"), and a pressed fibrous panel (designated "new Fiberglass"). The "new Class C" had the same specifications as the used Class C tile. The "new Class A" was a fire-resistant acoustical tile composed of 20 - 60% mineral wool fiber and 4 - 10% hydrous aluminum silicate. The "new Fiberglass" tiles were composed of 90% fiberglass and 10% urea extended phenol - formaldehyde resin cured. The side toward the occupied space was covered with a vinyl film facing.

All ceiling tiles were purchased as 30.5 x 61 cm boards and cut into 3.8 cm squares. The pieces of tile were sterilized by autoclaving before inoculation (on the non-white, unfinished side) with fungal spores.

2.3 *Moisture Content*

Two types of moisture measurements are commonly used to relate microorganism growth to material moisture - MC and water activity (a_w). MC, defined as mass of water per unit mass of dry material, is measured gravimetrically (West and Hansen, 1992; Foarde et al., 1993). It is necessarily a bulk measurement made on a sample of the material. Dry material, in this context, has had adsorbed (bound by molecular bonds) and absorbed (held loosely in capillary spaces) water removed through normal oven drying methods (105°C), but may retain strongly chemically-bound water (Richards et al., 1992; Flannigan, 1992). The equilibrium RH above a sample of a material, divided by 100% is defined as a_w. Therefore, the a_w of a material that has been equilibrated in a closed chamber having a fixed RH of 85% is 0.85.

Corry (1987) stated that a_w is the proportion of "available water for biological reactions." It has been used primarily to relate water content of foods to the ability of microorganisms to grow on them (Pitt, 1981). It is a useful laboratory measurement when RH conditions are known to be at equilibrium.

The term a_w, as used in the literature, is usually reported as the minimum a_w at which an organism can germinate at a given temperature. The minimum a_w for the test organisms employed in these experiments are: 0.78 (82 days, 25°C) for *P. chrysogenum* (Hocking and Pitt, 1979), 0.81 (20 days, 23°C) for *P. glabrum* (Mislivec and Tuite, 1970), and 0.77 (unknown days, 35°C) for *A. niger* (Pitt, 1981). Many factors can effect the minimum a_w for germination of an organism including nutrient availability and temperature (Block, 1953; Pitt, 1981; Flannigan and Miller, 1993).

For porous materials, MC and a_w are related through the water adsorption isotherm, and different relationships are obtained for different materials. Because nutrient content varies widely for various building materials (and between clean and dirty materials), no single moisture measurement value will unequivocally indicate whether microorganisms will grow in a particular building situation or on a class of materials. We have chosen to use MC and RH (equilibrium or non-equilibrium) in the present study because these terms are more common in the building industry and because when wetted materials were tested, material moisture conditions were not at equilibrium. However, for the experiments at equilibrium, a_w is easily calculated for the data presented in this paper because the laboratory data was obtained in closed chambers at various fixed RHs.

2.4 *Test Microorganisms*

Two of the predominant genera of allergenic fungi found in indoors problem environ-

ments are *Penicillium* and *Aspergillus*. Three species, representative of these two genera, were selected as test microorganisms. The specific test organisms were *P. glabrum*, *P. chrysogenum*, and *A. niger*. *P. glabrum*, purchased from the American Type Culture Collection (ATCC) as *P. aragonense* (ATCC #4228), was re-identified by R.A. Samson of the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. *P. glabrum* has been isolated from the indoor environment (Samson and van Reenen-Hoekstra, 1992), and has also been proposed as a causative agent of asthma in a saw mill (Comptois and Malo, 1990). *A. niger* (#6275) was also purchased from ATCC. *A. niger* has been isolated from contaminated materials and problem environments (Lewis et al., 1991; Light et al., 1989). *P. chrysogenum* was isolated from a contaminated building material and cultivated for the ceiling tile tests. The culture is being maintained in the University of Texas Medical Branch Fungus Culture Collection as UTMB3491. This organism has been also been isolated from a problem environment and contaminated materials, and proposed as a causative agent of allergic alveolitis (Fergusson et al., 1984).

2.5 *Experimental Procedure*

To prepare the test organisms for inoculation onto the test materials, the each organism was first inoculated onto solid media, Sabouraud dextrose agar (SDA). The cultures were allowed to grow for 5 - 10 days, until mature confluent growth covered the surface of the plate. A sterile swab wetted with sterile water was gently stroked across the surface of the petri dish to collect the growth. The material collected on the swab was eluted into sterile water. Sterile water was used to minimize the introduction of extraneous nutrients. Microscopic examination of the suspension during the development of the inoculation protocol demonstrated that the majority of the material was spores. Mycelial fragments were

identified in only 2 fields out of 15. The procedure was repeated until a reading of 15%T at 520 nm (Milton-Roy Spec 20D) was achieved (approximately 1 x 10^7 CFU ml^{-1}). The suspension was mixed well and 10μl was pipetted on each block of test material for a final inoculum of approximately 1 x 10^5 CFU per sample.

Each test for each organism on each test day included five blocks. Three blocks were inoculated as described above. Two uninoculated blocks were used as controls. All blocks for each experiment were placed in the chamber at the test RH on the same day.

For studies under equilibrium or near equilibrium conditions, ceiling tile blocks were chamber-conditioned for three days before inoculation. Material equilibration studies have shown that these particular materials reached equilibrium MC within 3 days, except for those in the 97% RH where approximately 70 - 75% of the MC was attained in the first 3 days (Foarde et al., 1994). For studies under non-equilibrium conditions, ceiling tiles blocks were wetted to approximately 40% MC with sterile water and chamber-conditioning was omitted. For the non-equilibrium studies, two test series were conducted. In the first test series, the air inside the static chamber was relatively quiescent (no fan). The second test series was conducted with the fan on to promote air mixing inside the static chamber and to accelerate water evaporation.

To quantify the fungal growth, triplicate inoculated and duplicate uninoculated blocks were removed, usually on days 0, 3, 7, 14, 21, and 28. The ceiling tile blocks were removed from the chambers, weighed, and placed in sterile receptacles containing phosphate-buffered saline 0.1% Tween 80. The blocks in buffer were shaken on a wrist-action shaker for 30 minutes, then the block/buffer suspension was diluted and plated on Sabouraud dextrose agar. Plates were incubated at room temperature for at least 1 week. CFUs were counted shortly after visible growth was first noted and again as moderate growth became apparent.

3.0 RESULTS AND DISCUSSIONS

3.1 *Equilibrium Moisture Content*

Figure 2 shows a plot of adsorption isotherms (MC data against RH at equilibrium) for the test ceiling tiles. Each data point was the mean of at least 3 measurements. Overall, relatively low (less than 8%) equilibrium MC was obtained for the four ceiling tiles tested. New fiberglass tiles were the least hygroscopic (had the lowest equilibrium MC) among the four. This is reasonable because clean glass fibers were not able to gain much moisture by themselves and had no contamination to add surface area and adsorption sites. Figure 2 also shows that the used Class C tiles were more hygroscopic than the new Class C tiles. Since the used Class C tiles were 10 years old, the aging could have caused some structural changes and made it more hygroscopic, or the formulation for the tile composition may have been changed. Also, a thin dust layer had accumulated on the back of used Class C tiles. Approximately 1.4g of dust/m^2 was collected with the high volume surface sampler (HVS3) (Leese et al., 1993). West and Hansen (1989) showed that deposits of fine soil, dried leaves and grass, and microbial matter could significantly enhance the water absorbing ability of a substrate. Therefore, the dusts that deposited on the used Class C tiles could have contributed to making this tile more hygroscopic than the new tiles. On the other hand, new Class A tile was more hygroscopic than both the new or the used Class C tiles when RH was greater than 75%. The higher moisture absorbing ability of Class A tiles may have been caused by their higher fiber content or other composition differences. Overall, these data show the relatively wide variation in moisture-holding capability even in a single type of building material.

3.2 *Fungal Growth on Chamber-Conditioned Tiles*

For this test series, the ceiling tile blocks were chamber-conditioned at the desired RH for 3 days before inoculation. Figure 3 shows the static chamber test results for new Class A tiles inoculated with *P. glabrum*. The error bars indicate the standard deviation of each data point. No growth (no increase of CFUs in the test period) of *P. glabrum* was obtained in chambers with a RH of 85% or less. Moderate fungal growth (an increase of CFUs by 1 to 2 orders of magnitude in the test period) was measured at 90% equilibrium RH. Significant growth (an increase of CFUs by more than 2 orders of magnitude in the test period) of *P. glabrum* was obtained in chambers with RHs at 94% or greater. Therefore, the minimum equilibrium RH at which growth of *P. glabrum* was initiated on used Class C tile blocks was between 85 and 90% (which is equivalent to 0.85 and 0.90 a_w). Correspondingly, the minimum MC for the proliferation of *P. glabrum* on new Class A tiles was between 3.4 and 3.8% based on Figure 2.

The results of the tests for all the ceiling tiles and test organisms evaluated are summarized in Table 1. The minimum MCs ranged from 2.2 - 2.4% on new Class C, 2.2 - 2.8% on new fiberglass for *P. chrysogenum*, and 4.3 -5.8% for *A. niger* on used Class C. The corresponding minimum equilibrium RHs (a_w) ranged from 85 - 90% (0.85 - 0.90) and 94 - 97% (0.94 - 0.97), respectively. Block (1953) reported that a minimum MC of 10% was required for fungal growth on substrates such as leather, wool, cotton, wood, and cheese. However, Table 1 indicates that the minimum MC for fungal growth on the four ceiling tiles tested was considerably less than 10%. As mentioned previously, the minimum equilibrium RH (or a_w) for germination of the test organisms have been reported to be 81% (0.81), 78% (0.78), and 77% (0.77) for *P. glabrum*, *P. chrysogenum*, and *A. niger*, respectively. The differences in minimum MC or equilibrium RH required for fungal growth on ceiling tiles

reflect the fact that the nature of the substrate materials can have significant impacts on the conditions required for fungal proliferation.

Table 1 shows that all three new ceiling tiles supported the growth of at least one of the test organisms. It is likely that some of the raw materials served as nutrients and facilitated fungal growth. For new fiberglass ceiling tiles, it was suspected that the chemical compounds applied by the manufacturer as fiberglass binders were used by fungi as nutrients.

Table 1 also indicates that the used ceiling tiles were more susceptible to fungal growth than the new ones. All three test organisms were able to grow on the used Class C tiles whereas the new Class C tiles supported the growth of only *P. chrysogenum*. In addition, while used Class C ceiling tiles supported the growth of *A. niger*, no growth of that organism was detected on any of the three new ceiling tiles. Since dust and debris can supply sufficient nutrients for fungal growth, one possibility was that the thin dust layer accumulated on the surface of the used Class C tiles provided a more substantial and complete supply of nutrients than those available intrinsically in the new ceiling tiles. Another possibility was that the dust increased the hygroscopicity of the used tiles by increasing the amount of water present or by making what was present more available to the microorganisms. Figure 2 shows that the used Class C tiles were more hygroscopic than the new Class C tiles. Block (1953) suggested that the more hygroscopic a substrate was, the easier it became for fungi to proliferate. Therefore, the increased hygroscopicity of used ceiling tiles could also make them more susceptible to fungal growth. The use of bulk moisture measurements such as MC and a_w do not provide information on the moisture adsorption characteristics of the various components of heterogeneous materials (ie.- dirt on ceiling tile). Realistically, a combination of both effects was responsible.

3.3 *Fungal Growth on Wetted Tiles*

Used Class C ceiling tile blocks were wetted with sterile water to contain approximately 40% moisture before inoculation with *P. glabrum*. The inoculated blocks were placed in two different 70% RH static chambers: one with the fan on and the other with it off. Table 2 summarizes the results and compares them to the data from previous tests with tiles conditioned at 70% RH.

Table 2 shows that the MC of the wetted blocks decreased slowly in the chamber with the fan off. However, due to the effects of air turbulence and better mixing, the MC of wetted blocks decreased rapidly in the chamber with the fan on. No growth of *P. glabrum* was detected on the rapidly dried blocks within the 10 d. period. Yet notable growth was obtained on the slowly dried blocks.

The RH of air does not directly impact the growth of fungi (Block, 1953; Pasanen et al. 1991); however, fungi may grow at very low levels of air humidity if water is available within or on the surface of the substrate (i.e. - due to condensation). Analysis of these fungal growth data for ceiling tiles from this project reinforces that conclusion. Table 1 showed that the minimum MC required for *P. glabrum* to grow on the used Class C tile blocks was between 3.0 and 3.6%. Correspondingly, the minimum equilibrium RH for the growth of *P. glabrum* was between 85 and 90%. If RH were directly controlling fungal proliferation, no growth should occur at RH below 85%. However, Table 2 (i.e.- wetted blocks, fan off) shows that, when the MC was maintained above the minimum level, significant fungal growth was detected at RH as low as 70%.

As shown in Table 1, the minimum MC for growth on used Class C tile was determined to be 3.0 - 3.6% (85 - 90 % RH). In addition, Table 2 shows that, when wetted blocks were dried to below that minimum MC within 3 days, no growth of *P. glabrum* occurred.

Microbial growth exhibits sigmoidal kinetics. Typically, this consists of a lag or latent period followed by log growth, and then deceleration, stationary, and decline phases. If the MC of a substrate is reduced to below a certain level during the latent period, the germination and subsequent growth of the fungi could be arrested. In other words, an episode of fungal proliferation may be avoided if ceiling tiles are dried quickly and thoroughly after they are accidentally wetted by leaks, floods, or spills.

4.0 CONCLUSIONS

The water adsorption isotherms for the four commercial ceiling tile materials had the same general shape, but were sufficiently different to expect differences in fungal growth properties, as were observed. All three new ceiling tiles tested were able to support the growth of at least one of the test organisms under appropriate conditions (e.g., with MC above the minimum level required). The materials intrinsically contained sufficient nutrients without aging or becoming soiled.

The used ceiling tile samples were more susceptible to fungal growth than the new ones. The used tiles were slightly more hygroscopic than the new ones thus favoring the growth of the test organisms. In addition, the dusts that settled on the surface of the used ceiling tiles probably provided additional nutrients and contributed to the overall increased hygroscopicity of the material.

As shown in Table 1, the minimum equilibrium RH at which growth occurred for these materials and organisms was always above the currently recommended 60% (ASHRAE Standard 55-92). Therefore, maintaining a building following this standard can help prevent the growth of the molds tested provided no other source of water (i.e. - from condensation or

leaks) is available. However, wetted ceiling tiles were able to support fungal growth even at 70% RH as long as the tile moisture content was greater than the minimum MC for that material.

In addition to MC, time is a critical factor determining fungal growth. Even under favorable conditions, there is usually a latent period before fungal proliferation. Current data indicate that fungal growth may be avoided by regulating the substrate conditions (e.g., moisture elimination) during the latent period. Thus, if a ceiling tile became wetted by a leak, spill, or flood, fungal growth may be avoided by quickly and thoroughly drying the tile and keeping it dry.

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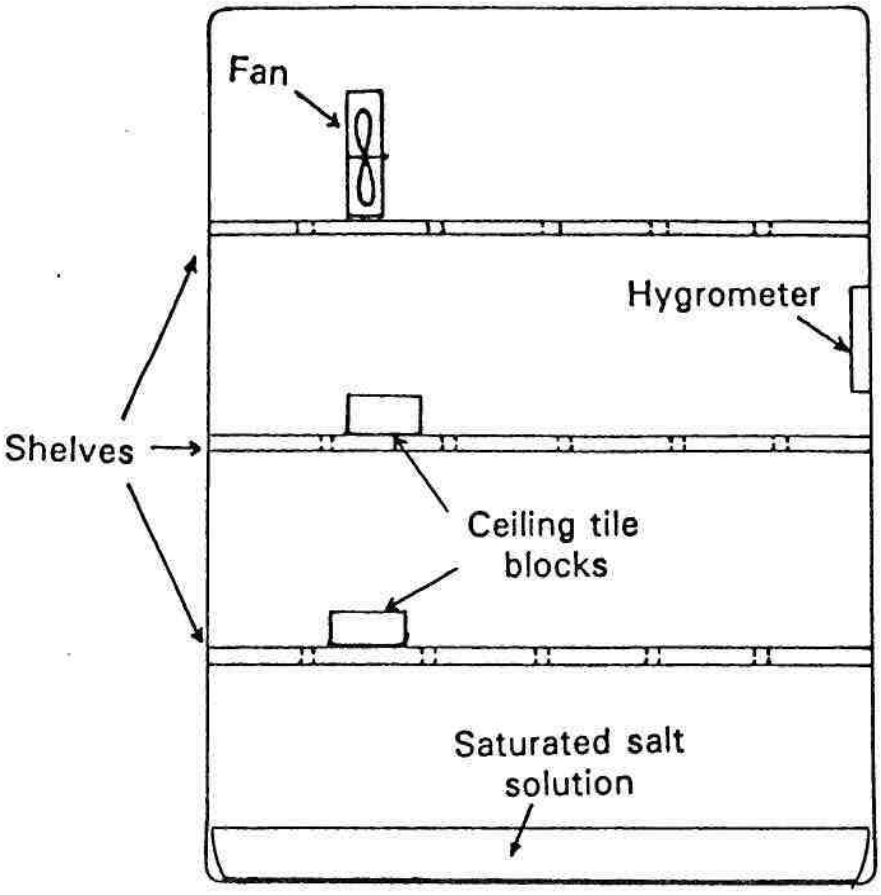
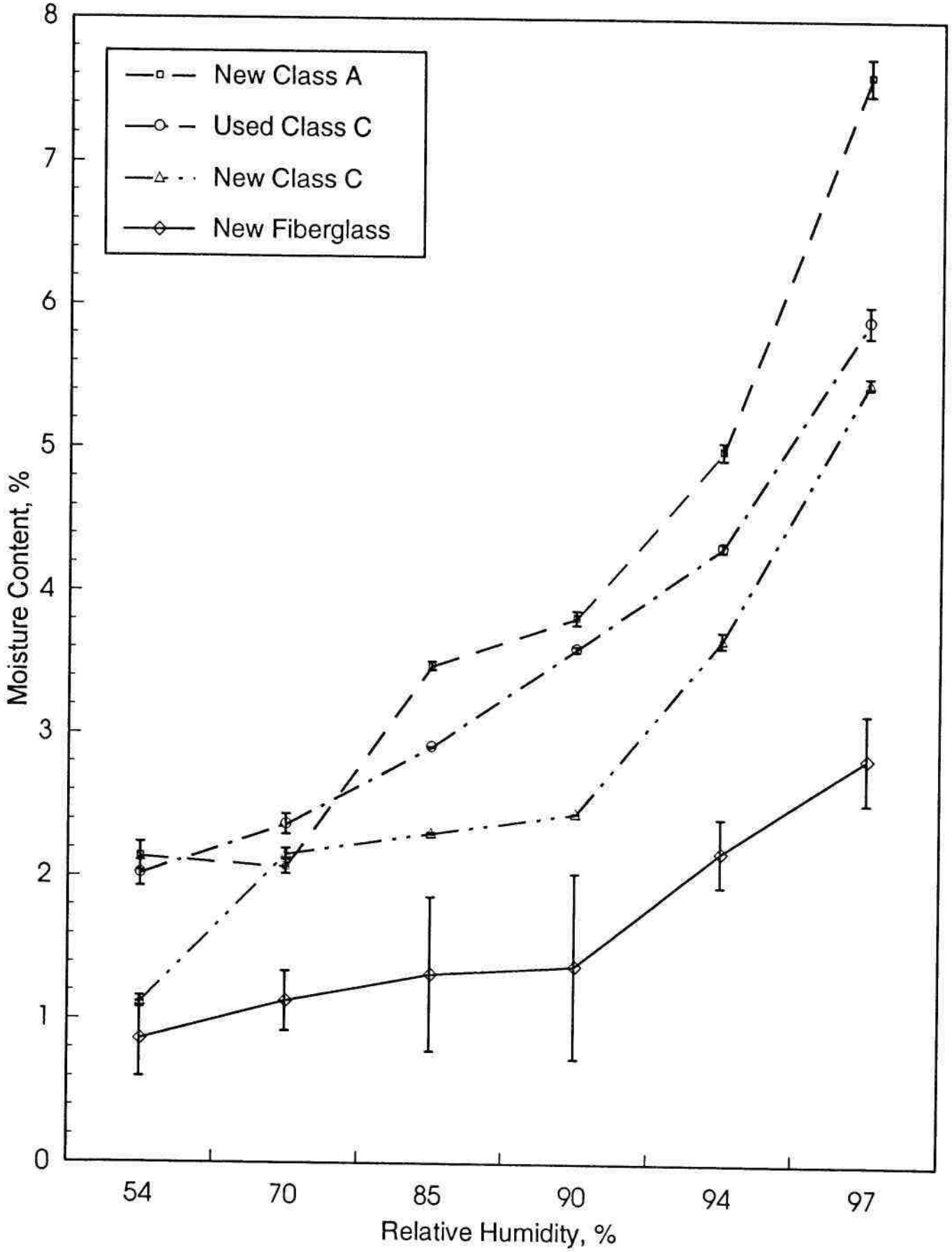

Fan
Hygrometer
Shelves
Ceiling tile blocks
Saturated salt solution

Table 1. Summary of Fungal Growth (28 days) Data from Ceiling Tiles Conditioned in Static Chambers

Ceiling Tile	Test Organism	Log Increase, CFUs	Minimum ERH, percent	Minimum Moisture Content, percent
Used Class C	*P. glabrum*	1 - 2	85 - 90	3.0 - 3.6
	P. chrysogenum	2 - 3	85 - 90	3.0 - 3.6
	A. niger	2 - 3	94 - 97	4.3 - 5.8
New Class C	*P. glabrum*	None	—	—
	P. chrysogenum	2 - 3	85 - 90	2.2 - 2.4
	A. niger	None	—	—
New Class A	*P. glabrum*	2 - 3	85 - 90	3.4 - 3.8
	P. chrysogenum	2 - 3	85 - 90	3.4 - 3.8
	A. niger	None	—	—
New Fiberglass	*P. glabrum*	1 - 2	94 - 97	2.2 - 2.8
	P. chrysogenum	1 - 2	94 - 97	2.2 - 2.8
	A. niger	None	—	—

Table 2. Comparison of Moisture Content and Fungal Growth of *P. glabrum* on Used Class C Ceiling Tiles Evaluated in 70 % RH Static Chambers

Day	Moisture Content, %	Log Increase, CFUs
Wetted Tiles, Fan Off		
0	43.0	None
3	21.0	1 - 2
7	4.7	1 - 2
10	2.6	2 - 3
Wetted Tiles, Fan On		
0	40.0	None
3	2.8	None
7	2.8	None
10	2.8	None
Conditioned Tiles		
0	2.2	None
3	2.3	None
7	2.4	None
10	2.2	None


New Class A
Used Class C
New Class C
New Fiberglass
Moisture Content, %
0
1
2
3
4
5
6
7
8
54
70
85
90
94
97
Relative Humidity, %

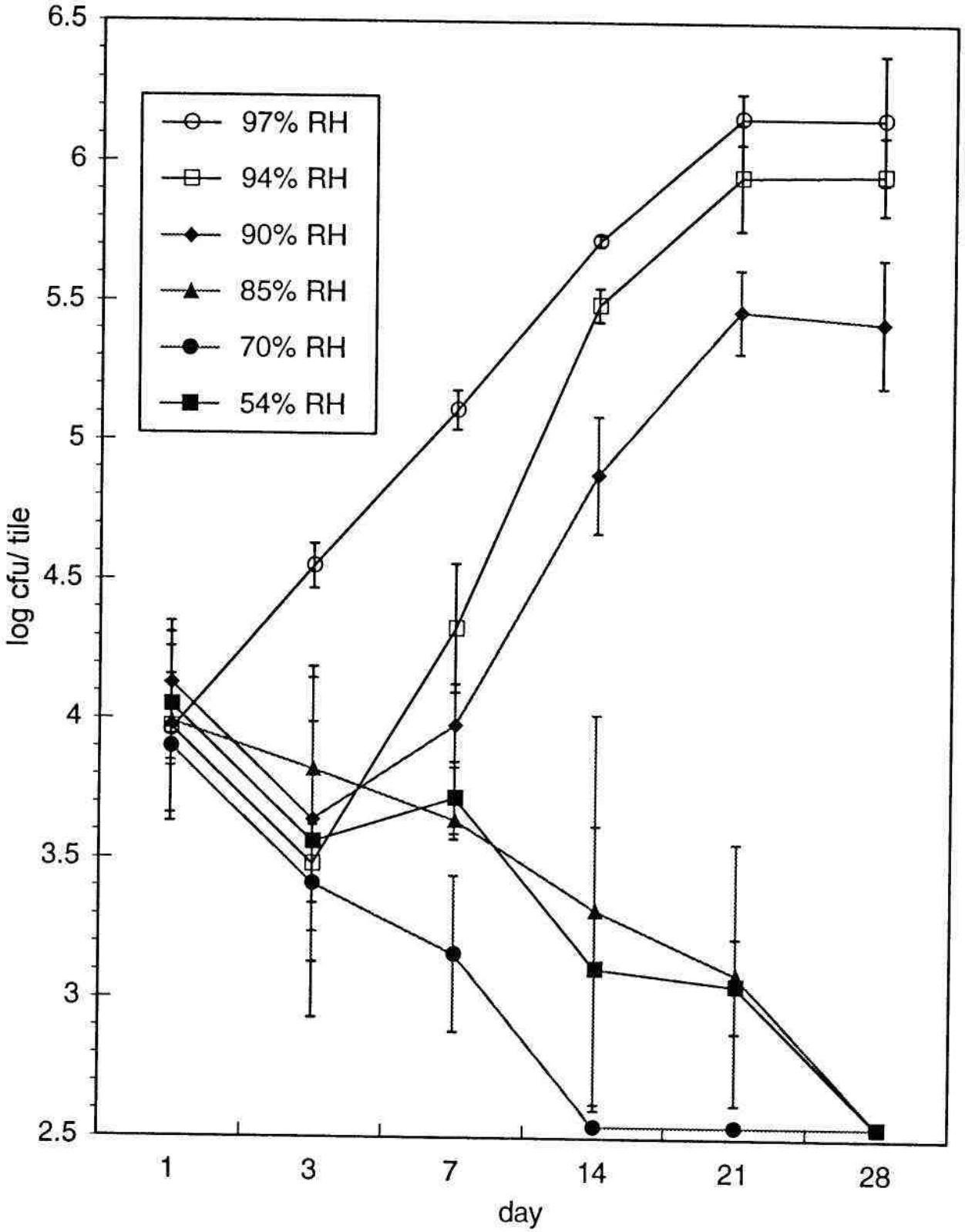

97% RH
94% RH
90% RH
85% RH
70% RH
54% RH
log cfu/ tile
6.5
6
5.5
5
4.5
4
3.5
3
2.5
1
3
7
14
21
28
day

Figure Captions

Figure 1. Schematic (side view) of a static chamber (Chang et al., 1994)

Figure 2. The adsorption isotherm of the ceiling tiles tested at different RH.

Figure 3. *Penicillium glabrum* growth on chamber conditioned new Class A ceiling tiles.