



## Effect of Humidity on Fungal Deteriogens of Ancient Monuments

Dubey S.<sup>1</sup> and Jain S.K.<sup>2</sup>

<sup>1</sup>Mata Gujri College of Professional Studies, Indore, MP, INDIA

<sup>2</sup>School of Studies in Microbiology, Vikram University, Ujjain, MP, INDIA

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### Abstract

Fungi have always found to be correlated with the deterioration of historical buildings. The present study is aimed to evaluate the effect of relative humidity on growth of fungi associated with Biodeterioration. The experimental work has been carried out using saturated salt slurry method. Five fungal deterioogens were investigated for their growth response over a wide range of relative humidity under laboratory conditions. Out of which, *Penicillium* sp, *Fusarium* sp and *Aspergillus* sp. showed growth between 93-100% RH, whereas *Alternaria* sp. and *Curvularia* sp. exhibited adequate growth between RH of 95-100%. Knowledge about fungal deterioogens will help to control the parameters causing Biodeterioration.

**Keywords:** Biodeterioration, relative humidity.

### Introduction

Biodeterioration may be defined as any undesirable change in the properties of material caused by vital activities of living organism. Deterioration of monuments has been reported by various micro organisms like bacteria, fungi, algae etc.<sup>1,2</sup>. Depending upon their ecological and physiological requirements, the biological deterioogens colonize the surface. The aptitude of material to be colonized by one or several groups of living organisms is known as Bioreceptivity<sup>3</sup>. Tropical and sub tropical regions all over the world are particularly prone to microbial deterioration than temperate regions as temperature and humidity favours the colonization of micro organisms<sup>4</sup>. Fungi has greater role in the biodeterioration of monuments<sup>5</sup>.

A number of matters which seem to be unrelated can play a role in proliferation of biological growth like surface inclination of stone, pollution levels, shade patterns of surrounding trees and amount of direct sunlight constitute microclimate. Horizontal surface may promote pooling of rainwater which increases the surface moisture. Also, parts which remain shaded for extended period of time remain humid and favour growth of fungi. The initial penetration of fungal hyphae into the pores of stone pave the path of various other microbes into the interior of stone.

Microbial colonization of stones depends on environmental factors such as water availability, pH, climatic exposure, nutrient sources, and parameters such as mineral composition, type of cement, as well as porosity and permeability of rock material<sup>6</sup>. Humidity and presence of organic residue on stone favor growth of fungi on monuments<sup>7</sup>. Water availability is crucial for various life forms and relative humidity can affect the growth of fungi. Ancient monuments and buildings have been a good source of relative humidity to favour the growth of

various microbes. The colonization of fungi on moistened building materials and resultant biodeterioration are very much evident from research work done on Bioreceptivity of stone materials. That is why it becomes necessary to evaluate the effect of relative humidity on fungal growth.

### Material and Methods

Fungal strains isolated from monument site ie. *Curvularia* sp, *Penicillium* sp, *Fusarium* sp, *Alternaria* sp and *A. nidulans* were maintained on Potato dextrose agar in Department of Microbiology, Mata Gujri College of Professional Studies, Indore (M.P.) Saturated solutions of different chemicals for maintaining relative humidity were selected as per literature<sup>8-10</sup>.

Use of saturated salt slurries has been a standard method for obtaining specified relative humidity values in closed chambers for obtaining isotherms. This method uses a saturated salt slurry of a known relative humidity value to bring a material to equilibrium with its surrounding environment<sup>11</sup>. A saturated salt slurry is composed of water and a saturated aqueous salt solution with salt crystals in a glass or plastic sealed container<sup>12</sup>. Varying levels of relative humidity were obtained using saturated salt solutions in form of moist chambers. The solutions used and their relative humidity are listed in table 2.1. Agar strips of 4 cm × 1 cm from solidified potato dextrose agar are transferred to plates with solutions and then incubated for seven days at 25±2<sup>0</sup>C to allow the agar strips to equilibrate with the water vapour. The tip of each agar strip was inoculated with test organisms then inoculated moist chambers were incubated at 25±2<sup>0</sup>C for five days.

### Results and Discussion

Saturated solution of different chemicals i.e. CaSO<sub>4</sub>. 5H<sub>2</sub>O (98%R.H.), KH<sub>2</sub>PO<sub>4</sub> (96.6% R.H.) Na<sub>2</sub>HPO<sub>4</sub>. 12H<sub>2</sub>O(95%

R.H.),  $\text{NH}_4\text{H}_2\text{PO}_4$  (93% R.H.),  $\text{ZnSO}_4$ (88.5% R.H.), KCl (85% R.H.),  $\text{NH}_4\text{Cl}$  (79% R.H.), NaCl (76% R.H.),  $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$  (52% R.H.) and water (100% R.H.) were used to provide relative humidity to fungal detriogens *Aspergillus nidulans*, *Fusarium sp*, *Alternaria sp* *Curvularia sp*, *Penicillium sp* to study the effect of humidity on their growth. At 79% humidity, only *Aspergillus sp* showed slight growth below the range all the fungal growth were retarded. In the range of 85-100% *Penicillium sp*, *Aspergillus sp* and *Fusarium* showed growth whereas *Curvularia* and *Alternaria sp* were able to grow in the range of 95-100% humidity.

**Table-2.1**  
**List of chemicals with known RH value**

S.No.	Saturated solutions	Relative humidity
1	NaCl	(76%)
2	$\text{NH}_4\text{Cl}$	(79%)
3	KCl	(85%)
4	$\text{NH}_4\text{H}_2\text{PO}_4$	(93%)
5	$\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$	(95%)
6	$\text{KH}_2\text{PO}_4$	(96.6%)
7	$\text{CaSO}_4 \cdot 5\text{H}_2\text{O}$	(98%)
8	Water	(100%)

**Discussion:** The growth of specific biological species on a particular surface depends on physical and chemical properties of surface (like stone) and various environmental conditions predominantly humidity in case of fungi. Various factors as Condensation, Building disaster (fire and flood), construction moisture contribute to moisture in buildings. Excessive moisture in building materials supports microbial growth<sup>13</sup>.

It has been reported that microorganisms, especially fungi, are capable of growing on almost any substrate when the ERH (Equilibria relative humidity) is more than 75 to 80%<sup>14</sup>. Low RH has been investigated as retarding factor for growth of fungi as *Eurotium herbariorum* died within 1 h when active hyphae were transferred from 94% RH to 32% RH<sup>15</sup>. In one of the investigation, the effect of wide range of RH at varied temperature for 28 weeks over wood-based materials, stone-based materials and insulation materials exposed to mould contamination was tested<sup>16</sup>. RH higher than 90% at temperature

above 15°C was found susceptible to mould growth. The condensation under the varying RH and temperature conditions caused only restrained fungal growth in the materials. RH 30% previously seemed to decrease the viability of fungi but later in the experiment, fungi adapted to tolerate fluctuating conditions and the drying at RH 50% had only a slight effect on the viability of fungi<sup>17</sup>.

However, airborne spore counts of *A. fumigatus* and *Penicillium sp*. were usually higher in dry than moist air, being minimal at RH above 70%. In another study it was found that species occurrence also vary with the moisture level. *Aspergillus versicolor*, *Stachybotrys*, and *Acremonium* were detected only in samples from moisture damaged buildings. In addition, the presence of *Oidiendron* as well as elevated concentrations of *Cladosporium* and *Actinobacteria* were associated with moisture damage in concrete schools<sup>18</sup>. Optimal selection of ground covers and ventilation help to achieve the acceptable moisture conditions<sup>19</sup>. In vitro conditions are not always favourable for growth of all residents of building due to lack of one or more essential nutrients even in humid conditions. In buildings, surfaces are normally covered with a thin layer of dust and organic debris which contains nutrients suitable for microorganisms<sup>20</sup>.

The aim of the current study was to determine whether fungal genera associated with damaged building materials is affected by moisture. At 76 to 93% RH, two of the five species were viable and could grow. Microbial colonization of building materials is potentially active process in which population composition changes in response to the equilibrium relative humidity (ERH) of the materials. Like above 75 to 80% ERH, Primary colonizers (*Penicillium*, *Eurotium*, and *Aspergillus* species) begin to grow whereas secondary colonizers (e.g., *Cladosporium* species) appear at an ERH of 80 to 90% and tertiary colonizers (such as *Fusarium* and *Stachybotrys* species, actinomycetes, and yeasts) begin to grow at an ERH above 90%<sup>14</sup>. In the present work *Aspergillus nidulans*, *penicillium sp* showed their growth between 79%-100% relative humidity *Fusarium sp* show growth above 90% humidity below which their growth was retarded. The findings of present study are in accordance with previous studies.

**Table-3.1**  
**Effect of Humidity on fungi**

Chemical compound and Relative humidity	Fungal isolates (Dry mycelial weight in mg)*				
	<i>Penicillium sp</i>	<i>Curvularia sp</i>	<i>Aspergillus sp</i>	<i>Fusarium sp</i>	<i>Alternaria sp</i>
NaCl (76%)	-	-	-	-	-
$\text{NH}_4\text{Cl}$ (79%)	-	-	+	-	-
KCl (85%)	+++	-	+++	++	-
$\text{NH}_4\text{H}_2\text{PO}_4$ (93%)	++++	-	++++	+++	-
$\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ (95%)	++	+++	+++	++++	+++
$\text{KH}_2\text{PO}_4$ (96.6%)	++++	++++	++++	++++	++++
$\text{CaSO}_4 \cdot 5\text{H}_2\text{O}$ (98%)	++++	++++	++++	++++	++++
Water (100%)	++++	++++	++++	++++	++++

= No growth, +=0-20%, ++= 21-40%, +++=41-60%, ++++=61-80%, +++++=81-100%

## Conclusion

Details of diverse parameters causing biodeterioration provides insight to restoration strategies to protect cultural heritages from being exploited by microbial intruders. As apparent from the above study, higher relative humidity favours the colonization of fungi causing deterioration of ancient buildings. Hence, measures to control the humidity in the environment of historical buildings will help to reduce the chances of deterioration by fungi.

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