

## Original Research Article

### Screening and indexing of dominance of fungal flora on sandstones with special reference to historical monuments

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#### A B S T R A C T

#### Keywords

Bio-deterioration; micro-organisms; bio-films; pollutants; microbial metabolites; pigments; cultural heritage.

Stone cultural heritage materials are at risk of bio-deterioration caused by diverse populations of micro-organisms living in bio-films. The microbial metabolites of these bio-films are responsible for the deterioration of the underlying substratum and may lead to physical weakening and discoloration of stone. Fungal ability in production of pigments and organic acids have crucial role in discoloration and degradation of different types of stone in cultural heritage objects. Additionally, stone objects may support novel communities of microorganism that are active in bio-deterioration process. This investigation is focused on mycological analyses of microbial bio-films from various stone structures of an archaeological monument namely Mahadev temple of Chhattisgarh, India. These structures are made of sand stone and are heavily colonized by fungi. The five fungal species have been isolated which have dominance over stone structures of the monument. *Aspergillus* sp. was common in stone structures of this monument. The identified micro-organisms cause discoloration as well as mechanical exfoliation of building stone material that was analyzed through mechanical hyphae penetration and production of dark pigments as well as organic acids.

#### Introduction

The west facing Mahadev temple situated in a small village called Tuman. This village is situated in Chhattisgarh state of India. This west facing temple built of sand stone consists of a Garbhgriha and a mandapa, presently both in dilapidated state of preservation. The square sanctum houses a shivlinga and is approached through an ornate entrance adorned with images of Ganga and Yamuna accompanied by Saiva dwarapalas. Images

of Brahma, Vishnu, Navagraha and Siva on the Lalatabimba and various incarnations of Vishnu on the door jambs are making the entrance more ornate. The jangha portion separated from the pada by vandhana mouldings has two segments embellished with geometrical and floral motifs, diamond designs, trampling lion, Bhara vahakas, marching elephants etc. A sand stone image of Nandi is situated outside the temple facing its entrance. The

temple is saptaratha on plan. It is commonly believed that the temple was built during the 10<sup>th</sup> century AD (Mishra *et al.*, 2012).

Microorganisms participate actively in the weathering of minerals (Banfield and Hamers, 1997). Microbial processes leading to the degradation of mineral may include microbial oxidation and reduction, creation and maintenance of appropriate physicochemical conditions, and production of acidic metabolites (Barker *et al.*, 1997). These microbially-mediated processes are partially responsible for the chemical and physical weathering of rocks, which lead, eventually, to the formation of soils. Micro-organisms may also contribute to the deterioration of stone artifacts such as historical monuments and statues. The production of organic and inorganic acids by micro-flora in the bio-film has been generally recognized as the predominant mechanism of stone deterioration (Eckhardt, 1978). Most authors have tested acid production by isolated microorganisms in laboratory cultures, in the absence of the stone substrate, extrapolating these results to the field situation.

## Materials and Methods

### Sampling and Isolation of Fungi

A total of 10 samples were collected from various portions of the monument (Fig 1-2) and were brought to the laboratory under aseptic conditions. The isolation of micro-organisms was done by culturing the samples and by direct incubation of samples in moist chamber. The purified fungal cultures were identified by using mycological techniques and were compared with the available authentic literature, reviews and mycological

manuals (Alexopoulos, 1978; Barnett & Hunter, 1987; Ellis, 1976 and Gilman, 1995).

## Calculations

Various myco-ecological characters have been calculated using the following formulae:

$$\% \text{ Frequency (F)} = \frac{\text{Number of samples in which specific organism occurred}}{\text{Total number of samples examined}} \times 100$$

$$\text{Relative Frequency (RF)} = \frac{\text{Frequency of an individual species}}{\text{Frequencies of all species}} \times 100$$

$$\text{Index of Dominance (D)} = \sum (n_i / N)^2$$

Where  $n_i$  = Relative value of individual species.

$N$  = Relative values of all species.

## Results and Discussion

During screening for search of mycoflora, total five species of fungal organisms were isolated from the monument (Photographs, Culture Plates & Table - 1). Composite result indicate that almost all i.e. nine (09) out of total ten (10) samples were mainly dominated by *Aspergillus niger*, followed by *Aspergillus sydowi* and *Penicillium citrinum* with seven (07) and six (06) out of ten samples respectively.

*Aspergillus niger* shows maximum frequency as well as relative frequency followed by *Aspergillus sydowi* and *Penicillium citrinum*. Some of the fungal species are confined to particular samples. For example *Memnoniella levispora* is confined to sample number two, three and seven only, *Fusarium* species is confined

**Figure. 1:** (a) Front view of Mahadev temple (b) Fungal growth on the surface of monument



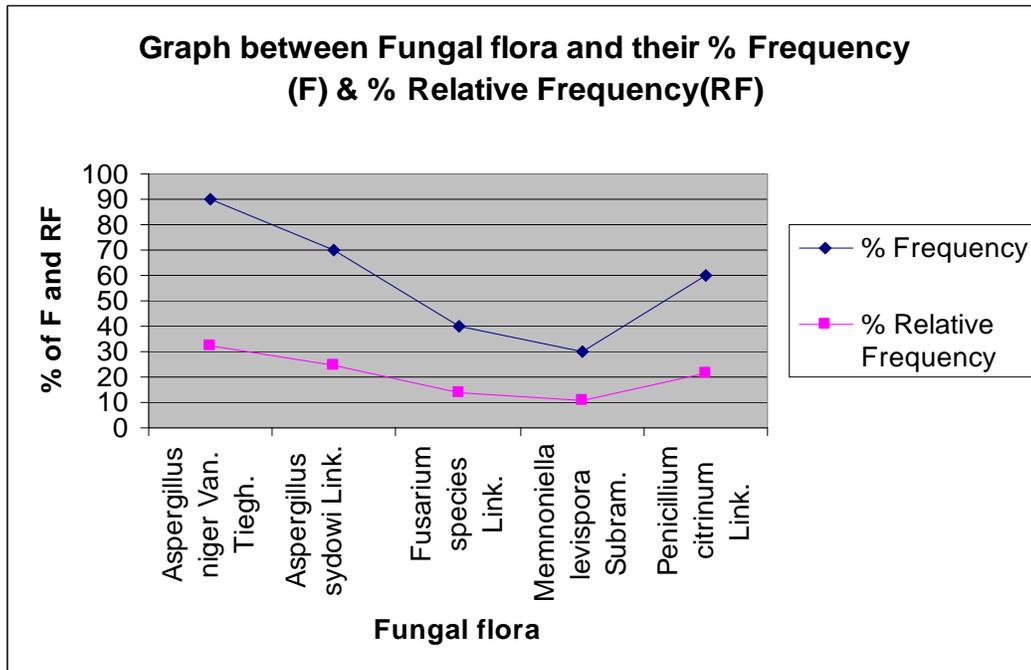
**Figure.2** (a) Fungal growth on the surface of monument (b) Sand stone image of Nandi



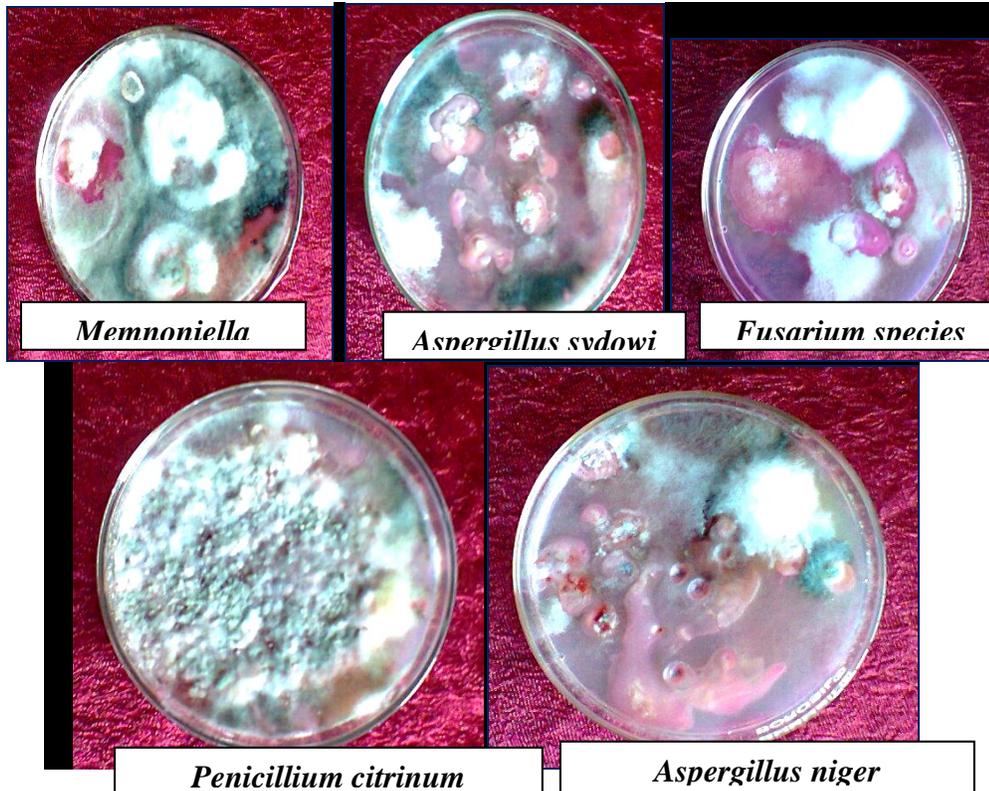
**Table.1** Observation of fungi from all the study area

Isolated Fungal Organism	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>	S <sub>7</sub>	S <sub>8</sub>	S <sub>9</sub>	S <sub>10</sub>	F%	RF%	D
<i>Aspergillus niger</i> Van. Tiegh.	+	+	-	+	+	+	+	+	+	+	90	32.1	<b>0.20</b>
<i>Aspergillus sydowi</i> Link.	-	+	+	+	+	-	+	+	-	+	70	25.0	
<i>Fusarium</i> species Link.	+	-	-	+	-	-	-	+	+	-	40	14.2	
<i>Memnoniella levispora</i> Subram.	-	+	+	-	-	-	+	-	-	-	30	10.7	
<i>Penicillium citrinum</i> Link.	+	+	+	-	-	+	-	+	-	+	60	21.4	
<b>Total</b>											<b>290</b>	<b>103.4</b>	

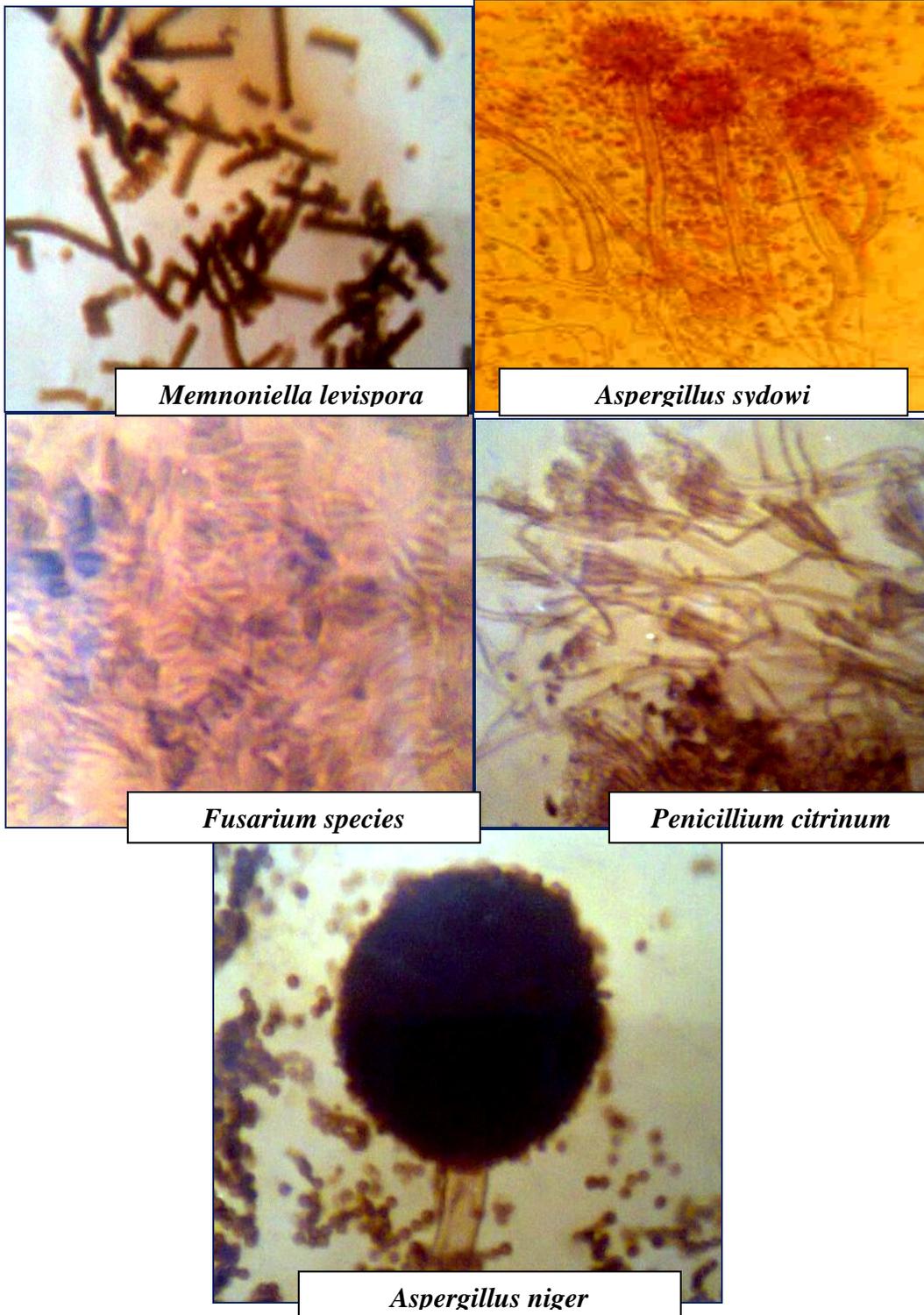
**Figure.1** Fungal flora and their frequency and Relative Frequency



**Plate.1** culture photographs of isolated fungi



**Plate.2** culture photographs of observed fungi



to sample number one, four, eight and nine where as *Penicillium citrinum* species is confined to sample number one, two, three, six, eight and ten. These confinements of fungal species depend on the specific environmental conditions of the area, which vary from one geographical area to another.

In each fungal community all the species are not equally important. There are relatively only few of these, which determine the nature of the community (Simpson, 1949). These few species exert a major controlling influence on the community and also play important role in deterioration of various substrates. Value of index of dominance i.e. 0.20 revealed that the studied fungal organisms were dominated significantly with reference to different samples.

The variation in the composition of fungal organism depends upon biochemical nature of host, degree of competition between the fungal organisms and the prevailing environmental conditions. The frequency and relative frequency are directly or indirectly correlated with meteorological data and climatic conditions (Chandel, 1990).

The toxic metabolites produced by various species of fungal organisms function as chelating agents that can leach metallic cations, such as Iron, Magnesium etc from the stone surface. Laboratory experiments have demonstrated that basic rocks are more susceptible to fungal attack than acidic rocks. It has also been shown in the laboratory that fungal species such as *Aspergillus niger* were able to solubilize powdered stone and chelate various metal ions in a rich glucose medium because they produce organic acids such as gluconic, citric, and oxalic acids (Lapidi

and Schipa, 1973). In the present study *Aspergillus* are the most common species found in the sites. *Aspergillus niger* releases certain metal ions from the rock samples (Boyle and Voight, 1973).

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### References

- Alexopoulos, C.J., 1978. Introductory Mycology (2<sup>nd</sup> ed.). Wiley Estern Ltd. New Delhi, Bangalore, Bombay.
- Banfield J.F., and Hamers R.J. 1997. Processes at minerals and surfaces with relevance to microorganisms and prebiotic synthesis. Rev. Mineral. 35: 81–122.
- Barker, W.W., S.A. Welch and Banfield, J.F. 1997. Biogeochemical weathering of silicate mineral, In: Banfield, J.F., K.H. Nealson (eds.), Interactions between microbes and minerals, Reviews in Mineralogy vol. 35. The Mineralogical Society of America, Washington, DC., 391–428.
- Barnett, H.C., and Barry Hunter, B. 1987. Illustrated Genera of Important Fungi. Macmillan Publishing Company. New York and Collier Macmillon Publishers, London.
- Boyle, J. R., and Voight G. K. 1973. Biological weathering of silicate

- minerals. Implications for tree nutrition and soil genesis. *Plant. Soil.* 38:191-201,
- Chandel, D.S., 1990). Studies of Phylloplane interaction of fungi from Soybean and Pigeon pea. Ph.D. Thesis. Pt. Ravishankar University, Raipur.
- Eckhardt, F.E.W.. 1978. Micro-organisms and the weathering of a sandstone monument. In: Krumbein, W.E. (ed.), *Environmental Biogeochemistry and Geomicrobiology.* Ann Arbor Science, Mich. USA. 675–686.
- Ellis, B.M., 1976. *More Dematiaceous Hyphomycetes*, CMI, Kew, England.
- Gilman, C. Joseph 1995. *A Manual of Soil Fungi.* Print well publication, Jaipur (India).
- Lapidi, A. A., and Schipa, G. 1973. Some aspects of the growth of chemotrophic and heterotrophic bacteria from decayed stone. In: *Proceedings of the 5th International Congress on Deterioration and Conservation of Stone.* (Ed) G. Felix Lausanne, Switzerland. 633-640.
- Mishra P.K., S.C. Srivastava, D.K. Khamari and Yadav, S.N. 2012. *Samskriti. Archaeological Survey of India, Raipur Circle, Raipur, 21.*
- Simpson, E.H., 1949. Measurement of diversity, *Nature.* 163 – 688.