



RESEARCH ARTICLE

BIODETERIORATION AT 15TH CENTURY INDIAN CULTURAL HERITAGE SITE: IDENTIFICATION, MICRO-ENVIRONMENT AND THEIR SCIENTIFIC PROTECTION

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ABSTRACT

Scientific preservation of cultural heritage is one of the major challenges for the new generation. Several biodeteriorating agents are affecting our cultural heritage, which is made with almost all types of materials produced by nature and used by men to realize several types of heritage buildings, from very simple mono-components to complex structures integrating inorganic and organic matters. Stone from cultural heritage buildings is usually affected by microflora and microclimate, mainly the loss of dimensional and structural stability. Environmental degradation was developed by fluctuation cycles of humidity and temperature. The present study focused on identifying the microenvironment-induced biodeteriogens that were growing on the fifteenth-century stone structure of Lat ki masjid and their scientific protection. In this study, *S. aureus*, *Actinomyces*, *A. niger*, *A. fumigatus*, *Cyanobacteria*, and lichens (*foliated with fruticose*) were identified as biodeteriogens and assessed the microenvironment parameters. Temperature, R.H., oxides of sulfur and nitrogen, particulate matter (2.5), and precipitation were measured in the ambience of the Lat ki masjid, Dhar, Madhya Pradesh, India. RH and temperature were reported maximum in the months of December and May, respectively, in a year.

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INTRODUCTION

Fifteenth-century cultural heritage, Lat Masjid (literally meaning "Pillar Mosque"), is located in the open-plan area of the historic city of Dhar, Madhya Pradesh, India. It is the famous Iron Pillar Mosque and Indo-Islamic architecture (4), which was built by Dilawar Khan, the Governor of Malwa province, in 1405 A.D. The preservation of cultural heritage buildings is a crucial concern worldwide, which represents the architectural, historical, and cultural legacies of societies. 1. The major challenges are to maintain such types of heritage buildings, which are affected by biodeterioration with environmental factors, particularly on the exterior surfaces of stone materials (2, 3, 5). Multispecies communities of microbes and lichen grow on the stone surface, and they influence the surface layers of stone matrices with their diverse metabolic functions (6, 7). Sandstone is used as the building material in the Lat ki masjid, which suffers from colonization of unique colorful microorganisms and flora that grow in the form of biofilms on the exterior stone surface. There are close relationships between the environment and microbial communities that are colonizing on these structures that are often ignored, but it is essential to understand the impact of microclimate on the biodegradation of exterior sandstone surfaces (8) because stone biodeterioration is a major challenge for the conservation and

protection of stone monuments and heritage buildings (9) that involves a complex ecological interplay among organisms, stone materials, changing climates, and specific environmental factors (10, 11). The present case study focused on identifying the biodeteriogens with the assessment of environmental conditions in the ambience of the fifteenth-century cultural heritage Lat Masjid and on protecting the heritage building using scientific preservation strategies.

MATERIALS AND METHODS

Study Area and Architecture: The study area, Lat Masjid, is located in GPS coordinates of 22° 36' 4.6512" N and 75° 18' 8.8812" E in Dhar town of Madhya Pradesh, India, which is a centrally protected monument by the Archaeological Survey of India (12) (Figure 1.). The 15th heritage building consists of a huge courtyard with post and lintel arcades on all edges. The north, south, and east side arcades are shallow and only one inlet deep. The hall of prayer has four bays and a dome over the mihrab. The raised platform inside the prayer hall likely served as a royal gallery used by the Sultan, comparable to the platform in the Adina Mosque in Pandua. Entrances feature a gatehouse built in the eclectic style favored in the fourteenth and fifteenth centuries (13). The Lat Mosque has drawn the

attention of visitors, scholars, and archaeological scientists due to its Persian inscription on the iron pillar (14).

Sample collection and identification of microflora:

Biological samples were collected from eight different locations of the heritage building according to standard protocol as demonstrated by Aitken 2023 (15). Images of collected samples were displayed in figure no. 2. The microbial culture from isolated samples was done by the spread plate method (16, 17, 18). Microbial analysis was done by the method demonstrated by Ibrahim 2018 (19) and Basak 2021 (82) with some slight modifications. Standard protocols were followed to identify the lichens and algal population as described by Rai et al. 2014, Wolseley 2015, Dobson 2018, and Sunoj 2021 (20, 21, 22, 23).

Monitoring and Assessment of Environmental Parameters:

The microenvironmental parameters data were recorded continuously for 12 months (Year 2023) with the help of Handy Gaseous and particulate matter sampler (APM821, Handy Sampler, Envirotech) and Digital Data logger (Make RS-Pro 5322 handy) for humidity, temperature, and particulate matter (PM2.5) precipitation in the premises of the heritage building. The assessment methods were described in Ullo et al. 2020 (24).

Scientific Protection methods: The conservation methods to protect and preserve the exterior stone surface of the heritage building of Lat ki masjid were adapted as described by Plenderleith 1979, Kumar et al. 2021, and ASI 2016 (25, 26, 27).

RESULTS AND DISCUSSION

Algae and lichens: Lichens represent the symbionts of fungi and algae or fungi and cyanobacteria. Algal layers were confirmed on the exterior stone surface on the monument, which is confirmed with comparison by the standard (79, 80). The presence of lichens on the exterior wall surface of the monument was visibly evident. Fructose and Folios Lichen were identified as per the standard procedure (16, 17, 18, 81). and their macroscopic structure was studied under the image analysis described in figure 2.

Microbes: The results of bacterial colonies indicate on nutrient agar media the presence of both Gram-negative bacilli and Gram-positive cocci *Staphylococcus aureus* having a total CFU of 1.3×10^7 , 2.92×10^8 , and 2.82×10^8 . The obtained yellow colonies confirmed the presence of *Staphylococcus aureus*, and white color colonies were Actinomycetes, as observed in figures 3(A) and 3(B). Brown and green-colored colonies of fungi were identified as *Aspergillus niger* and *Aspergillus fumigatus*, respectively, after the complete growth on the Dichloran Rose-Bengal Chloramphenicol (DRBC) agar media, which were observed during the examination as described in figs. 3(D) and 3(E). A visible appearance of cyanobacteria species growth on BG-11 nutrient broth was observed in figure 3(C) (15, 16, 17, 18, 19, 20, 72). The optimum conditions required for identified microbes for growth on the stone substrate are described in Table 1, which was reported in several studies.

Assessment of Environmental Parameters and correlation with microbial growth

Temperature: Measurements of temperature fluctuation are described in figure 4A, which shows that the temperature was lowest in the month of January and gradually increased up to

the month of May and then decreased up to the month of September and slightly increased in the month of October and then decreased up to the month of December. The greater deference also observed between the maximum and minimum temperature in all months that means fluctuation of temperature were very high. Increased temperatures can lead to the proliferation of various microorganisms, such as bacteria, algae, and fungi, which can colonize the surfaces of stone structures. These biological agents can contribute to the weathering and erosion of the stone, causing physical, chemical, and biological damage (1, 2, 28, 73).

Relative humidity: Measured data of relative humidity were described in figure 4B. Maximum RH was observed in the months of December, January, June, July, and September, whereas the minimum measurement of RH was shown in the month of April. During the average values, minimum RH was identified in the month of April and The RH was increased from the month of February up to the maximum RH, which was reported in the month of July, and then the values of RH decreased. High relative humidity can promote harmful chemical reactions, facilitate the growth of mold and fungi, and encourage insect activity, all of which can lead to the gradual breakdown of the stone and other materials that make up these structures (29). Conversely, extremely low relative humidity can also cause desiccation and embrittlement of materials, further exacerbating the problem (30, 73).

Particulate Matter 2.5 (PM2.5): The data of particulate matter 2.5 micron (PM2.5) were described in figure 4c. Maximum PM2.5 was identified in the month of May. The graph shows that the value of PM2.5 was minimum in the month of July and then gradually increased up to the month of January. The measured amount of PM2.5 was within the permissible limits. Particulate matter from different sources, such as long-range transport or local emissions, may have distinct impacts on biological growth on stone substrates. The relationship between PM2.5 concentration and biological growth on stone surfaces is an important aspect to consider, as stone-based structures and monuments can be susceptible to deterioration due to microbial colonization (31, 32, 33, 74).

Oxides of Sulphur and Nitrogen: Data of measurements of SO₂ are described in figure 4D and NO₂ in figure 4E. In the analysis, the maximum concentration of SO₂ was in the month of February, and the minimum was in the month of June, whereas the maximum concentration of NO₂ was in the month of December, and the minimum was identified in the month of April. All gaseous pollutants were within the permissible limits. Gaseous pollutants, such as sulfur dioxide and nitrogen oxides, can have a detrimental impact on stone substrates. These pollutants can react with water to form acidic solutions, leading to the dissolution and erosion of stone materials (1, 30). Additionally, the deposition of particulate matter on stone surfaces can further exacerbate weathering processes (75, 76).

Precipitation: Precipitation data were described in figure 4F. The maximum concentration of precipitation was in the month of September, and the minimum was in the months of February and October. Precipitation, whether in the form of rain, snow, or other forms of atmospheric water, can have a significant impact on the weathering and deterioration of the stone surface of heritage buildings, which interacts with the stone surfaces and facilitates the growth of various microorganisms, such as algae, lichens, and fungi, which can significantly contribute to the overall deterioration of the stone surface of the cultural heritage, as highlighted in Table 1 of various studies (1, 34, 77).

Table 1. Optimum conditions required for identified microbes for growth on the stone substrate

S. No.	Name of Microbes	Temperature	pH	Oxygen Requirement	Salt Tolerance	Nutrient Requirements	Moisture/ Humidity	References
1	<i>Staphylococcus Aureus</i> (Gram Positive Bacteria)	35-37°C	7.0 -7.5	Facultative anaerobe (grows in both oxygen-rich and oxygen-poor environments)	7.5% NaCl (optimal); It can survive up to 15% NaCl	Glucose, amino acids, and proteins	Prefers Humid environment (up to 60%) but can tolerate in dry conditions.	35,36,37,38,39,40
2	<i>Actinomyces</i> (Gram positive Bacteria)	Optimal 25-30°C; For Thermophilic species 45-55°C. For Psychrotrophic species: Below 20°C.	6.5-8.0	Primarily aerobic (require oxygen for growth).	Most are sensitive to high salt, but some can tolerate up to 5% NaCl.	Need complex nutrients like proteins, amino acids, glucose and cellulose as carbon sources, organic nitrogen sources for nitrogen and carbohydrates.	Prefer moist environment (60-80%). It can withstand drier conditions due to spore formation.	41,42,43,44,45,46
3	<i>Aspergillus Niger</i>	Optimal: 30-35°C. Can tolerate a broader range from 15°C to 40°C.	4.0 to 5.0. Can tolerate a pH range from 2.0 to 8.0	Strictly aerobic, requiring oxygen for growth (well aerated conditions).	Sensitive to high salt, but some can tolerate up to 15% NaCl.	Glucose, starch, ammonium, sucrose for carbon, and nitrogen for growth.	Prefers high humidity conditions (up to the 60 %). Moist substrates facilitate mycelial growth.	47,48,49,50,51
4	<i>Aspergillus Fumigatus</i>	Optimal: 37-42°C. Can grow in a range of 12-55°C.	Optimal 6.5-7.5. Can tolerate a broader pH range of 4.5 to 8.0.	strict aerobic. Grows best in well-aerated environments	Tolerates low to moderate salinity, but high salt concentrations inhibit growth	glucose and sucrose, various organic and inorganic nitrogen sources such as ammonium salts or peptone.	Prefers high humidity (up to 60 %) and moist environments. Typically found in decaying organic matter with high moisture content	52,53,54,55,56,77,78
5	<i>Cyanobacteria</i> (Gram negative bacteria)	Optimal: 25-30°C. Growth can occur in a range from 15°C to 35°C, depending on the species. Require moderate light intensity (e.g., 30-60 μmol photons/m ² /s).	7.5-10 (alkaline conditions). Cyanobacteria can tolerate a broader pH range of 6.0 to 11.	Cyanobacteria are aerobic and require oxygen for respiration. Adequate aeration supports growth and prevents anoxic conditions	Freshwater species prefer low salinity, while marine species thrive at 35 ppt (typical seawater salinity). Halophilic cyanobacteria grow in hyper saline environment with salinities exceeding 100 ppt.	Need phosphorus, magnesium, calcium, potassium, and trace elements. Phosphate is a critical nutrient, and its limitation can restrict growth	Freshwater species required high water content	57,58,59,60,61,62
6	<i>Fruticose Lichen</i>	10-25°C. Growth can occur across a wide temperature range, from -5°C to 30°C, depending on the species. Most fruticose lichens are adapted to moderate temperatures but can tolerate extreme cold in alpine or arctic regions.	Optimal pH: 4.0 to 6.5. Fruticose lichens prefer slightly acidic substrates. They grow well on acidic rocks, tree bark, or soil in acidic environments.	Oxygen and Aeration: Fruticose lichens are aerobic and require good aeration. Pollution Sensitivity: Highly sensitive to air pollution, particularly sulphur dioxide and heavy metals.	Generally, prefer slightly acidic to neutral substrates	Require minimal nutrients, primarily relying on atmospheric inputs and rainwater. Nitrogen is supplied through atmospheric sources or, in some cases, through cyanobacterial photobionts that fix nitrogen.	Prefer high humidity environments (50-70 %).	63,64,65,66,67,68,
7.	<i>Foliose Lichen</i>	Optimal: 15-25°C. Growth can occur over a range of 5-30°C,	Optimal pH: 4.5-6.0.	Foliose lichens are aerobic and require good air circulation. Grow best in well-aerated environment.	Generally, prefer slightly acidic to neutral substrates	Require minimal nutrients, primarily derived from atmospheric inputs (rainwater, dust, etc.).	Prefer humid environments (40-60%) with frequent moisture availability	69,70,71

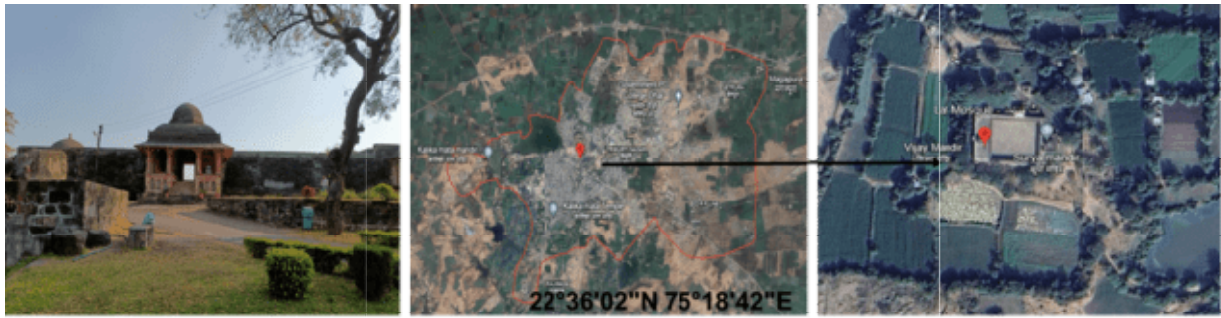


Figure 1. General view and GPS coordinates of Lat Masjid, Dhar, Madhya Pradesh a Fifteenth century cultural heritage

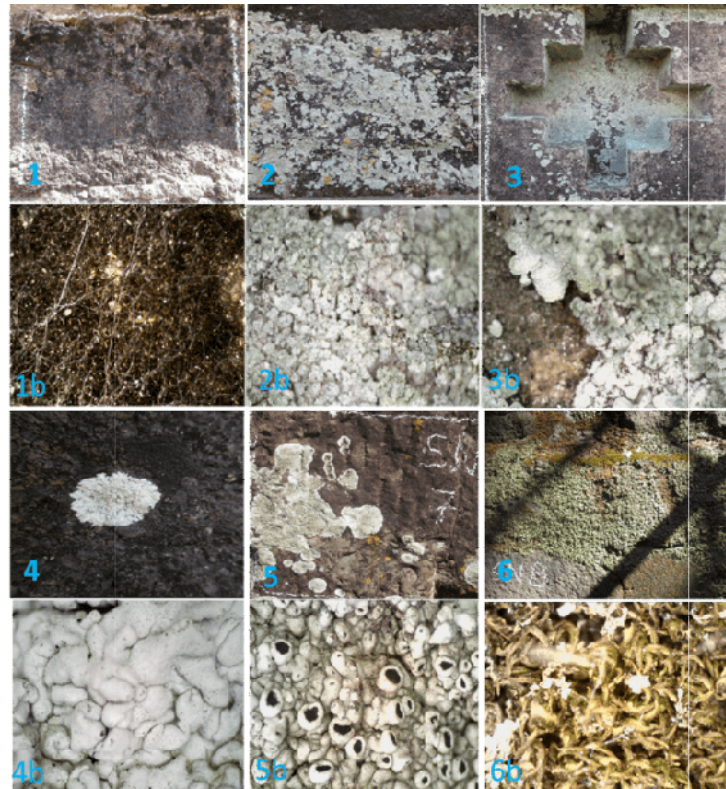


Figure 2. The Macroscopic and microscopic view of the biological growth on the stone substrate of Lat ki masjid, Dhar, Madhya Pradesh

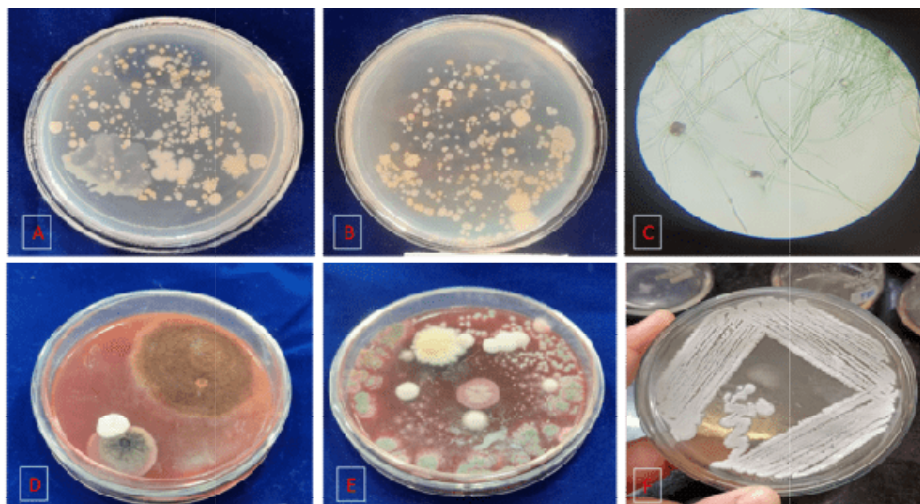


Figure : 3 Colonies of microflora present in the biological growth on the stone surface of Lat Ki masjid heritage building

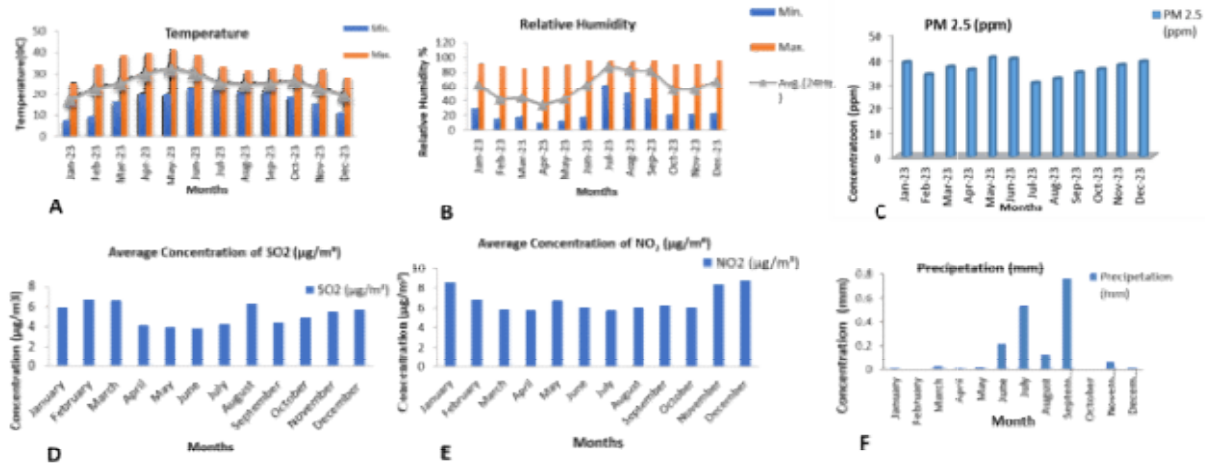


Fig. 4. Measurement data of environmental parameters in the ambience of Lat ki masjid 15th century cultural heritage

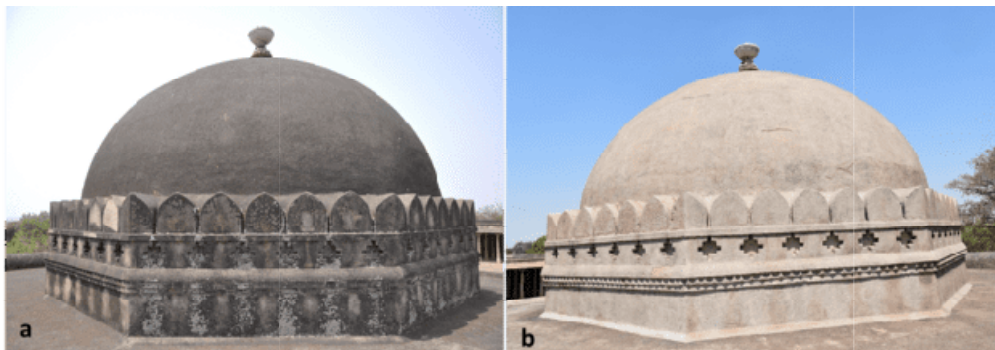


Figure 5 Scientific Preservation of Dome of Lat Ki Masjid, Dhar, Dist: Dhar a: before conservation, b: after conservation.

Conservation issues and Scientific preservation: The exterior stone surface of the 15th-century heritage building Lat ki masjid had become blackish due to the deposition of dust, dirt, and dried vegetation and micro-vegetation growth under hot and humid weather conditions as measured in this study. Thick dust layers were redeposited on the exterior surface of the heritage building over the period of time (Figure 5a). These depositions provided favorable conditions for the growth of microbes and initiated the biodeterioration of the stone surface. They also tarnished and reduced the aesthetic value of the heritage building. Such biogenic and micro-vegetation growth secretes plant acids that digest major oxides of the stones and cause weathering of the stone surface (26, 27). The layers of dust, dirt, and micro-vegetation growth were removed by applying a 2–3% solution of a suitable mild base with a non-ionic detergent with the help of soft jute and organic brushes. After the removal of layers, 1% of an aqueous biocidal solution was sprayed on the clean and dried surface of the heritage building of Lat ki masjid to prevent further growth of micro-vegetation (25, 26, 27); finally, a hydrophobic treatment (solution of silane siloxane) was applied on the dried surface of the heritage structure to stop the excess atmospheric water from entering the stones, as described in figure 5b (77, 78).

CONCLUSION

As per the planned study, *S. Aureus*, *Actinomycetes*, *A. Niger*, *A. fumigatus*, cyanobacteria, and lichens foliose with fruticose were identified as biodeteriogens that were grown on the surface of the 15th-century heritage monument Lat ki masjid.

The measured data environmental parameters (temperature, the measured data environmental parameters (temperature, R.H., SO₂ and NO₂, and precipitation) indicate that the microenvironment was given the suitable atmosphere to grow the identified biodeteriogens. As per the conservation problems, the heritage building has been preserved to protect it from biological growth and environmental factors, particularly the impact of microclimates on the exterior surfaces of stone materials for long-term preservation of the 15th-century ancient heritage building of national importance

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