Research Article

Fungal and Mixed Microbial Colonization on Heritage Stone Surfaces in Gujarat: A Comparative Study of Queens Mosque, The Associated Tomb, and Rani-Ki-Vav

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Abstract

Biodeterioration of heritage stone surfaces is a natural process that occurs when fungi, cyanobacteria, algae, lichens, and bryophytes become ecological interfaces between the stone and the environment and interact with the substrate under varying conditions. Recent studies in heritage microbiology demonstrate that these colonizers are not single entities but form mixed microbial communities, affecting moisture retention, staining, mineral changes, and long-term stone decay. In the present study, fungal and mixed microbial colonization on three important monuments of Gujarat, Queens Mosque, Sarangpur, Ahmedabad (associated tomb near Queens Mosque), and Rani-ki-Vav, Patan, was compared. The goals were to determine the predominant colonizers, compare colonization patterns among the monuments, and evaluate the impact of environmental and architectural factors on biodeterioration. A descriptive, analytical, and diagnostic methodology was applied, including field survey, visual documentation, selective sampling, laboratory isolation of culturable fungi, and comparative ecological interpretation. The results revealed significant differences in fungal and mixed microbial colonization between the urban mosque-tomb complex and the humid stepwell setting. Dark fungal staining and dust-associated mixed films were the dominant features at Queens Mosque and the associated tomb, Sarangpur, Ahmedabad whereas greater development of green films, biofilms, lichenic incrustation, and bryophytic patches in shaded and damp recesses was observed at Rani-ki-Vav, Patan. Fungal isolates from the mosque-tomb complex comprised Aspergillus niger, Aspergillus sydowii, Penicillium citrinum, Fusarium sp., and Memnoniella levispora, with Aspergillus niger being the most common taxon cultured. The study concludes that biodeterioration of Gujarat's heritage stone is community-based, microhabitat-dependent, and materially significant, requiring site-specific conservation planning.

Introduction

During the past decade stone heritage has been increasingly investigated as an active surface environment, instead of being merely an inert construction material. Studies of monument microbiomes have revealed the presence of mixed communities of bacteria, cyanobacteria, algae and fungi on biodeteriorated stone, and that these communities vary with exposure, season and moisture regime. Experimental investigations of subaerial biofilms have also shown that supply of water can directly impact the development, persistence and interaction of biofilms with the stone surface. Moisture is one of the most significant factors for the interpretation of microbial stone deterioration nowadays [1, 2]. Fungi are particularly significant as one of the major biological colonizers, as they are found to be persistent on nutrient impoverished, dusty and intermittently wet stone surfaces. Fungi are found to be significant both visually and materially due to recent reviews of fungal colonization, blackening, pigment deposition, hyphal penetration, biochemical weathering, and biomineral interaction. However, during the same period, fungal colonization of monuments frequently is accompanied by colonization by other creatures, especially phototrophic films. This means that visible staining may be a component of a microbial succession, not a single-species phenomenon. The surface mineralisation and/or the biofingerprint on monuments also indicate the possibility of the preservation of evidence from deeper biological and physicochemical processes [3, 4]. The lessons learnt are of particular significance in Gujarat where a number of monuments are constructed using porous stone and are vulnerable to seasonal humidity, dust deposition, urban pollution, architectural shelter and microhabitat variation. In the present study, the focus is on the monuments of the Queen's Mosque with its adjacent tomb near the Queen's Mosque Sarangpur, Ahmedabad and Rani-ki-Vav Patan as they exhibited visible signs of fungal patches, green film, lichenic growth, deposits associated with mosses, roughening and powdering. The three sites—the tomb, Queens Mosque, Sarangpur, Ahmedabad and the stepwell of Rani-ki-Vav, Patan—indicate different kinds of ecological environments, with the stepwell of Rani-ki-Vav, Patan having a profile different from the tomb or the Queens Mosque, Sarangpur, Ahmedabad setting, both of which are characterized by urban and semi-sheltered sandstone environments where dust and intermittent wetting are factors [5, 6]. Queens Mosque at Sarangpur, Ahmedabad, is an important late-medieval monument attributed to Malik Qiwam-ul-Mulk Sarang, a noble associated with the court of Sultan Mahmud Begada. The mosque is characterized by a multi-domed architectural arrangement, arched entrances, and flanking minarets. Its architectural complexity, extensive stone surfaces, enclosed interior

zones, and sheltered recesses create varied microhabitats that can retain dust, organic deposits, and moisture. These attributes make the monument particularly relevant for investigating the relationship between environmental exposure, biological colonization, and stone deterioration. This paper is intended to make a comparison between the colonization of fungi and mixed microbiological communities in these three monuments and to correlate the colonization with the environmental exposure, architectural form and visible damage. The paper puts forth a field and laboratory diagnosis of the systems of colonization to argue for conservation of the stone heritage of Gujarat based on ecological diagnosis, and not by sweeping the visible growth.

Methodology

The research design of the investigation was descriptive, analytical, and diagnostic. The descriptive component recorded the visible evidence of biodeterioration, the analytical component investigated the relationship between colonizers and site conditions, and the diagnostic component provided evidence for conservation planning. The selected sites were Queens Mosque, the neighbouring tomb at Sarangpur (Ahmedabad), and Rani-ki-Vav, Patan.

The field survey was conducted systematically on plinths, lower wall zones, pillars, joints and fissures, carved recesses, perforated screens, sheltered corners, and deep shaded areas. The symptoms observed included black/brown stains, green films, crust-like deposits, lichenic cover, bryophytic patches, powdering, pitting, roughening, flaking, and loss of surface clarity. The growth was correlated with architectural placement and conditions, including shade, dampness, dust retention, and lack of ventilation, through photographs, field notes, and site coding.

Representative samples were taken aseptically from biodeteriorated surfaces with minimal disturbance of the substrate using sterile blades, spatulas, swabs, and forceps. Fungi were isolated primarily on PDA and by moist chamber incubation to obtain viable propagules from stone fragments and crust-associated material. Purified isolates were characterized by colony morphology and microstructures, while cyanobacteria, microalgae, lichens, and bryophytes were evaluated mainly based on field appearance, attachment, and habitat association. Dominant fungal taxa were identified using frequency and relative frequency, and the final interpretation was based on comparison of the three monuments according to the type of colonization, microhabitat vulnerability, and significance for deterioration.

In addition to field diagnosis and microbiological assessment, the Queens Mosque site was evaluated in relation to an implemented scientific conservation programme. The condition assessment documented substantial accumulation of superficial dust, dirt, organic deposits, and bat excreta on the façade and interior stone surfaces, including pillars, ceilings, and flooring. The presence of bats within the monument was associated with persistent soiling and an unpleasant interior odour, creating an additional conservation concern alongside visible microvegetational growth.

The conservation intervention comprised: (i) mechanical removal of loose dust, dirt, and superficial accretions using soft brushes; (ii) cleaning of microvegetational growth and adherent deposits using a 2–3% ammonia solution with non-ionic detergent in a 3:1 ratio, applied using soft nylon brushes; (iii) fungicidal treatment of exterior surfaces using a 2–3% aqueous sodium pentachlorophenate solution; (iv) consolidation of weak and fragile stone at side-wall locations using Wacker OH-100; and (v) hydrophobic treatment of external stone surfaces using a Wacker BS-290 and MTO mixture in a 1:14 ratio, applied wet-on-wet in two coats. The intervention was documented photographically before and after treatment.

Results and Discussion

The study revealed that the biodeterioration on the selected monuments was a result of mixed biological communities and not a single visible agent. All fungi, cyanobacteria, microalgae, lichens and bryophytes were also recorded occurring in relation to micro habitat variations in moisture, shade, roughness and dust build up. This pattern of the monument is compatible with the recent research that has suggested that the biofilms found on cultural heritage should be diagnosed on a case-by-case basis due to the different taxa that will be predominant depending on the environment. It also concurs with recent research indicating that the communities of micro-organisms on monument stone are structured communities, not random contaminants of the stone surface [7, 8].

Table 1. Comparative distribution of high-risk microhabitats, dominant colonization forms, and environmental controls on selected heritage stone monuments of Gujarat.

Monument	High-risk microhabitats	Dominant visible colonization	Main controlling factors	Comparative reading
Queens Mosque, Sarangpur, Ahmedabad	Pillar bases, façade elements, lower wall zones, semi-sheltered surfaces	Black or dark fungal patches, dust-associated mixed films, localized staining	Urban dust, seasonal wetting, partial shelter, surface roughness	Stronger fungal staining on exposed and semi-exposed sandstone than at Rani-ki-Vav
Associated Tomb near Queens Mosque, Sarangpur, Ahmedabad	Jali screens, inner supports, gallery spaces, shaded interior zones	Persistent fungal growth, localized microbial deposits, mixed colonization in low-ventilation areas	Shade, retained dust, limited ventilation, localized moisture	Intermediate microenvironment between the open mosque and the humid stepwell
Rani-ki-Vav, Patan	Recessed carvings, stepped corridors, niches, damp lower zones	Green films, biofilms, lichenic incrustations, bryophytic patches, mixed colonization	Depth, persistent humidity, shade, water association, rough carved surfaces	Stronger lower plant and phototrophic colonization than the mosque-tomb complex



Figure 1. Before and after photos of Queen's mosque, Sarangpur, Ahmedabad scientific conservation work

Table 1 shows that the three monuments were not only colonized to different degrees but also in different ways, so that the expression of the deterioration was mostly one biological type on the one, another type on the other, and a third on the third monument. The dark fungal staining and mixed microbial films (MFM) associated with dust were a common feature in the Queens Mosque and its associated tomb due to their recessed, humid, water-connected architecture, whereas green phototrophic films, lichens and bryophytes were common in Rani-ki-Vav. This is in accordance with the recent evidence that the composition and function of biofilms are closely related to the microclimatic stress and local water dynamics [9, 10].

One of the most significant groups that colonised were the fungi, particularly in the mosque-tomb complex. Aspergillus niger, Aspergillus sydowii, Penicillium citrinum, Fusarium sp. and Memnoniella levispora have been isolated from the surfaces of monuments that are stained and deteriorated. The reoccurrence of these taxa indicates a stable and well developed fungal community under the conditions of the dusty rough, often damp, stone. This interpretation is in agreement with updated studies of fungal colonizers on stone heritage and their role in the process of blackening, pigment production and interaction with minerals [11, 12].

Table 2. Comparative characteristics of fungal and mixed microbial colonizers on heritage stone surfaces in Gujarat, including field appearance, ecological role, and biodeteriorative significance.

Colonizer group	Typical field appearance	Most associated monument condition	Ecological role in colonization sequence	Deterioration significance
Fungi	Black, brown, grey, or whitish patches; powdery or spot-like staining	Shaded, dusty, rough, and moist sandstone surfaces, especially in Queens Mosque and the associated tomb	Major heterotrophic colonizers that persist on conditioned stone surfaces	Associated with staining, pigment deposition, possible acid production, and weakening of stone
Cyanobacteria	Bluish-green to dark green films on damp surfaces	Moist and shaded zones with persistent wetness	Pioneer phototrophs initiating surface conditioning and moisture retention	Promote early biofilm formation and prepare substrate for later colonizers
Microalgae	Thin green surface films	Moist exposed or semi-shaded surfaces, especially in Rani-ki-Vav	Early phototrophic colonizers contributing to biofilm development	Increase moisture retention and support mixed succession
Lichens	Crustose or small foliose grey, yellowish, or greenish thalli	Rough, stable, weathered stone surfaces	Persistent long-term colonizers on already bioreceptive surfaces	Cause aesthetic masking, strong attachment, and physical-chemical interaction with stone
Bryophytes	Soft green cushion-like or velvety growth	Damp corners, joints, depressions, and low-ventilation niches, especially in	Advanced colonizers dependent on prolonged moisture	Retain water, intensify dampness, and support continued weakening

Table 2 reveals that the distribution of monument biodeterioration was not random, but rather established an ecological sequence. The pioneer phototrophs, cyanobacteria and microalgae, were more common on moist surfaces; fungi, lichens and bryophytes were more common on conditioned, roughened and moisture-retentive stone. This is consistent with studies that have shown that subaerial biofilms alter the behavior and surface

chemistry of water, which promotes later and more persistent colonizers [13]. The results also indicated that Aspergillus niger was predominant cultured fungus in the mosque-tomb complex with Aspergillus sydowii and Penicillium citrinum in the second and third ranks, respectively. This is ecologically significant as it means that the organism has colonized the sample repeatedly and not just been accidentally introduced. Fungus conservation studies in recent years also report that in exposed contexts, such as monuments, dark and stress-tolerant fungi can become stable parts of the surface community [14]. Material consequences of colonization were evident all across the study sites. Powdering, pitting, roughening, crust-like alteration, dullness, and masking of carved details were often observed in colonized areas and were linked to a weakening of the substrate due to the growth of biological organisms. This interpretation is corroborated by recent studies of biomineral deposits and stone surface fingerprints, which indicate that visible coatings and discolored deposits may reflect current processes of interaction between microorganisms, mineral phases and environmental inputs. The primary factor in all three monuments was environmental regulation. Moisture was the most significant driver as wet joints, recesses, plinth zones and protected corners encouraged the development of microbial and lower plant growth whilst fast drying exposed surfaces did not. This effect was enhanced by the presence of shade, lower ventilation rates, dust and stone surface roughness, which prolonged the wetness period and provided particle lodgment that allowed the recolonization of the stone. These findings, then, help validate the use of microhabitat diagnosis as a tool for conservation, particularly because seemingly identical signs can be observed from various colonization systems at different monuments [15].

Conclusion

he study reveals that fungal and mixed microbial growth on Gujarat's heritage stone surfaces is monument-specific, environment-specific, and materially significant. Fungal staining was the main colonization feature at Queens Mosque and the associated tomb, Sarangpur, Ahmedabad while phototrophic, lichenic, and bryophytic colonization was most prominent at Rani-ki-Vav, Patan due to its humid, shady, and water-associated architecture, together with dust-associated mixed colonization in the urban and semi-sheltered sandstone environment. Fungal isolates from the mosque-tomb complex, particularly the prevalence of Aspergillus niger, indicated that dark staining was biologically significant and supported by a viable fungal community. The study also demonstrated that powdering, pitting, crusting, roughening, and loss of surface clarity were closely linked to biological growth, showing that biodeterioration is not merely an aesthetic issue but a dynamic and ongoing interaction between organisms, the environment, and the stone substrate. Therefore, moisture-prone and shaded areas should be prioritized during conservation, dominant colonizers should be identified at the site level, and environmental conditions that enable recolonization should be controlled. A monument-specific conservation approach is more appropriate than uniform cleaning or generalized treatment methods.

References

1. Liu, X., Koestler, R. J., Warscheid, T., Katayama, Y., & Gu, J. D. (2020). Microbial deterioration and sustainable conservation of stone monuments and buildings. Nature Sustainability, 3(12), 991-1004. https://doi.org/10.1038/s41893-020-00602-5
2. Li, Q., Zhang, B., Yang, X., & Ge, Q. (2018). Deterioration-Associated Microbiome of Stone Monuments: Structure, Variation, and Assembly. Applied and Environmental Microbiology, 84(7), e02680-17. https://doi.org/10.1128/AEM.02680-17
3. Yadav, S., & Purchase, D. (2025). Biodeterioration of cultural heritage monuments: A review of their deterioration mechanisms and conservation. International Biodeterioration & Biodegradation, 201, 106066. https://doi.org/10.1016/j.ibiod.2025.106066
4. Sanjurjo-Sánchez J, Alves C, Freire-Lista DM. Biomineral deposits and coatings on stone monuments as biodeterioration fingerprints. Sci Total Environ. 2024 Feb 20;912:168846. doi: 10.1016/j.scitotenv.2023.168846.
5. Sanjurjo-Sánchez J, Alves C, Freire-Lista DM. Biomineral deposits and coatings on stone monuments as biodeterioration fingerprints. Sci Total Environ. 2024 Feb 20;912:168846. doi: 10.1016/j.scitotenv.2023.168846.
6. Prasad, Gupta & Kumar, Agnihotri. (2024). Bio-Deterioration and Scientific Preservation of Queen's Mosque. Journal of Environmental Science and Pollution Research. 10. 498-500. 10.30799/jespr.247.24100301.
7. Ma C, Zhang X, Wu F, Liu X. Identifying keystone taxa and metabolisms of epilithic biofilms is crucial to the conservation of stone heritage from biodeterioration. Front Microbiol. 2025 May 27;16:1600865. doi: 10.3389/fmicb.2025.1600865

8. Li QZhang BYang X, Ge Q.2018.Deterioration-Associated Microbiome of Stone Monuments: Structure, Variation, and Assembly. Appl Environ Microbiol84:e02680-17.https://doi.org/10.1128/AEM.02680-17
9. Prieto, B., Vázquez-Nion, D., Fuentes, E., & Durán-Román, A. (2020). Response of subaerial biofilms growing on stone-built cultural heritage to changing water regime and CO2 conditions. International Biodeterioration & Biodegradation, 148, 104882. https://doi.org/10.1016/j.ibiod.2019.104882
10. Villa F, Ludwig N, Mazzini S, Scaglioni L, Fuchs AL, Tripet B, Copié V, Stewart PS, Cappitelli F. A desiccated dual-species subaerial biofilm reprograms its metabolism and affects water dynamics in limestone. Sci Total Environ. 2023 Apr 10;868:161666. doi: 10.1016/j.scitotenv.2023.161666.
11. Paiva DS, Fernandes L, Portugal A. Fungi and Stone Heritage Conservation: Friend, Foe, or a Bit of Both. J Fungi (Basel). 2026 Feb 11;12(2):128. doi: 10.3390/jof12020128.
12. Celi, D., Marvasi, M., & Perito, B. (2025). Conventional and innovative approaches to black fungi control for stone heritage preservation. Iubmb Life, 77(3), e70010. https://doi.org/10.1002/iub.70010
13. Villa F, Ludwig N, Mazzini S, Scaglioni L, Fuchs AL, Tripet B, Copié V, Stewart PS, Cappitelli F. A desiccated dual-species subaerial biofilm reprograms its metabolism and affects water dynamics in limestone. Sci Total Environ. 2023 Apr 10;868:161666. doi: 10.1016/j.scitotenv.2023.161666.
14. Vázquez-Nion D, Rodríguez-Castro J, López-Rodríguez MC, Fernández-Silva I, Prieto B. Subaerial biofilms on granitic historic buildings: microbial diversity and development of phototrophic multi-species cultures. Biofouling. 2016 Jul;32(6):657-69. doi: 10.1080/08927014.2016.1183121.
15. Sanjurjo-Sánchez J, Alves C, Freire-Lista DM. Biomineral deposits and coatings on stone monuments as biodeterioration fingerprints. Sci Total Environ. 2024 Feb 20;912:168846. doi: 10.1016/j.scitotenv.2023.168846.