Journal of Faculty of Archaeology (Qena)

Microbial Deterioration of Sandstone in the Temple of Isis at Shenhur, Qena, Egypt

Aya Hussein Mohamed [1], Mahgoub A. Ahmed[2], Abd-Elkareem, E. A[2] , Khalaphallah R.[3]

1. A postgraduate student, faculty of Archaeology, South valley university, Egypt.

2. Conservation Department, Faculty of Archaeology, South Valley University, Egypt.

3. Microbiology Department -Faculty of Agriculture - South Valley University, Egypt.

Corresponding author: Mahgoub A. Ahmed (mahgoub@arch.svu.edu.eg)

Microbial Deterioration of Sandstone in the Temple of Isis at Shenhur, Qena, Egyp

Abstract:

This study aimed to investigate the effects of microbes on the composition of archaeological sandstone at the Temple of Isis in Qus city, Egypt. Six samples of degraded sandstone were collected to evaluate the deterioration process and analyzed by using X-Ray diffraction (XRD), Scanning electron microscopy (SEM), and USB digital microscope. Microbial isolation revealed the presence of microorganisms such as Aspergillus niger, Penicillium sp, Fusarium sp, and Bacillus sp. These microorganisms play a main role in the degradation of sandstone. X-ray diffraction analysis identified minerals like quartz, calcite, anhydrite, and gypsum, influencing its susceptibility to microbial deterioration. Scanning electron microscopy (SEM) showed the microbial populations, microstructure, and the formation of biofilms and erosion on the sandstone. The USB digital microscope examination provided detailed insights into the extent of degradation, including pigmented spots, micro cracks, and granular disintegration. The research highlights the complex interactions between microorganisms and the sandstone substrate, contributing to the understanding of biodeterioration in cultural heritage preservation.

Keywords: Sandstone, Temple of Isis, Biodeterioration, cultural heritage, preservation.

1. Introduction:

The Temple of Isis, located in the city of Qus, Egypt, stands as a testament to the ancient Egyptian civilization and its religious practices(Widmer and Jasnow 2017). Dedicated to the goddess Isis, one of the most revered deities in the Egyptian pantheon. Temple of Isis dates back to the Greco-Roman period, constructed between the

2nd century BCE and the 3rd century CE(Widmer and Jasnow 2017). It comprises various structures, including a sanctuary, colonnades, halls, and courtyards, adorned with wall relives and religious inscriptions. The temple is vulnerable to environmental factors, such as temperature variations, humidity fluctuations, and exposure to ground water. These factors created a conducive environment for the growth and proliferation of microorganisms, leading to the degradation of the temple's surfaces and structural elements (Gambino, Ahmed et al. 2017) (Viles and Cutler 2012, Cappitelli, Cattò et al. 2020, Wu, Zhang et al. 2021).

Microbial deterioration of archaeological sandstone is a significant concern in the field of cultural heritage conservation (Liu, Meng et al. 2018, Gu and Katayama 2021, Li, Deng et al. 2021). Sandstone, a commonly used material in ancient architecture, is susceptible to colonization and degradation by a wide range of microorganisms (Urzì and Realini 1998, Gaylarde and Morton 1999, Lan, Li et al. 2010, Ravikumar, Rao et al. 2012, C. Gaylarde 2020, Liu, Koestler et al. 2020). Bacteria, fungi, algae, and cyanobacteria have been found to colonize sandstone surfaces, forming complex biofilms that can have detrimental effects (Gaylarde and Morton 1999, Gaylarde and Baptista-Neto 2021). Microorganisms contribute to the deterioration of sandstone through various mechanisms, including the production of acids, enzymes, and extracellular polymeric substances (EPS)(Gadd 2010, Gong, Wu et al. 2018, Negi and Sarethy 2019). These metabolic by-products can chemically and physically degrade the sandstone matrix, leading to the loss of structural integrity, granular disintegration, and the formation of micro-cracks. Moreover, the pigments produced by certain microbial species can cause discoloration and aesthetic alterations in the sandstone, further compromising its visual appeal

(Ranalli, Matteini et al. 2000, Warscheid and Braams 2000, Tiano 2002, Andreotti 2019).

The primary objective of this study is to identify the types of microorganisms responsible for the deterioration of the sandstone and to understand the underlying factors that promote their growth and activity. The study aims to discern the roles of different microorganisms in the decay process. the study seeks to bridge the gap between scientific analysis and cultural preservation, offering valuable insights into the complex interplay between microorganisms, environmental conditions, and sandstone deterioration within the unique context of the Temple of Isis.

2. Material and methods:

2.1. Microbial isolation:

Shenhur, situated in Upper Egypt between the prominent religious centers of Koptos and Thebes, was shaped by the theological influences of both regions. However, the primary influence on the temple at Shenhur was derived from Koptos. Consequently, the temple's decorative elements played a significant role in the local religious landscape, reflecting the predominance of the Koptos theological system in the region (as show in figure 1). Sandstone samples collected from the Temple of Isis at Shenhur to investigate the microbial communities present on the deteriorated surfaces. Sampling locations include visibly degraded, such as discolored patches, samples assigned a unique identifier for accurate tracking and documentation. three Microbial samples were collected using sterile cotton swabs. performed by gently rolling the swabs across the designated areas (as show in figure 2). Pressure applied uniformly to ensure consistent microbial collection. Prepared samples inoculated onto potato dextrose agar plates and incubated at 37°C for up to 7 days.

2.2. X-ray diffraction analysis:

X-ray diffraction analysis proves to be an invaluable asset in the realm of stone preservation, as it aids in both the identification of minerals within the stone and the evaluation of its degradation. For our analysis, we employed a Bruker D8 advance X-ray diffractometer, utilizing CuKα radiation and calibrating it against a quartz standard with a wavelength of 1.789. Mineralogical interpretations were made possible using PANalytical X'Pert High Score software.

2.3. Scanning electron microscope examination:

Four Specimens intended for scanning electron microscopy (SEM) were allowed to air-dry, then coated with a layer of gold using Balzers Union SCD 030. Subsequently, they were analyzed using the Hitachi Scanning Electron Microscope S-3200N.

2.4. USB digital microscope examination:

A portable USB digital microscope was employed to observe and analyze the surfaces of in site archaeological bio deteriorated sandstones. The USB digital microscope, specifically the PZ01 model, featured an image sensor with a resolution of 0.3 megapixels. It offered adjustable magnification settings spanning from 20X to 500X, with photo capture resolutions of 640x480 and 320x240. The intensity of the LED illumination light could be controlled using the provided adjustment wheel.

3. Result and discussion:

3.1. Microbial isolation:

The isolation of microorganisms from a microbially damaged sandstone surface has revealed the presence of distinct microbial entities, including Aspergillus niger, Penicillium sp, Fusarium sp, and Bacillus sp. These microorganisms play a main role in the degradation processes affecting the sandstone(Burford, Fomina et al. 2003). As is evident in the Temple of Shenhur in Qus. (as show in figure 3). Aspergillus niger and Penicillium sp, known for their metabolic versatility and enzymatic capabilities, potentially contribute to the deterioration through the secretion of organic acids and enzymes that initiate the biogenic dissolution of mineral components within the sandstone matrix (Franco-Castillo, Hierro et al. 2021). Fusarium sp, recognized for its resilience and ability to thrive in adverse conditions, can exacerbate deterioration through hyphal penetration and mechanical disruption of the substrate. Bacillus sp, known for its metabolic versatility and resistance, might influence sandstone degradation through biofilm formation, secretion of metabolites, and physical stress exertion. The collaborative action of these microorganisms, fueled by their varied metabolic activities and colonization strategies, underscores their intricate role in the degradation of the sandstone structure at the Shenhur Temple (Sáiz-Jiménez 1995, Negi and Sarethy 2019).

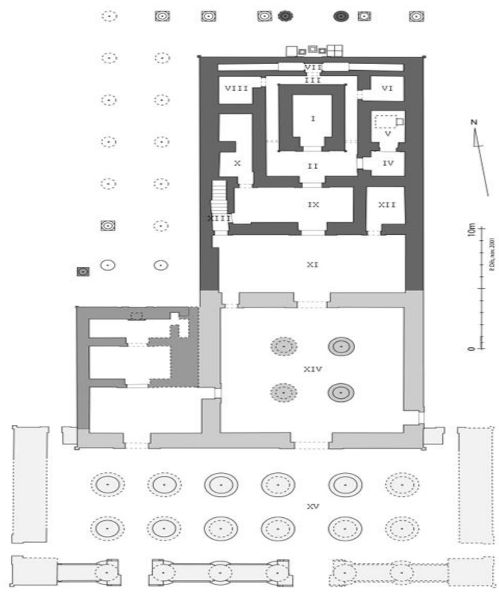

Figure 1. Ground plan of Shenhur Temple (Wendrich, W. et al. 2012).

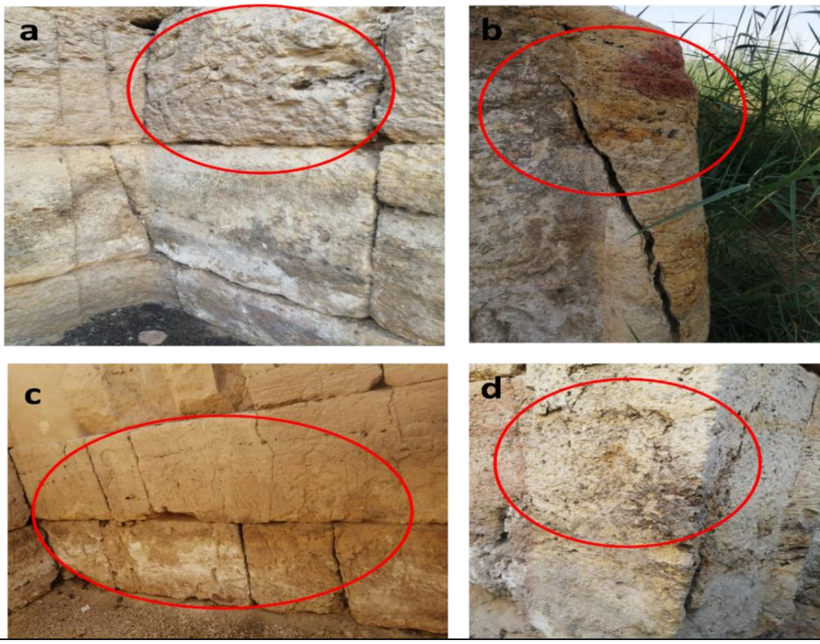

Figure 2. Biodegradation on the inner wall of the Temple of Isis at Shenhur (a: granular disintegration of sandstone. B: detachment of stone due to microbial degradation. C: salt crystallization and biofilm formation and d: the biogenic dissolution of mineral components).

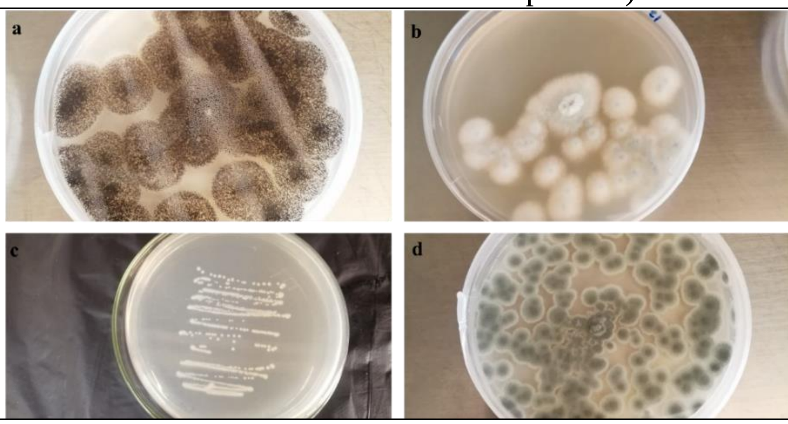

Figure3. microbial samples incubated at 37°C for up to 7 days, (a: aspergillus niger, b:fusarium sp , c: bacillus sp and d: pencillium sp).

3.2. X-ray diffraction analysis:

The analysis of the microbially damaged sandstone sample revealed a varied mineral composition, primarily consisting of quartz, calcite, anhydrite, and gypsum

(Bader 2014). These minerals significantly shape the sandstone's physical and chemical attributes, impacting its vulnerability to microbial deterioration. The presence of these minerals suggests complex geological processes that influenced the sandstone's formation and alteration. This mineral identification forms the basis for comprehending the sample's broader mineralogical context (as show in figure 4). The mineral composition of sandstone, which can include minerals such as quartz, calcite, anhydrite, and gypsum, holds a crucial connection to the stone's vulnerability to biodeterioration. These minerals exert influence on the sandstone through multiple mechanisms. Some microorganisms can utilize these minerals as a nutrient source, hastening mineral dissolution and weakening the stone's structure. Furthermore, the diverse mineral content creates distinct microenvironments that attract specific microbial communities, which can produce acidic byproducts, accelerating mineral decay. Additionally, minerals like anhydrite and gypsum undergo hydration and rehydration processes, causing volume changes within the stone, leading to physical stress and crack formation. These cracks, in turn, offer favorable sites for microbial colonization, further contributing to mineral breakdown. Overall, comprehending the intricate relationship between mineral composition and biodeterioration is vital for formulating effective preservation and conservation strategies to mitigate the impact of microbial deterioration on sandstone structures. (Fahmy, Molina-Piernas et al. 2022) (AG 2023).

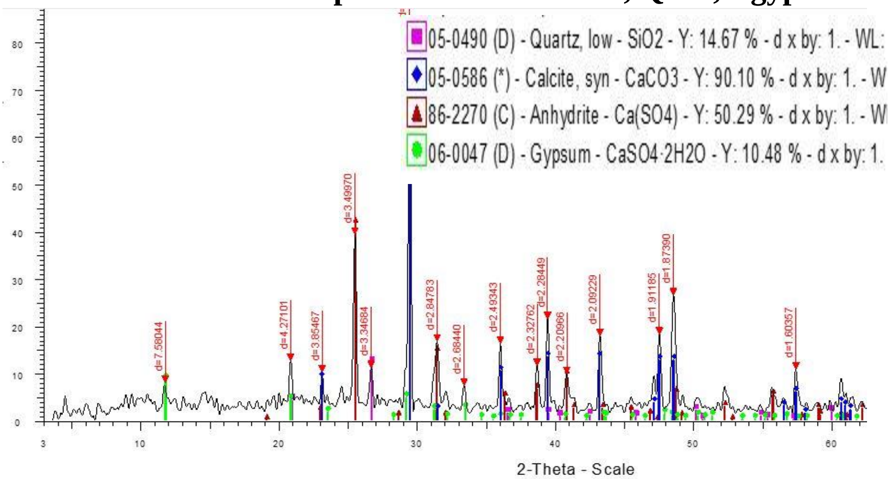

Figure 4. XRD diffractogram of the microbially damaged sandstone sample revealed a varied mineral composition, primarily consisting of quartz, calcite, anhydrite, and gypsum.

3.3. Scanning electron microscope examination:

Results showed that SEM is an effective tool for identifying the microbial populations present in bio deteriorated sandstone samples and determining their distribution and colonization patterns. Additionally, SEM was used to gain insights into the microstructure of the sandstone samples, including the presence of microfossils, cracks, and pores. Furthermore, research has proven that microbial degradation led to the formation of biofilms, the erosion of the surface, and the formation of micro-pits (as show in figure 5). the research demonstrate that SEM is a valuable tool for studying the microbial degradation of sandstone and can provide new insights into the biotic processes that affect sandstone monuments and artifacts(Kakakhel, Wu et al. 2019). The presence of a biofilm is particularly indicative of microbial activity, as biofilms are formed by communities of microorganisms adhering to a surface and producing extracellular polymeric substances (EPS) that can help to protect them from environmental stresses (as show in figure 5 c) and antibiotics(Kokare, Chakraborty et al. 2009).

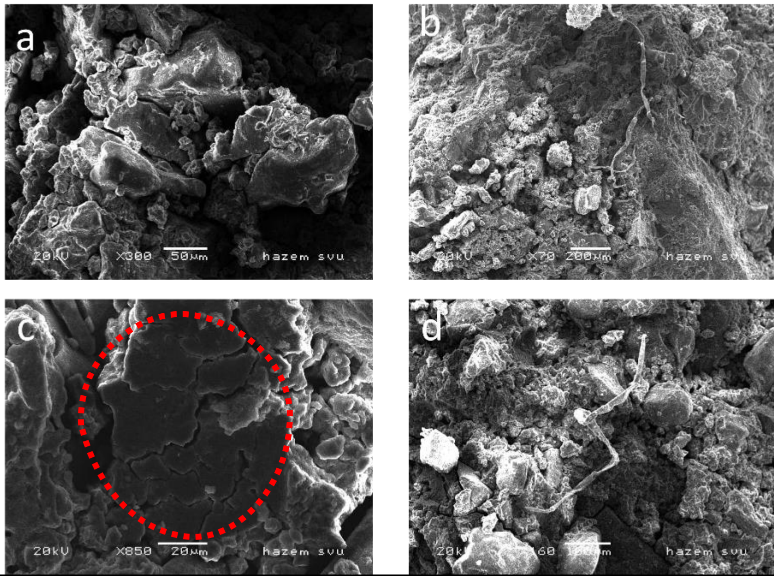

Figure5. SEM micrographs of deteriorated stone at Shenhur Temple (a: microcracks, b: growth of fungal hyphae, C: microbial biofilm and D: granular disintegration)

3.4. USB digital microscope examination:

The sandstone's surface, when scrutinized through the USB digital microscope's lenses, unveiled the presence of distinctive green and black pigmented spots, suggestive of microbial colonization. The observed occurrence of micro cracks traversing the surface underscored the mechanical impact of these microorganisms. Furthermore, granular disintegration patterns came into focus, highlighting the disruptive influence of microbial activities on the structural integrity of the sandstone. A remarkable phenomenon observed was the separation of individual sand grains within the sample, indicating a loss of intergranular cohesion attributed to microbial-induced processes (as show in figure 6). The combined observations from this s USB digital microscope investigation provide valuable insights into the complex interactions between microorganisms and the sandstone substrate, fostering a deeper understanding of the underlying mechanisms of degradation.

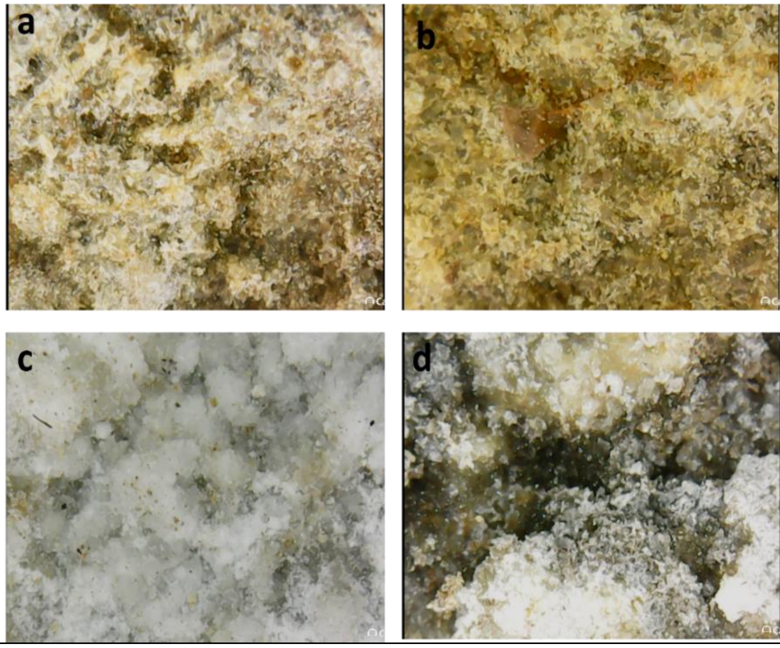

Figure 6. USB digital microscope examination of the deteriorated sandstone surfaces (a, b: growth of microbial biofilm. C: loss of intergranular cohesion attributed to microbial-induced processes, d: pigmented spots, suggestive of microbial colonization).

4. Conclusion:

The extensive investigation into the degradation processes affecting the sandstone of the Shenhur Temple has yielded significant insights through a comprehensive, multidisciplinary approach. The study incorporated microbial isolation, X-ray diffraction analysis, scanning electron microscope (SEM) examination, and USB digital microscope investigations. Microbial isolation revealed the presence of various microorganisms, including Aspergillus niger, Penicillium sp, Fusarium sp, and Bacillus sp, and highlighted their diverse roles in the sandstone's deterioration. X-ray diffraction analysis provided critical information about the sandstone's mineral composition, which includes quartz, calcite, anhydrite, and gypsum, emphasizing their influence on the sandstone's physical and chemical properties and susceptibility to microbial decay. SEM examination offered insights into microbial populations' distribution, colonization patterns, and the sandstone's microstructure, revealing features like microfossils, cracks, and erosion. The USB digital microscope examination detailed degradation features such as pigmented spots, micro cracks, and granular disintegration, shedding light on the intricate interactions between microorganisms and the sandstone substrate. In summary, this integrated study underscores the complexity of microbial-driven degradation in the context of cultural heritage preservation, with microorganisms' actions, mineral composition, and structural vulnerabilities of the sandstone all playing significant roles. These insights contribute to the broader scientific understanding of biodeterioration and provide a foundation for developing effective preservation strategies for sandstone-based heritage sites and artifacts, safeguarding them from microbial degradation.

5. Recommendations for treatment process:

Biocides are chemicals employed to manage and eradicate fungi and various microorganisms, serving as effective tools in preventing fungal growth and safeguarding archaeological sandstone from deterioration. While fungicides are specifically engineered to target and eliminate fungi, biocides have a broader spectrum, capable of eradicating a wide range of microorganisms, including fungi. However, caution must be exercised when using both fungicides and biocides, as their application may negatively impact stone preservation and the surrounding environment. Some chemicals can lead to discoloration or alter the stone's surface, while others may harm other microorganisms within the environment. Hence, it is crucial to employ these chemicals at the appropriate concentrations and seek guidance from professional conservators to ensure their responsible use. (Abd-Elhalim, Hemdan et al. 2023).

Nanoparticles have emerged as a promising and innovative approach in the realm of antimicrobial agents (Gambino, Ahmed et al. 2017, Fernando, Gunasekara et al. 2018). These minuscule structures, often on the nanometer scale, possess unique physical and chemical properties that make them highly effective against various microorganisms. The utilization of nanoparticles as antimicrobial agents involves their interaction with the cell membranes or internal components of bacteria, viruses, and fungi. This interaction can disrupt crucial cellular functions, leading to cell death or inhibition of replication(Nisar, Ali et al. 2019). Additionally, nanoparticles can exhibit a broad spectrum of activity, targeting a wide range of pathogens. Silver nanoparticles, for instance, have displayed potent antimicrobial properties by releasing silver ions that disrupt microbial cell membranes and inhibit enzymatic processes. Similarly, zinc oxide nanoparticles have shown efficacy against pathogens by producing reactive oxygen species that damage cellular structures. The controlled size and surface characteristics of nanoparticles can be tailored to enhance their antimicrobial effects while minimizing potential harm to human cells.

6. References:

1. Abd-Elhalim, B. T., et al. (2023). "Enhancing durability and sustainable preservation of Egyptian stone monuments using metabolites produced by Streptomyces exfoliatus." Scientific reports **13**(1): 9458.

2. AG, M. (2023). "CONDITION ASSESSMENT OF THE OSIRIS CHAPEL WALL PAINTINGS IN SETI-I TEMPLE, ABYDOS-EGYPT." Egyptian Journal of Archaeological and Restoration Studies **13**(1): 75-90.
3. Andreotti, S. (2019). "Biopolymers-based treatments for the protection of stone in the cultural heritage."
4. Bader, N. A. (2014). "The deterioration problems of the wall reliefs of Komir temple, Esna, Egypt." Mediterranean Archaeology and Archaeometry **14**(1): 201-219.
5. Burford, E., et al. (2003). "Fungal involvement in bioweathering and biotransformation of rocks and minerals." Mineralogical Magazine **67**(6): 1127-1155.
6. C. Gaylarde, C. (2020). "Influence of environment on microbial colonization of historic stone buildings with emphasis on cyanobacteria." Heritage **3**(4): 1469-1482.
7. Cappitelli, F., et al. (2020). "The control of cultural heritage microbial deterioration." Microorganisms **8**(10): 1542.
8. Fahmy, A., et al. (2022). "Salt weathering impact on Nero/Ramses II Temple at El-Ashmonein archaeological site (Hermopolis Magna), Egypt." Heritage Science **10**(1): 125.
9. Fernando, S., et al. (2018). "Antimicrobial nanoparticles: applications and mechanisms of action."
10. Franco-Castillo, I., et al. (2021). "Perspectives for antimicrobial nanomaterials in cultural heritage conservation." Chem **7**(3): 629-669.
11. Gadd, G. M. (2010). "Metals, minerals and microbes: geomicrobiology and bioremediation." Microbiology **156**(3): 609-643.

12. Gambino, M., et al. (2017). "Zinc oxide nanoparticles hinder fungal biofilm development in an ancient Egyptian tomb." International Biodeterioration & Biodegradation **122**: 92-99.
13. Gaylarde, C. C. and J. A. Baptista-Neto (2021). "Microbiologically induced aesthetic and structural changes to dimension stone." Npj Materials Degradation **5**(1): 33.
14. Gaylarde, C. C. and L. G. Morton (1999). "Deteriogenic biofilms on buildings and their control: a review." Biofouling **14**(1): 59-74.
15. Gong, B., et al. (2018). "Differential regulation of phenanthrene biodegradation process by kaolinite and quartz and the underlying mechanism." Journal of hazardous materials **349**: 51-59.
16. Gu, J.-D. and Y. Katayama (2021). "Microbiota and biochemical processes involved in biodeterioration of cultural heritage and protection." Microorganisms in the Deterioration and Preservation of Cultural Heritage: 37.
17. Kakakhel, M. A., et al. (2019). "Controlling biodeterioration of cultural heritage objects with biocides: A review." International Biodeterioration & Biodegradation **143**: 104721.
18. Kokare, C., et al. (2009). "Biofilm: importance and applications."
19. Lan, W., et al. (2010). "Microbial community analysis of fresh and old microbial biofilms on Bayon temple sandstone of Angkor Thom, Cambodia." Microbial ecology **60**: 105-115.
20. Li, J., et al. (2021). "The active microbes and biochemical processes contributing to deterioration of Angkor sandstone monuments under the tropical climate in Cambodia–a review." Journal of cultural heritage **47**: 218-226.

21. Liu, X., et al. (2018). "Water is a critical factor in evaluating and assessing microbial colonization and destruction of Angkor sandstone monuments." International Biodeterioration & Biodegradation **133**: 9-16.
22. Liu, X., et al. (2020). "Microbial deterioration and sustainable conservation of stone monuments and buildings." Nature Sustainability **3**(12): 991-1004.
23. Negi, A. and I. P. Sarethy (2019). "Microbial biodeterioration of cultural heritage: events, colonization, and analyses." Microbial ecology **78**(4): 1014-1029.
24. Nisar, P., et al. (2019). "Antimicrobial activities of biologically synthesized metal nanoparticles: an insight into the mechanism of action." JBIC Journal of Biological Inorganic Chemistry **24**: 929-941.
25. Ranalli, G., et al. (2000). "Bioremediation of cultural heritage: removal of sulphates, nitrates and organic substances." Of microbes and art: the role of microbial communities in the degradation and protection of cultural heritage: 231-245.
26. Ravikumar, H., et al. (2012). "Biodegradation of paints: a current status." Indian Journal of Science and Technology **5**(1): 1977-1987.
27. Sáiz-Jiménez, C. (1995). "Deposition of anthropogenic compounds on monuments and their effect on airborne microorganisms." Aerobiologia **11**: 161-175.
28. Tiano, P. (2002). Biodegradation of cultural heritage: decay mechanisms and control methods. Seminar article, new university of Lisbon, Department of Conservation and Restoration.
29. Urzì, C. and M. Realini (1998). "Colour changes of Notos calcareous sandstone as related to its

colonisation by microorganisms." International Biodeterioration & Biodegradation **42**(1): 45-54.
30. Viles, H. A. and N. A. Cutler (2012). "Global environmental change and the biology of heritage structures." Global Change Biology **18**(8): 2406-2418.
31. Warscheid, T. and J. Braams (2000). "Biodeterioration of stone: a review." International Biodeterioration & Biodegradation **46**(4): 343-368.
32. Widmer, G. and R. Jasnow (2017). "Illuminating Osiris: Egyptological Studies in Honor of Mark Smith." Illuminating Osiris: 1-498.
33. Wu, F., et al. (2021). "Community structures of bacteria and archaea associated with the biodeterioration of sandstone sculptures at the Beishiku Temple." International Biodeterioration & Biodegradation **164**: 105290.