

Biodeterioration of cultural heritage monuments: A review of their deterioration mechanisms and conservation

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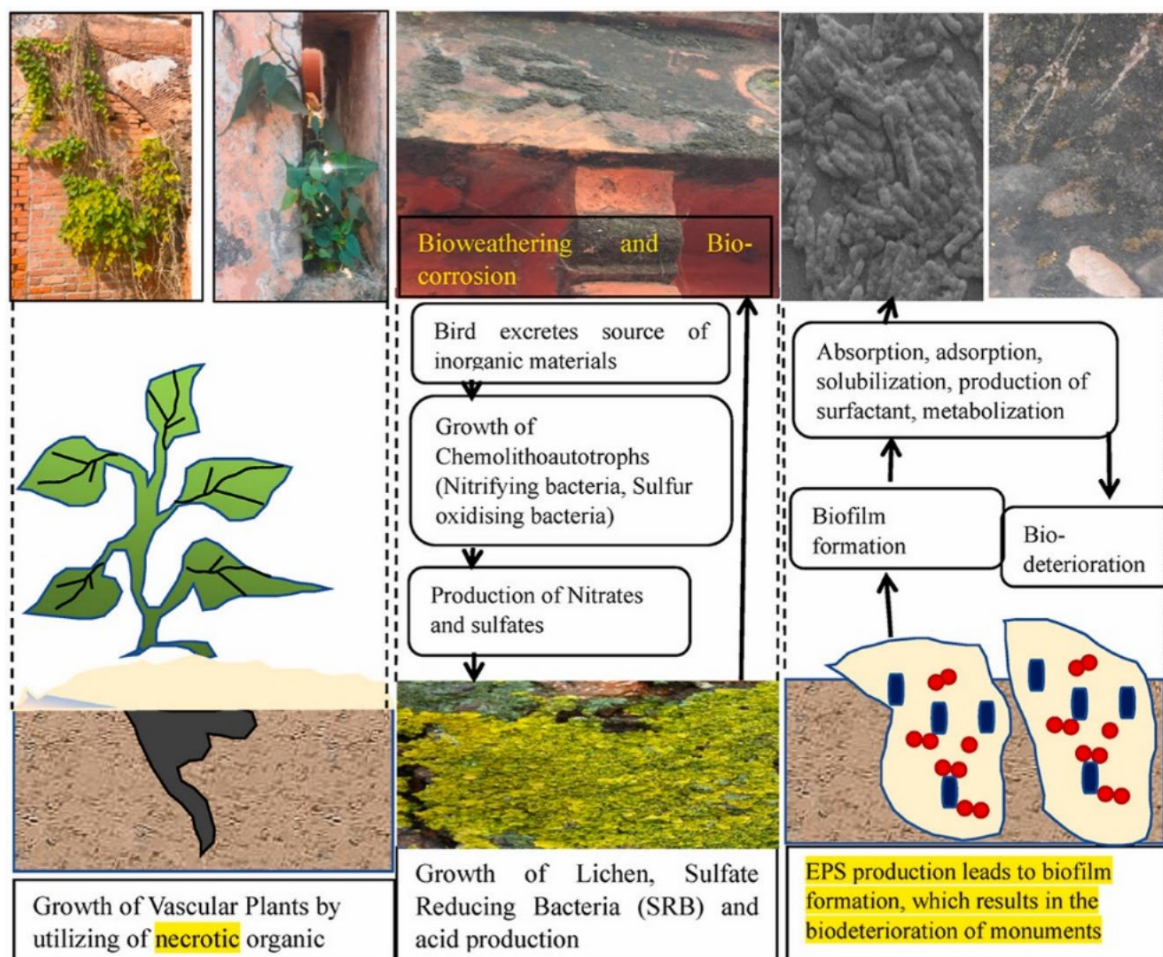
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Highlights:

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- Cultural heritage biofilms consist of phototrophs, chemolithotrophs and chemoorganotrophs
- Biofilm play important role in bio-deterioration of cultural heritages.
- Metal nanoparticles, oxides and hydroxides form protective layer for biodeterioration

Graphical abstract:



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Abstract

Geochemical cycles result in the chemical, physical, and mineralogical modification of rocks, ultimately leading to the formation of soil. However, when stones and rocks form part of historic buildings and monuments, the effects are deleterious. In addition, microorganisms colonize these monuments over time, resulting in the formation of biofilms, while microbial metabolites cause physical weakening and discoloration of the stone. This process, known as biodeterioration, results in a significant loss of cultural heritage. Purification and 16S rDNA sequencing of bacteria growing on heritage monuments revealed that the most prominent taxa were closely related to *Bacillus* spp., *Arthrobacter* spp., *Staphylococcus* spp. and *Paenibacillus* spp. In addition to bacteria, some fungal strains of the genera *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria* were also reported. To formulate effective conservation strategies to prevent biodeterioration and restore monuments, it is important to identify the microorganisms colonizing the substrate and the energy sources they utilize for sustenance. With this perspective, this review focuses on studies that explored the process of biodeterioration, the mechanisms by which microbes colonize and impact monuments, the techniques used to assess biodeterioration and conservation strategies designed to preserve the monuments.

Keywords: Biocorrosion, Biodeterioration management, Bioweathering, Cultural heritage, Encrustation, Reduction of biodeterioration, Patina

1. Introduction

Cultural landmarks, such as monuments, are major attractions for tourists and hence have a significant economic impact. Many historical monuments are composed of various materials including stones and other man-made materials. Various stones such as granite, marble, sandstone, limestone, slate and quartzite, which are formed from volcanic, metamorphic and sedimentary rocks, are used in historical monuments. Many of these stones contain various minerals, including Cu, Ni, Ca, Na, Mg, Fe and Al. The production and construction of blocks, structural slabs, flooring slabs, monuments, tombstones, sculptures, cobblestones, pebble stones and landscape garden stones are all now a part of India's stone industry. However, environmental factors, and man-made and microbial activities can lead to the degradation of monuments. For example, environmental processes such as temperature fluctuations, wind, water, capillary action, and solar radiation can damage the material structure (Branysova et al.,

2022). From an aesthetic perspective, encrustation or soiling resulting from the presence of organisms, their metabolic products or dead bodies, which form a microbial layer on the surface known as biofilm, has the most significant effects. The colonisation of microbes (biofilms) on heritage monuments is influenced by environmental conditions (Rather et al., 2021; Skipper et al., 2022). The majority of organisms can grow in warm and humid weather (Negi and Sarethy, 2019). Due to microbial activity and biofilm formation on the surface, these monuments deteriorate, leading to discolouration and a reduction in their aesthetic value.

The degradation of monuments due to biological activity is known as biodeterioration. Over time, stones are converted into sand and soil. This type of transformation is a major cause of permanent loss of heritage (Negi and Sarethy, 2019). Biological deterioration, which damages cultural heritage, involves animals, plants, bacteria, archaea, fungi, phototrophic organisms, particularly cyanobacteria (Liu et al., 2020; Skipper et al., 2022). In general, there are two mechanisms of biodeterioration, or categories into which deterioration can be divided: physical and chemical. The physical process is associated with growth processes and the migration of living things (e.g. root damage, rodent gnawing). Chemical deterioration, on the other hand, occurs within the stone materials of the monuments. There are two distinct processes involved in chemical biodeterioration: assimilatory and dissimilatory. By altering the material's properties during assimilation, organisms use the structural component of heritage monuments as a nutrient source, whereas during dissimilatory processes, organisms secrete waste materials or other substances that negatively affect the material quality. According to the findings of several researchers, stone deterioration may be due to the release of inorganic and organic acids by the microbes (Cappitelli et al., 2020; Negi and Sarethy, 2019). Metabolic acids produced by microorganisms cause solubilization and can promote biodeterioration (Maharana et al., 2021).

The mechanisms by which microbes deteriorate stone and metal are oxidation, reduction, acid dissolution, and elemental cycling. Natarajan et al. (2022) showed that autotrophs first grow on heritage buildings. When autotrophs die, they release carbon, ammonia and phosphate, creating an environment favorable for heterotrophs. These heterotrophs growing on heritage buildings accelerate the physico-chemical deterioration (Stanaszek, 2020). Photocyanotrophic microorganisms leave behind essential organic matter that heterotrophs require to flourish. The degradation of the stone is quickly accelerated by the colonisation of fungi and other heterotrophic organisms (Negi and Sarethy, 2019).

Purification and 16S rDNA sequencing of bacteria growing on heritage monuments revealed that the most prominent taxa were closely related to *Bacillus cereus*, *B. thuringiensis*, *B. anthracis* and *B. pumilus*. In addition to bacteria, some fungal strains of the genera *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria* have also been isolated from discolored building paints in Lagos, Nigeria (Obidi and Okekunjo, 2017).

Several reviews have been published on the deterioration of heritage monuments by individual organisms, but only a few parameters have been taken into consideration. This review aims to address this gap in knowledge by examining the general causes and sources of deterioration, as well as the factors influencing microbial alteration of the surface of cultural heritage buildings. Finally, the challenges, prospects and precautions for mitigating the deterioration process are discussed. This review also explores specific techniques and provides recommendations for preserving valuable cultural heritage buildings.

2. Mechanisms of biodeterioration of cultural heritage buildings

Many different types of stone have been used for the construction of monuments over the years, with the most common ones being marble and limestone (calcareous type), sandstone (primarily composed of iron oxide, quartz, and feldspar) and granite (siliceous type). They differ in porosity, alkalinity and hardness which influence their susceptibility to biodeterioration to varying degrees. A depiction of monument's biodeterioration and its mechanism is shown in Fig. 1.



Fig. 1. Biofilms of cyanobacteria and algae accompanied by the growth of vascular plants on a brick wall of the cultural heritage.

The primary biodeterioration mechanisms include the uptake of ions as nutrients, which are used in vital functions or incorporated into cell walls (Branysova et al., 2022) and the weathering of minerals caused by acidic or alkaline compounds produced by organisms. Acidolysis, which involves carbonic acid formed from the dissolution of CO_2 produced by microbial respiration, is a key factor in this process (Zhang et al., 2023). Other mechanisms include cation exchange, which has been linked to the release of K, Fe, Mg, and Al from minerals that are minerals that compose monuments.

Microorganisms involved in nitrogen (N) and sulfur (S) cycles also contribute to biodeterioration (Ding et al., 2020; Liu et al., 2020). Chemolithoautotrophic bacteria, which can be classified as epi-, hypo-, or endolithic microbiota, utilize CO_2 or HCO_3^- as their carbon source and inorganic compounds as electron donors. Their primary inorganic energy sources include sulfur compounds (such as $\text{S}_2\text{O}_3^{2-}$ and H_2S) and nitrogen compounds (such as NO_2^- and NH_3). The metabolic activities of these bacteria result in the production of inorganic acids, including nitric acid (HNO_3), nitrous acid (HNO_2), and sulfuric acid (H_2SO_4), which contribute to the dissolution of the host material. Cations are released in significant quantities from mineral particles in stone, and their high solubility in water leads to the formation of

secondary minerals, such as calcium oxalate. The microbial metabolization of these metal ions, facilitated by siderophores, weakens the stone structure.

Additionally, microorganisms in biofilms can produce a variety of organic molecules with multiple functions, such as pigments, phycobiliproteins for light harvesting, extracellular polymeric substances (EPS), lipids, proteins, and low-molecular-weight organic acids. These molecules are commonly identified in biodeterioration studies and on stone surfaces (Negi and Sarethy, 2019). Bio-pitting is a process in which bioerosion leads to the formation of pits on the rock surfaces of monuments, contributing to their deterioration (Sterflinger et al., 2013). Several fungal genera, including *Acremonium*, *Curvularia*, *Cladosporium*, *Penicillium*, and *Aspergillus* are involved in the process of bio-pitting (De Leo et al., 2022; Wenxia et al., 2020). These fungi contribute to the deterioration of stone surfaces through bioerosion.

Furthermore, cyanobacteria and algae, through their metabolic activities, contribute to the accelerated formation of crusts and biogenic coloration on stone surfaces, leading to a loss of aesthetic value. Dark-colored crusts on natural stone in buildings or monuments increase physical stress on the structural material by enhancing specific heat uptake, altering thermal-hygrometric expansion behavior, and increasing moisture retention (Ennis et al., 2021; Zhang et al., 2018). In addition to salt swelling, penetration by filamentous fungi also causes mechanical stress. Cyanobacteria can attach to calcareous stones, absorb water, and induce mass expansion, exerting internal pressure on the substrate. The death of these cyanobacteria and other autotrophic microbes provides organic matter that supports the growth of heterotrophic organisms.

2.1 Bioweathering or stone dissolution

Bioweathering refers to the process by which microbial communities contribute to the dissolution of rock surfaces through the secretion of metal-binding ligands and corrosive organic and inorganic acids. This microbial activity gradually breaks down the surface minerals of the rock. The interaction between microbes and minerals provides a valuable framework for researching the role of microbes in the geochemical transformation of monumental stones and artworks. This interaction highlights various strategies employed by bacteria to extract nutrients from mineral surfaces. Microbial communities require trace metals, phosphates, sulfates, and other essential metabolites to grow and thrive, all of which are sourced from the dissolution of stone.

The release of metabolic acids is one of the most well-known biogeochemical destructive mechanisms at rock surfaces (Gaylarde and Little, 2022). This process leads to the leaching of rock-binding materials and the consequent weakening of the crystal structure (Gu and Katayama, 2021; Liu et al, 2022). The net outcome of this type of biodeterioration is the physical and mechanical breakdown of the rock. Microbes produce organic acids whose anions react with the cations present in the stone to form salts that come to the surface from the pores, a process called efflorescence. Efflorescence creates a suitable environment for the growth of halophiles (Branysova et al., 2022). These salts cause physical stress leading to scaling, flaking, blistering, and granular disintegration. The subsequent hydration and swelling of salt within the stone pores ultimately result in cracking. Some organisms frequently isolated from this type of environment are species of *Bacillus*, *Staphylococcus*, *Kocuria*, *Micrococcus*, *Paenibacillus* and *Arthrobacter*.

Lichens and fungi contribute to the bioweathering process through the release of organic acids, chelating agents, and highly corrosive inorganic acids onto stone surfaces. These substances inadvertently accelerate the dissolution of stone, thus playing a significant role in bioweathering.

Lactic and acetic acids also react with calcium hydroxide in the stone to produce highly soluble calcium salts. As these salts leach out, they lower the pH within the stone's pores, weakening the binding agents in the cement and ultimately causing disintegration. Additionally, salts that precipitate inside the pores can recrystallize, exerting pressure on pore walls and causing physical damage by widening pores and cracks. Climatic factors such as rainfall, temperature, relative humidity, air pollution and biological activity all influence the degradation processes of stone (Otto et al., 2019).

2.2. Biocorrosion

The biocorrosion of monumental stone surfaces is primarily attributed to the release of inorganic and organic acids by various microorganisms, a process known as biogenic secretion. This release contributes to the breakdown of stone surfaces through acidolysis - the dissolution of stone materials by inorganic acids, such as sulfuric and nitric acids, produced by chemolithotrophic bacteria. These acids react with the stone, leading to its degradation. In addition, complexolysis occurs when the organic acids released by microorganisms break down stone materials. Mechanisms of biocorrosion in stone include acid production, exopolysaccharide production, and oxidative and reductive processes

involving sulfur-oxidizing bacteria, iron-oxidizing bacteria, and nitrifying bacteria. Lichens and mosses release carbon dioxide during cellular respiration, which contributes to the corrosive environment.

Organic acids like oxalic acid, malic acid, citric acid, formic acid, propionic acid, fumaric acid, and sulfuric acid produced by microorganisms, combine with air pollutants like NO₂ and SO₂, depositing on stone surfaces and providing nutrition to colonizing nitrifying bacteria (*Nitrosomonas sp.* and *Nitrobacter sp.*) and sulfur-oxidizing bacteria (*Thiobacillus sp.*). These microorganisms further produce nitric acid and sulfuric acid, respectively, accelerate the process of biodeterioration. Sulfate-reducing bacteria, known for their ability to convert sulfate into sulphites, are well-documented for damage to metals. *Desulfovibrio vulgaris* and *Desulfotomaculum nigrificans* produce H₂S, which induces corrosion on various surfaces.

Chemolithoautotrophic bacteria, such as *Nitrosomonas spp.*, *Nitrobacter spp.*, and *Acidithiobacillus spp.*, can release acids like nitrous acid, nitric acid and sulfuric acid, thereby altering the local pH. Chemoorganotrophic bacteria and fungi may release chelating organic compounds or weaken the mineral lattice by oxidizing metal cations such as Fe²⁺ or Mn²⁺ (Elzbieta, 2020; Kaur et al., 2014). Scanning Electron Microscopy (SEM) has revealed the presence of bacterial and fungal biofilms on the crystalline structure of substrates.

2.3. Stone encrustation

Stone encrustation refers to the deposition of mineral layers or crusts on stone surfaces, often associated with biofilms. Increased industrialization and fossil fuel combustion have led to elevated levels of SO₂ and NO₂ in the atmosphere, contributing significantly to bioweathering. When SO₂ and other particulate matter settle on stone surfaces, they cause the stones to darken and develop a yellowish-brown hue. A specific group of bacteria, including sulfur-oxidizing bacteria (*Thiobacillus sp.*) and nitrifying bacteria (*Nitrosomonas sp.* and *Nitrobacter sp.*), can thrive on marble surfaces. These bacteria oxidize sulfur compounds and atmospheric ammonia into sulfuric and nitric acids, respectively. These acids are highly corrosive and accelerate the dissolution and alteration of stone surfaces, a process known as biocorrosion.

When these acids react with the carbonates in the stone, they produce nitrate and sulfate salts. Upon contact with rainwater, these sulfates dissolve and form hydrated salts like gypsum. The formation of

gypsum often traps dust, diesel particulate matter, and carbonaceous particles (such as fly ash), resulting in a black or brown sulfated crust on the stone surface. The effects of sulfate encrustation on marble extend beyond aesthetic damage. When sulfates precipitate inside the pores of the stone and recrystallize, they exert considerable stress on the pore walls, leading to structural damage. The chemical composition of these sulfated crusts can vary based on their age and the specific air pollutants in the area.

Sulfur-reducing bacteria support sulfur-oxidizing bacteria by converting sulfates into sulfides, providing an additional energy source for the latter. The interaction of organic and inorganic materials produced by these microbes significantly impacts the stone's surface. As salt crusts and deposits accumulate in the pore spaces of limestone, they can lead to the formation of blisters. Eventually, these crusts burst and separate from the stone material, causing further detachment of the surface layer. In addition, monumental stones and artworks often contain both extremely halophilic and moderately halophilic microorganisms. These halophiles thrive on surfaces covered in hygroscopic salt deposits or efflorescences, which form when salt-containing water evaporates from the stone's surface.

2.4. Patina formation and calcium oxalate

Lichens and fungi produce oxalic acid, which reacts with calcium carbonate in the stone to form calcium oxalate, resulting in colored patinas on the stone surface (Attiya and Jones, 2022; De Deckker, 2019). The two primary forms of calcium oxalate found in nature are weddellite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) and whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$). These compounds often appear as patinas on the stones of ancient monuments and artifacts. Raman spectroscopy has identified calcium oxalate dihydrate in lichens such as *Dirina massiliensis* f. *sorediata*, *D. massiliensis* f. *massiliensis* and *Tephromela atra* and calcium oxalate monohydrate in *Lecanora sulfurea* and *Aspicilia calcarea*. Additionally, historical stonemasonry treatments and air pollution are considered partial contributors to patina development. The mechanisms of biodeterioration and patina formation are shown in Fig. 2 and 3.



Fig. 2. A summary of biodeterioration mechanisms.

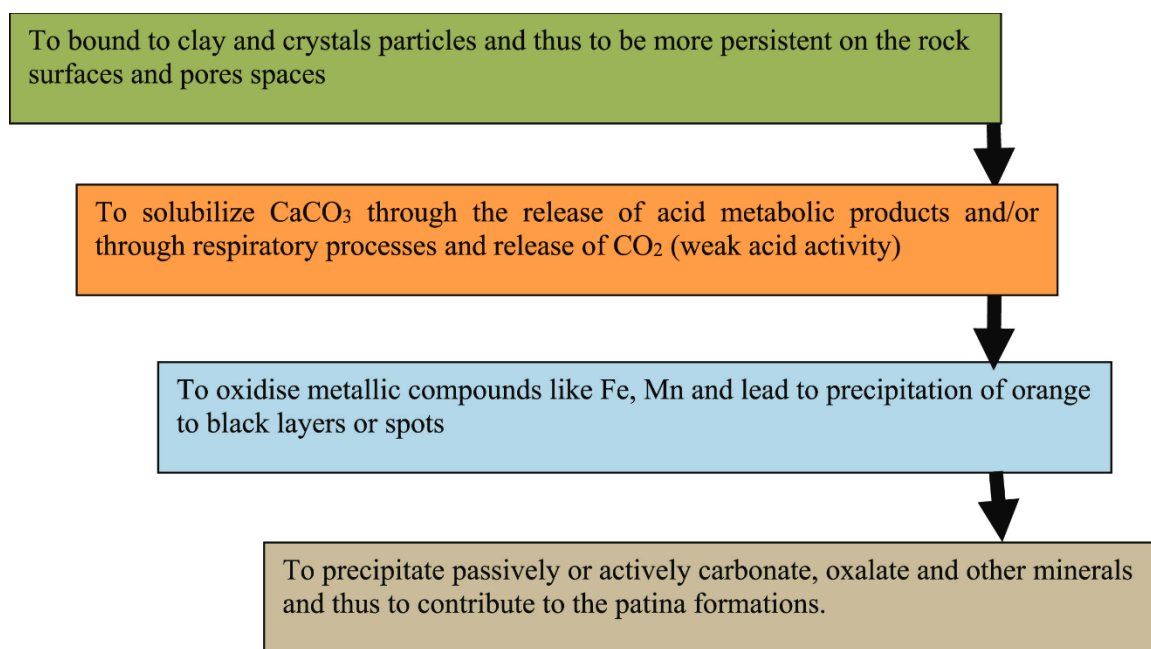


Fig. 3. Mechanisms of patina formation by chemoorganotrophic microorganisms.

2.5. Extracellular enzymes

The production of enzymes by living cells is crucial for specific biochemical reactions required for metabolic processes, with many reactions requiring a sequence of enzymes that vary in substrate specificity. These enzymes can differ across species and strains and are involved in processes such as hydrolysis, oxidation or reduction of substrates. Biofilms, which are dense aggregates of microorganisms, often contain extracellular enzymes excreted by the microbes. Extracellular polymeric substances (EPS) within biofilms provide protection to the enzymes against UV light, biocides, and cycles of desiccation and rehydration (Di Martino, 2018). Fungi like *Aspergillus niger* and *A. oryzae* produce a range of enzymes, including β -glucosidases and β -mannosidases, which break down polysaccharides such as xylan and cellulose into smaller, absorbable molecules. Several researchers have highlighted that the enzymatic activity of microorganisms colonizing rock surfaces contributes to the blackening of stone crusts (Kodarkar et al., 2024; Pozo-Antonio and Pereira, 2017). Furthermore, Warscheid and Braams (2000) proposed that tyrosinase released from dead microorganisms could lead to melanin synthesis during mineralization.

2.6. Biofilm on cultural heritages monuments

Bacteria on the surfaces of cultural heritage monuments can exist in two forms: as biofilms or as free-floating planktonic cells. Biofilms are structured microbial aggregates encased in a self-produced polymeric matrix that offers protection and enables survival and interactions among cells. Biofilms facilitate quorum sensing, a mechanism that enables microorganisms to communicate and coordinate their activities. The extracellular polymeric substances (EPS) produced by biofilms play several crucial roles, including enhancing cell adhesion, trapping airborne particles, synthesizing pigments, producing metal ions, and creating mineral and organic compounds. These processes contribute to monument discoloration and structural degradation (Skipper et al., 2022; Mehta and Shah, 2021). The effects of biofilms on monument degradation, including their role in trapping pollutants and altering microenvironments, are illustrated in Fig. 4.

Biofilms significantly impact the structural integrity of the materials where microbes colonize because they provide protection against harsh environmental conditions. Certain microorganisms, such as chasmolithic (which grow within structural cavities) and cryptoendolithic species (which inhabit fissures and cracks), can endure extreme environments by producing mucilaginous layers composed of extracellular polymeric substances (EPS).

Epilithic biofilms, commonly found on outdoor stone monuments, pose substantial conservation challenges. Shanshan et al. (2023) reported the presence of diverse microbial communities in biofilms on five outdoor stone sculptures. Among the identified taxa, *Pseudomonas*, *Deinococcus*, *Sphingomonas* and *Leptolyngbya* exhibit pigmentation capabilities, contributing to discoloration. In addition, *Pseudomonas*, *Bacillus* and *Beijerinckia* were implicated in nitrogen cycling, while *Acidiphilium* played a role in the sulfur cycle. These functional roles suggest that such taxa significantly influence biodeterioration processes. Furthermore, the study demonstrated that epilithic biofilms absorb stone minerals, as evidenced by strong correlations between metal concentrations in the stone and biofilm communities. The primary mechanism driving biodeterioration appeared to be corrosion caused by biogenic sulfuric acids. Geochemical analyses revealed elevated concentrations of sulfate ions (SO_4^{2-}) compared to nitrate ions (NO_3^-) and slightly acidic microenvironments on the stone surfaces. The presence of *Acidiphilium*, a sulfur-metabolizing microorganism, is particularly noteworthy, as it serves as an indicator of biodeterioration. These findings demonstrate the significance of

microenvironments in shaping biofilm community structures and their influence on monument deterioration. The role of biofilms in trapping environmental pollutants, modifying surface chemistry, and inducing microenvironmental acidity highlights the complexity of their impact on cultural heritage conservation.

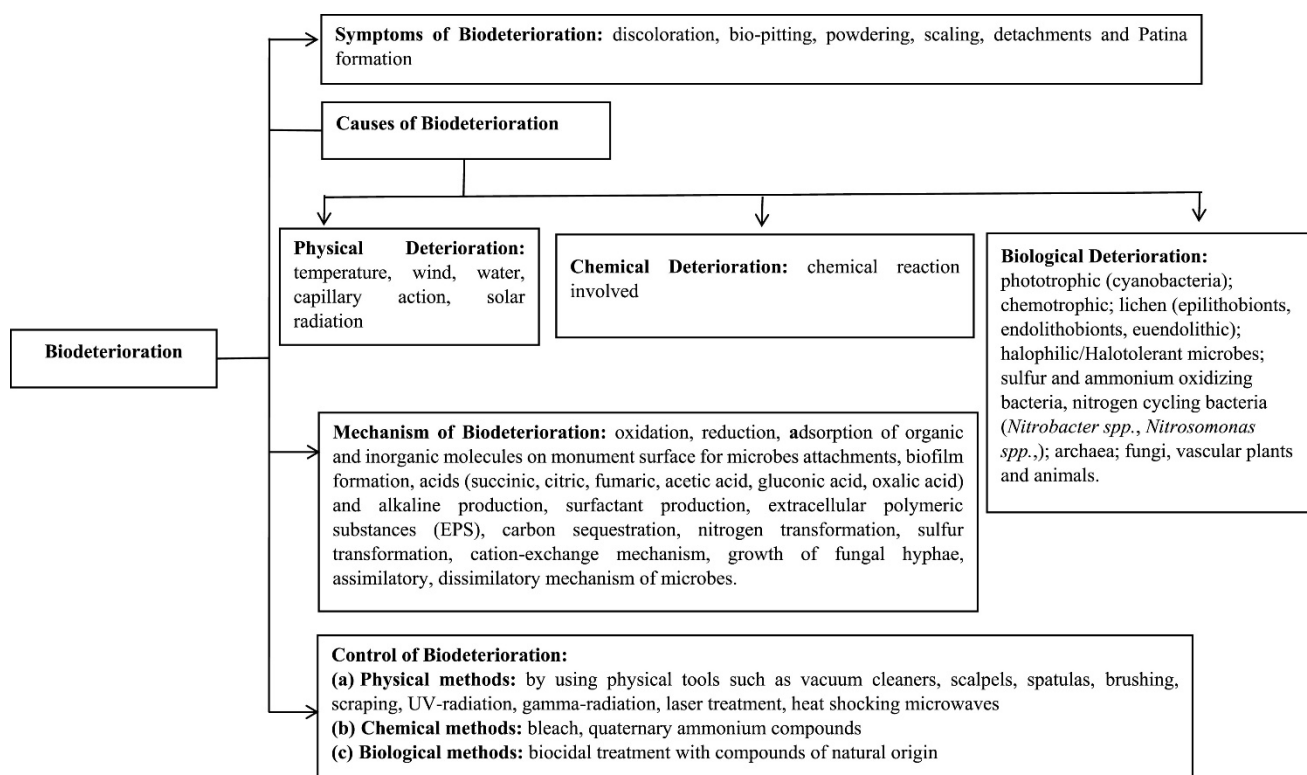


Fig. 4. Symptoms, causes and control mechanisms of biodeterioration of monuments.

3. Major groups of microorganisms responsible for biodeterioration

3.1. Deterioration caused by bacteria and cyanobacteria

Pioneer species, including cyanobacteria (*Gloeocapsa*, *Phormidium*, *Chroococcus*) and green algae (*Chlorella*, *Stichococcus* and *Chlorococcum*), play a significant role in the degradation of cultural heritage materials (Liu et al., 2022; Parulekar-Berde et al., 2020). These organisms thrive in diverse terrestrial habitats such as rocks, desert crusts, and building surfaces. Their ability to colonize various

environments leads to biofilm and crust formation, which appear bright in humid conditions and deep black during dry periods.

Cyanobacteria and microalgae are phototrophic organisms that produce colored pigments, leading to visible discoloration on heritage surfaces. Beyond aesthetic effects, they excrete chelating agents such as organic acids (e.g., oxalic and carbonic acids) that initiate the chemical and physical degradation of stone surfaces. For instance, these acids can solubilize minerals, causing micro-perforations that compromise the integrity of stone monuments (Bharti et al., 2017). Recent studies have highlighted the role of environmental factors, such as light exposure and surface geometry, in determining microbial colonization. For example, a study on an outdoor sculpture in Australia revealed distinct microbial communities based on exposure patterns (Catto, 2023). Cyanobacteria dominated well-lit, northwest-facing areas, while actinobacteria and proteobacteria were prevalent in shaded zones.

The deterioration of stone monuments may be attributed to the process carbonatogenesis (Rodriguez et al., 2015). For example, Li et al. (2018) isolated 85 bacteria among which 64% bacteria showed precipitation of carbonate (i.e. carbonatogenesis activity) during biomineralization assay. Microbial activity and growth result in the formation of biofilms on the surface, these monuments deteriorate leading to discolouration and a reduction in their aesthetic value.

The precipitation of CaCO_3 is also a key mechanism in cyanobacterial activity. During daylight, photosynthetic organisms deposit CaCO_3 , while at night, its solubility increases due to shifts in carbonate concentration. This cycle results in the deposition of vaterite and calcite, forms of CaCO_3 that contribute to stone biodeterioration. Analytical techniques such as Scanning electron microscopy (SEM) with energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD) and fluorescence imaging were employed to detect these precipitation processes during biodeterioration.

Biofilms on heritage monuments often include a mixture of algae and cyanobacteria, which dominate the phototrophic biomass (Albertano, 2012). In a study by Keshari and Adhikary (2014), 24 species of cyanobacteria from 11 genera were isolated from the surfaces of monuments and building facades in India. Molecular phylogenetic analysis using partial 16S rRNA gene sequencing of these species, along with those of other species isolated from stone surfaces in subaerial habitats and hypogeal environments in different parts of the world, revealed that genera *Chroococcidiops* clustered together (Keshari and Adhikary, 2014). Khaled et al. (2019) investigated the biodeterioration activity of bacteria on limestone

monuments from ancient Egypt and their treatments. They collected samples from affected parts of seven distinct ancient locations throughout Egypt and identified *Bacillus safensis* (MH370265) and *Streptomyces rochei* (MH370266) as major contributors to CaCO_3 degradation, with degradation rates of 20.9% and 25.6%, respectively. In addition, samples from various monuments contained populations of sulfur-oxidizing bacteria (*Thiobacillus* spp.) and nitrogen-cycling bacteria (*Nitrobacter* spp., *Nitrosomonas* spp., *Nitrospira* spp.).

Numerous *Streptomyces* species strains have been identified on stone and mural paintings in ancient Egypt (Abd-Elhalim et al., 2023). These species are primary agents of color changes in tomb paintings due to their production of hydrogen sulfide, biopigments (such as melanin) and various acids (e.g. oxalic, citric and succinic acid).

Heterotrophic bacteria, particularly from the Phylum Firmicutes, were commonly found within and on stone monuments. Among these, *Bacillus* spp. were notable for their diverse nutrient versatility and spore-forming ability. Halotolerant bacteria including *Kocuria* spp., *Micrococcus* spp., *Arthrobacter* spp., *Bacillus* spp., *Staphylococcus* spp. and *Paenibacillus* spp. were notable isolates contribute to stone deterioration by producing acids and pigments. Within the epi-, hypo-, or endolithic microbiota, chemolithoautotrophic bacteria utilize CO_2 or HCO_3^- as a carbon source and inorganic compounds as electron donors. Sulfur compounds ($\text{S}_2\text{O}_3^{2+}$, H_2S) and nitrogen compounds (NO_2^+ , NH_3) are the two primary categories of inorganic energy sources. The inorganic acids HNO_3 , HNO_2 , and H_2SO_4 , produced as a result of their metabolic activity, can dissolve the hosting material. *Thiobacillus* and *Nitrobacter* spp. as well as *Nitrosomonas* and *Nitrospira* spp. are sulfur-oxidizing and nitrogen-cycling bacteria, respectively, found in various monuments. Numerous *Streptomyces* species have been identified from ancient Egyptian mural paintings and monuments. *Streptomyces* species are known to be the main producers of hydrogen sulfide, biopigments (melanin) and different acids (oxalic, citric and succinic acid), which are responsible for color shifts in tomb paintings.

A study conducted by Mehta and Shah (2021) showed the microorganisms growing on specific monuments within the Champaner-Pavagadh complex in Gujarat, India, a UNESCO World Heritage Site. Microalgae and cyanobacteria were separated from biofilms gathered using non-destructive techniques and identified by 16S rRNA gene sequencing. High-performance thin-layer chromatography (HPTLC) was used to extract, hydrolyze and analyze the exopolysaccharides of each isolated strain.

The study reported distinct strains from the genera *Desmonostoc*, *Nostoc*, *Leptolyngbya*, *Chroococcidiopsis* and *Asterarcys*, comprising five cyanobacteria and one microalga. Maximum parsimony analysis was employed to assess the relationships between the substrate specificity of these isolated biofilm organisms and those found worldwide, aiming to produce a consensus phylogenetic tree. The five isolated cyanobacteria strains were found to be closely related to cyanobacteria from tropical regions. However, no correlation was found at the generic level between the species and substrate specificity. Exopolysaccharide analysis revealed seven monosaccharides in the isolated strains. All examined species contained glucose with high concentrations of either fructose or arabinose. Ammonia-oxidizing archaea (AOA) play an important role in the nitrogen cycle and can lead to the biodeterioration of monuments, as reported by Liu et al. (2020). *Nitrosococcus*-like AOA such as *Nitrosococcus tajmahalensis*, can produce nitric acid (HNO_3) and other nitrogenous compounds by oxidizing ammonia (NH_3) to nitrite (NO_2^-). Marble and other calcareous materials can deteriorate due to a lowered pH caused by the production of nitric acid and other acidic compounds. AOA can also play a role in biological weathering by generating extracellular polymeric substances (EPS) that can adhere to mineral surfaces and cause the formation of biofilms and the deterioration of the underlying materials. On the other hand, sulfur-reducing bacteria convert sulfate ions (SO_4^{2-}) into the hazardous and caustic gas hydrogen sulfide (H_2S). Sulfur reducing bacteria produced hydrogen sulfide, which reacts with metal ions, including iron and copper, to form metal sulfides, which can cause corrosion and material degradation. Sulfur reducing bacteria secrete enzymes such as thiosulfate reductase and sulfite reductase that can degrade materials that contain sulfur. SRB can form biofilms on surfaces, which can shield the bacteria from the environment and speed up material deterioration. Examples of organic matter that chemoheterotrophs break down into simpler compounds to release energy and nutrients from include proteins, carbohydrates and lipids. Amylases, proteases and lipases are examples of complex organic molecule-degrading enzymes that are produced by chemoheterotrophs. The oxidizing agents hydrogen peroxide and superoxide, which are produced by chemoheterotrophs, have the ability to oxidize and degrade various materials.

Chemoheterotrophs can discolor materials such as stone and concrete because they generate pigments such as melanin and encrustations. Acids and oxidizing agents produced by chemoheterotrophs have the potential to structurally damage materials like concrete and mortar. Microbes that colonize surfaces produce osmotic protectants such as polyols. Glycerol contributes to the expansion of micaceous

minerals, along with other polyols like glucose. The polyols present in these microbes protects them against freezing, desiccation, and excess salt by forming hydrogen-bond linkages with the polysiloxane crystals in the mica components. Microorganisms and mechanisms involved in the biodeterioration of monuments are shown in Table 1 and Fig. 2.

Table 1 Microorganisms and mechanisms involved in the biodeterioration of monuments.

Family	Species	Mechanisms of Biodeterioration	References
Bacteria			
Cyanobacteria, <i>Chlorella</i> spp or <i>Chlorococcum</i>	<i>Hassallia</i> , <i>Tolypothrix</i> , <i>Scytonema</i> , <i>Lyngbya</i> , <i>Calothrix</i> , <i>Aulosira</i> , <i>Nostoc</i> , <i>Camptylonema</i> , <i>Dichothrix</i> , <i>Chlorogloeopsis</i> , <i>Westiellopsis</i> , <i>Pleurocapsales</i> spp, <i>Chlorella</i> sp or <i>Chlorococcum</i> sp	Aesthetic and chemical deterioration: biofilm formation, color alteration, patina formation, crust formation, bioweathering as a consequence of calcium uptake, precipitation of calcium salt and formation of secondary minerals i.e. calcium oxalate, acidification, extracellular polymeric substances (EPS), siderophore formation, sulfur reduction, sulfur oxidation,	Li et al., 2018; Stratigaki et al., 2024
Chemoautotrophic bacteria	Sulphur oxidizing bacteria, Nitrifying bacteria include <i>Nitrosomonas</i> and <i>Nitrobacter</i>	Chemical deterioration: Black crust formation, conversion of sulfate into sulfite which act as a source of nutrition for sulfur-oxidizing bacteria	Catto et al., 2023; Khaled et al., 2019
Chemoorganotrophic bacteria	Sulfur-reducing bacteria	Sulfate reduction, formation of metal sulfides, Acid production, sulfite reductase, thiosulfate reductase	Li et al., 2018; Mian Adnan Kakakhel et al., 2019
Chemoheterotrophs	<i>Bacillus</i> spp. <i>Bacillus safensis</i> 9K (MH370265) and <i>Streptomyces rochei</i> 50 (MH370266)	Crust formation: patina, exfoliation, color alteration, decomposition of	Madigan and Martinko, 2006

	Halotolerant bacteria (<i>Kocuria</i> spp., <i>Micrococcus</i> spp., <i>Arthro-bacter</i> spp., <i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Paenibacillus</i> spp.)	organic matter, production of oxidizing agents (hydrogen peroxide and superoxides)	
Ammonia-oxidizing archaea	<i>Nitrosococcus tajmahalensis</i>	Conversion of ammonia to nitrite, producing acidity and damaging building materials	Han Meng et al., 2016
Fungi			
Trichocomaceae	<i>Aspergillus fumigates</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Cladosporium herbarum</i> <i>Cladosporium</i> sp., <i>Capnobotryella</i> sp., <i>Cordyceps bassiana</i> , and <i>Didymella bryoniae</i>	Aesthetic, chemical, physical and mechanical deterioration: Fungal diagenesis, color alteration, oxalate formation, bioweathering by secreted acids, chelating property of secreted acids; physical intrusion or penetration of fungal hyphae and destabilization of stone texture	Diniz et al., 2012; Farooq et al. 2015
Hymenochaetaceae family	<i>Phellinus igniarius</i> , <i>Inonotus hispidus</i> , <i>Fomitopsis pinicola</i> , <i>Hymenochaete rubiginosa</i> , <i>Coltricia perennis</i>	Decay of stone, discoloration, softening of stone, production of enzymes, biofilms	Nelson et al., 2023; Kumar and Kumar, 2017a
<i>mucoral</i> and <i>imperfecti</i> groups	<i>Alternaria</i> sp., <i>Aureobasidium</i> sp., <i>Cladosporium</i> sp., <i>Mucor</i> sp., <i>Rhizopus</i> sp., <i>Epicoccum</i> sp., <i>Rhizopus stolonifer</i> , <i>Absidia corymbifera</i> , <i>Penicillium chrysogenum</i> , <i>Penicillium expansum</i>	Production of mycotoxins, softening of materials, production of enzymes that break down materials	Singh and Kumar 2015
Basidiomycota	<i>Acanthophysellum fennicum</i> , <i>Artomyces pyxidatus</i> ; <i>Postia fragilis</i> , <i>Postia guttulate</i> , <i>Postia tephroleuca</i> , <i>Stereum sanguinolentum</i> , <i>Trichaptum fuscoviolaceum</i> , <i>Exidiopsis calcea</i> , <i>Schizophyllum commune</i> Fr., <i>Tapinella panuoides</i> , <i>Trichaptum fusco-violaceum</i> , <i>Tomentella</i> cf. <i>cinerascens</i> , <i>Tomentella terrestris</i> , <i>Trechispora farinacea</i>	Production of cellulase, hemicellulase, and ligninase; biofilm formation, change in pH	Ortiz et al. 2014; Han et al., 2021; Goodell, 2020
Ascomycota	<i>Dasyscyphus</i> sp., <i>Tapesia</i> sp., <i>Trichoderma</i> sp.	Whitish grey powder, patinas, white salt efflorescence	Stratigaki et al., 2024

Lichens	<i>Bacidia arnoldiana</i> , <i>Buellia</i> sp., <i>Caloplaca pseudopoliotera</i> , <i>Caloplaca cupulifera</i> , <i>Diploschistes</i> sp., <i>Dirinaria aegialita</i> , <i>Lecanora pseudistera</i> , <i>Lecidella enteroleucella</i> , <i>Lepraria lobificans</i> , <i>Parmotrema praesorediosum</i> , <i>Peltula euploca</i> , <i>Pertusaria</i> sp, <i>Phycia</i> sp., <i>Pyxine cocoes</i> , <i>Trapelia coarctata</i>	Chemical and mechanical deterioration: Extraction of nutrients from stone surface, oxalate formation, carbonic acids production associated bio weathering, physical intrusions in small pore etc Extraction of nutrients from stone surface; oxalate formation; carbonic acids production associated bioweathering; incursion of fungal mycelium into cracks inside rock formations that are generally stirring, ambient warmth and humidity enlargement or reduce the lichen thalli, formation of organic salts, breaking up and assimilating sandstone debris via lichen thalli	Thakur et al., 2022; Nayak et al., 2017
Bird, Insect and arthropods	Bird (<i>Columba livia</i> , <i>Passer domesticus</i>), Insect (<i>Isoptera</i> , <i>Anobium punctatum</i>) and arthropods (Diplopoda, Chilopoda, Araneae)	Webbing, burrowing, nesting. Arthropod exoskeletons can contain acidic compounds that can etch and discolor stone surfaces	Singh et al., 2019
Plants	<i>Clematis</i> spp., <i>Wisteria</i> spp, <i>Ficus</i> spp., <i>Parthenocissus tricuspidata</i>	Mechanical deterioration: Intrusion of roots inside the cracks and pores; collapse and detachment of stone structure	Cozzolino et al., 2022
Algae	<i>Chlorophyta</i> , <i>Bacillariophyta</i>	Aesthetic and chemical deterioration: Biofilm formation; color alteration; black crust formation; Enzymatic activity, pH modification, production of organic acids	Sikha and Jnanendra, 2013
Mosses and Liverworts		Aesthetic and chemical	Cozzolino, Alessia et

		deterioration: Discoloration; green-grey patches; extraction of minerals from stone surface	al., 2022
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3.2. Biodeterioration caused by fungi

Fungi are among the most destructive organisms, capable of deteriorating both organic and inorganic materials. Their metabolic versatility enables them to effectively colonize and damage a wide variety of substrates, including wood, glass, stone and books. Fungi are major agents of biodeteriation in stone monuments exposed to environmental conditions, as they can erode the mineral integrity and aesthetic appearance of these structures.

In recent years, black meristematic fungi have gained attention for their prevalence on rock surfaces, especially in sun-exposed Mediterranean regions and in extreme climates such as in dry and cold environments. Their presence not only causes discoloration, such as black spots on monuments, but also leads to severe physical damage. This includes the formation of crater-shaped lesions, chipping, and exfoliation, resulting in material loss. Black meristematic fungi, often referred to as rock-inhabiting fungi, are a diverse group characterized by their melanin content, which is present in swollen cells, hyphae, and spores. Filamentous fungi further contribute to stone weathering by secreting organic acids that lower the pH and dissolve various materials.

Fungal-induced stone biodeterioration involves both biophysical and biochemical processes. Biochemically, fungi produce extracellular mucilaginous substances rich in metabolites, acids, and metal-chelating compounds, which disrupt and dissolve minerals (Gu and Katayama, 2021). On carbonate substrates, fungal activity can also result in secondary biomineralization, altering the original minerals composition (Gu and Katayama, 2021).

Physically, fungi damage the stone matrix through the expansion and contraction of their hyphae. Their melanin-rich cell walls, coupled with their ability to form subaerial biofilms, exacerbate aesthetic alterations. Reports have identified biodeterioration primarily caused by fungi from the phyla Basidiomycota, Ascomycota, and Myxomycota (Protozoa). Key genera include *Antrodia*,

Gloeophyllum, *Athelia*, *Hyphoderma*, *Hyphodontia*, *Phanerochaete*, *Postia*, and *Botryobasidium*, all of which exhibit enzymatic activities that facilitate the breakdown of substrates.

Fungi produce hemicellulase, cellulase, and lignin-degrading enzymes that target organic materials, especially cellulose and lignin, often leading to irreparable damage. This enzymatic activity poses a significant threat to historical wooden artefacts. For example, Farooq et al. (2015) identified 19 fungal species contributing to the biodeterioration of stone monuments at Dharmarajika, Taxila, Pakistan. These species, which include *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium herbarum*, *Curvularia lunata*, *Dematium* spp., *Fusarium oxysporum*, *Mucor hiemalis*, *Penicillium chrysogenum*, *Penicillium frequentans*, and *Rhizopus oryzae*, were found to produce organic acids such as fumaric, acetic, gluconic, succinic, citric, itaconic and oxalic acids. High-performance liquid chromatography (HPLC) analysis linked these acids to stone corrosion (Farooq et al., 2015).

In the Leizhou Peninsula, China, famous for its ancient basalt sculptures like the Leizhou Stone Dogs, fungal and lichen contamination has caused considerable damage. Wang et al. (2021) analyzed biofilms on these sculptures and identified dominant fungal genera including *Caloplaca*, *Clitopilus*, *Acanthostigma*, *Tolypocladium*, *Aspergillus*, and *Toxicocladosporium*. Energy dispersive X-ray spectrometry (EDS) analysis revealed that the basalt composition primarily consisted of silica (53% by weight), followed by Al₂O₃ (14.4%), Fe₂O₃ (10.1%), CaO (8.4%), and MgO (7.4%), which are involved in the corrosion process caused by biogenic acids.

Wooden heritage artifacts are similarly vulnerable to biodeterioration. Fungi produce hemicellulase, cellulase, and lignin-degrading enzymes that target organic materials, especially cellulose and lignin, often leading to irreparable damage. This enzymatic activity poses a significant threat to historical wooden artifacts. For example, Farooq et al. (2015) identified 19 fungal species contributing to the biodeterioration of stone monuments at Dharmarajika, Taxila, Pakistan. These species, which include *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium herbarum*, *Curvularia lunata*, *Dematium* spp., *Fusarium oxysporum*, *Mucor hiemalis*, *Penicillium chrysogenum*, *Penicillium frequentans*, and *Rhizopus oryzae* were found to produce organic acids such as fumaric, acetic, gluconic, succinic, citric, itaconic and oxalic acids. HPLC analysis linked these acids to stone corrosion (Farooq et al., 2015). Sulfur-oxidizing fungi can release sulfuric acid which reacts

with calcium carbonate to form calcium sulfate, leading to damaging black crusts. Studies have shown that wood-decaying fungi isolated from wooden heritage samples, such as those in the Hymenochaetaceae family, initiate ecological succession by creating favorable conditions for colonization by more destructive fungi (Flyen and Thuestad, 2022; Kumar et al., 2022).

Ecological succession is pivotal in fungal biodeterioration. Initial colonizing fungi alter the substrate's bioreceptivity, paving the way for secondary settlers such as wood-decay fungi. This dynamic process leads to structural and dimensional instability in wood, necessitating timely conservation efforts. Several Hymenochaetaceae species, including *Phellinus igniarius*, *Phellinus tremulae*, *Inonotus hispidus*, *Inonotus obliquus*, *Fomitopsis pinicola*, *Fomitopsis palustris*, and *Hymenochaete rubiginosa* have been implicated in the biodeterioration of monuments (Li et al., 2018; Mihajlovski et al., 2017), including the Taj Mahal (*Phellinus igniarius*), the Great Wall of China (*Inonotus hispidus*), and Stonehenge (*Coltricia perennis*) (Nelson et al., 2023; Kumar and Kumar, 2017a).

Additionally, birds, particularly pigeons, can cause significant aesthetic and chemical damage through the deposition of guano. Guano not only disfigures surfaces but also creates an ideal growth medium for chemoorganotrophic microorganisms. These microorganisms can subsequently produce acid metabolites that accelerate the corrosive deterioration of materials.

3.3. Biodeterioration caused by lichen

A lichen thallus is a simple structure resulting from the symbiotic relationship between a photosynthetic organism (such as cyanobacteria or green algae) and a fungal component known as the mycobiont (typically Ascomycetes and occasionally Basidiomycetes). The upper surface of the thallus is covered by a cortical layer of organized hyphae with the medulla underneath consists of interwoven hyphae and an algal zone. The mycobiont, which directly contacts the substrate, plays a significant role in the lichen's ability to cause deterioration. Many epilithic lichen species have been observed to contribute to the degradation of various types of stone, a phenomenon that has been extensively studied (Matteucci et al., 2019). Lichens that colonize rocks are generally classified into three distinct types: crustose (flat), foliose (leafy), and fruticose (shrubby) (Nayaka et al., 2017). Crustose lichens weather more rapidly and adhere more firmly to the substrate compared to fruticose and foliose lichens. While epilithic lichens, which grow on the exterior, generally cause less damage to rocks, endolithic lichens, which grow within the rock, can hollow out and fracture the stone.

Crustose lichens can be categorized into two main groups: epilithic lichens, which grow on the exterior surfaces of rocks, and endolithic lichens, which inhabit the interior of rocks. Within endolithic lichens, there are further classifications: euendolithic forms, which aggressively bore into the rock; cryptoendolithic forms, which occupy structural cavities; and chasmoendolithic forms, which live in fissures and cracks within the rock. Lichens are lithophilous, photoautotrophic, chemiolithotropic organisms that thrive on strictly mineral surfaces, often in environments devoid of organic matter. As lichens grow and decay, they contribute eutrophic growth factors and organic matter to the stone and atmospheric accumulation of ammonia and phosphate compounds further supports the development of heterotrophic microorganisms. This process gradually fosters the growth of additional microorganisms on the stone surfaces. Thakur et al. (2022) reported that the chemical degradation of stone surfaces on various monuments is primarily due to lichen-produced carbonic and oxalic acids, as well as water-soluble chelating agents. Lichens also lead to physical rock weathering through fungal mycelium penetrating cracks or gaps. In addition, ambient temperature and humidity fluctuations cause the expansion or contraction of the lichen thalli, facilitating the production of organic compounds (Thakur et al., 2022). Various lichen species, including *Bacidia arnoldiana*, *Buellia* sp., *Caloplaca pseudopoliotera*, *Caloplaca cupulifera*, *Diploschistes* sp., *Dirinaria aegialita*, *Lecanora pseudistera*, *Lecidella enteroleucella*, *Lepraria lobificans*, *Parmotrema praesorediosum*, *Peltula euploca*, *Pertusaria* sp., *Physcia* sp., *Pyxine cocoes*, and *Trapelia coarctata*, have been reported to contribute to the deterioration of Sun temple of Konark situated in Odisha, India (Thakur et al., 2022).

To address conservation strategies, Matteucci et al. (2019) investigated lithobiontic colonization at the stone cultural heritage site of the Tomb of Lazarus, located in semi-arid region. Their study not only assessed the effectiveness of biocidal treatments for controlling these colonizations but also examined their dispersion and interactions with the lithic substrate. The findings revealed that both ancient and contemporary carbonate rock surfaces at the Tomb of Lazarus are colonized by lithobiontic communities. Bryophytes, cyanobacterial biofilms, and black fungi were found to primarily colonize wind-sheltered architectural elements, while epilithic and endolithic chlorolichens have adapted well to the site's semi-arid climate. These lichens, whether endolithic or epilithic, contribute to lithobiontic succession and cause unsightly changes to the rock surface through both chemical and physical alterations, thus acting as agents of biodeterioration. Consequently, the removal of these lichens is

recommended, especially from antique surfaces that have already suffered prolonged exposure to biotic and abiotic weathering agents.

4. Various factors affected the biodeterioration of heritage monuments

Biodeterioration is affected by both the physical properties of building materials and regional climate conditions. Factors such as mineral composition, pH, texture and moisture content of the material, as well as environmental conditions like temperature, relative humidity, light, wind and atmospheric oxygen and nitrogen levels, play significant roles (Table 2). In addition, the specific biodeteriogens present on monuments significantly influence the rate and extent of deterioration. High porosity in building materials facilitates the penetration of water, ions, and microbes, causing extensive damage. The microflora on monuments typically comprise a diverse ecosystem, including lower and higher plants, mites, bacteria, lichens, and algae. Microbial growth and enzyme activity are among the key contributors to the biodeterioration of monuments (Branysova et al., 2022).

Table 2. Factors affecting the biodeterioration of heritage sites.

Various parameters	Components		References
Inorganic Pollutants	Acids (sulfuric, nitric, and acetic acids), Particulate matter: PM ₁₀ and PM _{2.5} particles, Heavy metals, chlorides and nitrates	Crystallization, efflorescence, corrosion, alter the pH and damage to stone	Dakal and Cameotra, 2012; De Clercq, H. (2015).
Organic Pollutants	Volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), agricultural pollutants	Fungi and algae-promoting substances	Gherardi and Mazzoni, (2015)
Surface bioreceptivity	Nature of material, conservation, length of exposition	Rough surfaces provide a larger area for microbial attachment, Surfaces with nutrient-rich deposits or proximity to nutrient sources are more bioreceptive	Rosado et al., 2018
Treatments	Biocides/ Surfactants/ Hydrophobic compounds	(i) Chemicals like polyvinyl acetate (PVA), acrylic polymers, or epoxy resins silicone, polyurethane, or acrylics can attract moisture, creating a favorable environment for microbial growth (ii) Development of resistant microbial strains, reducing their effectiveness and potentially harming the environment.	De Clercq, (2015).
Atmospheric Salinity	Desalination treatments	Inadequate desalination can leave residual salts, which can attract moisture and promote	De Clercq, (2015).

		microbial growth	
Moisture	High humidity (above 60%)	Favors the growth of fungi, bacteria, and algae	Lefevre (2015).
Temperature	High temperatures (above 25°C/77°F): favor the growth of bacteria, fungi, and algae, leading to increased bio-deterioration (1).	Favor the growth of bacteria, fungi, and algae	Mikhailovskii (2013); Liu et al., 2020
	Low temperatures (below 10°C/50°F): inhibit microbial growth, slowing down bio-deterioration (2)	Inhibit microbial growth	Saiz-Jimenez (2018).
Solar radiation	High light intensity: can inhibit microbial growth, but also promotes photodegradation of materials		Kumar and Kumar, 2017b)
	Low light intensity: favors the growth of microorganisms, leading to increased bio-deterioration		Kumar and Kumar, 2017b
Air movement and pressure		Can disperse microorganisms, promoting bio-deterioration	Gorbushina (2007)
Precipitation	Rainfall, fog and dew, snow and ice, acid rain	moisture accumulation, salt crystallization, and erosion	Li et al (2018)
Chemical and biochemical attack	Acidification, hydrolysis, oxidation, complexation, enzymatic degradation, redox reactions	Chemicals like sulfur dioxide, nitrogen oxides, and ozone can react with moisture to form acids, leading to acid hydrolysis of materials ozone, nitrogen dioxide, and sulfur dioxide can oxidize materials, leading to the formation of reactive oxygen species (ROS) Chemicals like water, acids, and bases can hydrolyze materials, breaking down their molecular structure Siderophores formation	Liu et al (2020)
Intrusion of macro-microorganism	Insects, Rodents, Birds	physical damage and creating pathways for microorganisms	Tiano, and Cantisani, (2012)
Plants	Plant roots	can penetrate materials, causing physical damage and creating habitats for microorganisms	Tiano, and Cantisani, (2012)

In arid climates, microorganisms thrive within stones, where conditions are more favorable for their growth. These microbes can contribute to the powdering of the stone materials, a significant aspect of monument biodeterioration. This powdering occurs as microorganisms physically grind or crumble the stone, leading to its degradation. Scaling is another microbial alteration process affecting cultural

heritage. Furthermore, many microorganisms rely on airborne inorganic and organic particles that settle on monument surfaces as nutrients or energy sources. The interaction between microorganisms and their environment can lead to the precipitation or dissolution of monument materials, accelerating the degradation process. Understanding the factors influencing the biodeterioration of cultural heritage is crucial for the conservation of historical assets. Analyzing these factors provides valuable insights in developing effective conservation strategies.

4.1. The role of moisture in biodeterioration

Moisture is a crucial factor in the biodeterioration of materials (Cappitelli et al., 2020). It forms when surface materials interact with adsorbed atmospheric water. Relative humidity is a significant meteorological factor influencing outdoor material degradation, particularly metal corrosion. In the absence of atmospheric moisture, pollutants, whether biological or non-biological, have minimal corrosive effects. Moreover, microorganisms can further exacerbate biodeterioration by depositing surfactants, which alter a material's water permeability. These surfactants modify the interaction between the stone surface and moisture, potentially accelerating the biodeterioration process.

4.2. The role of temperature in biodeterioration

Temperature is another key factor that significantly influences the rate of material deterioration. Temperature variations create thermal gradients between the inner and surface layers of materials, particularly those with low thermal conductivity. These thermal gradients can lead to the formation of fine cracks, which are linked to a loss of structural strength, increased porosity, and reduced chemical resistance. Temperature also plays a critical role in the activity of enzymatic and microbial metabolic processes, often accelerating these reactions. In addition, specific wavelengths of solar radiation are necessary for photochemical reactions that contribute to material deterioration. Continuous exposure to solar radiation not only affects the deterioration of stone and wooden materials but also facilitates metal corrosion (Cappitelli et al., 2020).

5. Reduction of biodeterioration for heritage conservation

The primary challenge in restoring and conserving heritage structures is to return them to their original state or as close to it as possible, using environmentally friendly materials that have minimal negative

effects on human health and the environment. Effective conservation begins with the use of analytical techniques to identify and assess microbial presence related to biodeterioration. Factors influencing biodeterioration include the material's characteristics - such as porosity, permeability, and crystalline structure as well as surrounding environmental conditions like moisture, temperature and pollution levels.

Reducing moisture is a crucial and effective remedy for biodeterioration. To further control microbial growth, techniques such as high-pressure water cleaning, gentle pat-dry cleaning, and periodic application of disinfectants like hydrogen peroxide and isothiazole derivatives can be employed. Building materials and protective agents should possess attributes such as mineral composition, moisture absorbency, diffusivity and alkalinity to resist microbial growth.

Painting buildings can also help prevent microbial infection. Mineral calcareous plaster and silicate paints are commonly used for this purpose. Additionally, using organo-silanes on inorganic materials can help lower humidity levels, restricting microorganisms' access to nutrients and their ability to transport soluble salts through materials. Chemical treatments, such as gas fumigation and biocide application, are also utilized to address microbial issues.

Chemical treatments, such as fumigation and biocide application can play a critical role in controlling microbial colonization on heritage structures. Biocides, which are antimicrobial agents used for this purpose, include a range of synthetic organic products such as alcohols, aldehydes, organic acids, carbonic esters and phenols, as well as their derivatives. Extensive testing of these biocides has been conducted on concrete sewage pipelines. For example, Alum et al. (2008) investigated various biocide formulations incorporating materials like silica fume, zinc oxide, copper slag, ammonium chloride, sodium bromide and cetyl-methyl-ammonium bromide. Their research focused on 10% ZnO concrete mixtures, in both field and laboratory settings. The findings indicated that these mixtures exhibited biocidal properties comparable to commercially available biocides.

In a separate study, Arino et al. (2002) compared the efficacy of biocide (Algophase) and a silicone treatment (Hydrophase) on walls heavily covered with phototrophic communities at Guadalupe Monastery in Cáceres. Their findings revealed that the biocide effectively removed the biofilm. However, chemical methods may potentially damage the monument's structure and remove its outer

layer. As a result, research has explored alternative methods, such as employing electromagnetic fields, which have shown promising results for biofilm removal without causing structural harm.

Deák et al. (2021) synthesized and characterized a sustainable material designed to address the biodeterioration of stone monuments. The material, calcium zincate nanoparticles, was created using a precipitation method from waste eggshells. The Scherrer equation indicated that the crystallite size of the calcium zincate nanoparticles was approximately 23 nm. Elemental analysis using Energy-Dispersive X-ray Spectroscopy (EDX) and elemental mapping confirmed that calcium (Ca) and zinc (Zn) were the primary elements present in the sample.

5.1. Physical methods for reduction of biodeterioration

Protective coatings or overlayers made from inorganic nanoparticles with a chemical and crystallographic affinity to building materials play a crucial role in preventing further deterioration of cultural assets. Metal nanoparticles (Au, Ag, Pt) and preparations of metal oxides and hydroxides (TiO_2 , ZnO , Ca(OH)_2 , Mg(OH)_2 , Sr(OH)_2) has been used as protective additives (Ion et al., 2018; Caner et al., 2018). Colloidal silica particle suspensions have been effectively applied to monuments, preventing erosion-related deterioration and strengthening deteriorated stone. After interacting with a calcium carbonate component, silica nanoparticles penetrate pores and create a protective layer that shields calcareous materials.

When silica nanoparticles averaging 10-15 nm in size were added to historic mortars and tuff stone, they interacted with the calcium carbonate to form xerogels upon water loss, resulting in satisfactory consolidation (Sierra-Fernandez, 2017; Stuff, 2020; Tesser, et al., 2018). Promising prospects for safeguarding monuments have been demonstrated by the application of biopolymers and siloxane materials that modify surface properties (Helmi et al., 2022; Manoudis et al., 2017). Efstathia et al. (2023) prepared two different types of nanoparticles (NPs) to treat building materials that have calcite as a major component. By carefully hydrolyzing dimethyl carbonate (DMC) in the presence of sufficiently high calcium concentrations, amorphous calcium carbonate (ACC) was created. The resulting spherical nanoparticles had sizes ranging from 200 to 500 nm. For a minimum of one month, the particles remained stable at low temperatures. Tetraethyl orthosilicate (TEOS) was hydrolyzed in ethanol and ammonia to create amorphous silica (am-SiO_2) nanoparticles. The resulting suspensions

were stable and the particle size ranged from 10 to 30 nm for more than 8 months. Before the ACC and am-SiO₂ hydrolysis processes began, powdered Pentelic marble and a historic mortar from the Roman arena in Patras were suspended in the solutions. After the hydrolysis process was completed, significant coverage of the treated specimens was observed, indicating that the treated specimens were shielded from dissolution in aqueous acidic solutions.

Nanoparticles represent an emerging technology for monument preservation. For instance, calcium and strontium nanoparticles have been utilized for the consolidation of calcarenite (Michalopoulou et al., 2020). Various nanomaterials, including nanosilica, nanoclay, titanium dioxide, nano iron oxide, alumina, silver, copper, zinc oxide, and carbon nanotubes have been reviewed for their efficacy in preventing biodeterioration (Dhiman et al., 2022).

Nano-sized TiO₂ is primarily utilized as a photocatalyst, though some aspects of its application in cultural heritage conservation remain unresolved. Crupi et al. (2018) investigated the use of polydimethylsiloxane (PDMS) combined with TiO₂ nanoparticles to protect monuments from biodeterioration. Their findings indicated that TiO₂ influences the adsorption of water molecules. TiO₂ is a particularly effective nanomaterial due to its high chemical stability, non-toxicity, and strong photo-reactivity. TiO₂ nanoparticles can be applied as nanosols or dispersions, with or without organic binders. Coatings of these nanoparticles provide a protective layer that enhances resistance to pollutants, biological growths, and water damage. Additionally, TiO₂ and SiO₂ have been combined to create hybrid coatings for monument protection. Ultraviolet treatment ($\lambda < 400$ nm) of TiO₂ generates electron-hole pairs; the holes can convert hydroxide anions or water into hydroxyl radicals ($\bullet\text{OH}$), which decompose organic compounds. Cappitelli et al. (2007) investigated the impact of synthetic coatings, specifically poly-laurylmethacrylate and polyisobutylmethacrylate, on the Cathedral of Milan. They found that these coatings could cause damage to the monument by providing an organic substrate conducive to biological growth. In contrast, Ion et al. (2016) emphasized the importance of understanding the material composition of monuments before applying nanoparticle treatments. They characterized the materials of ancient building walls, identified potential decay products resulting from pollution, and proposed a new conservation method using hydroxyapatite (HAp) nanoparticles. Various analytical techniques were employed, including energy-dispersive X-ray fluorescence (EDXRF) to assess elemental composition, FTIR and Raman spectroscopy for stratigraphic analysis of layered

materials, and optical microscopy (OM) for studying sample morphology. The application of HAp nanoparticles by spraying reduced capillary water uptake, minimally altered the sample color, and decreased mass loss in treated samples. Additionally, siloxanes are widely used for treating different stone substrates due to their excellent chemical stability, strong Si–O bonds, low surface tension, and resistance to thermal stress. Nanosized calcium hydroxide and silica are also used as protector against biodeterioration, because of their high reactivity and deep penetration into the building materials (Rodriguez and Ruiz, 2017; Pozo-Antonio et al., 2019).

Coatings on stone materials incorporating nanoparticles have demonstrated enhanced performance, particularly in resisting biological attack, water damage, and pollution. These coatings generally fall into two categories based on their functionalities: superhydrophobic coatings and photocatalytic coatings. Superhydrophobic coatings are designed to repel water, creating a self-cleaning effect. The high surface energy of early microbial colonies and dirt is reduced, allowing them to be washed away more easily. This is achieved through nanoparticles that create a surface with low surface energy, causing water droplets to form a high contact angle and roll off the surface, carrying contaminants with them. Photocatalytic coatings utilize nanoparticles to oxidize organic matter upon exposure to light. These coatings have a photoactive surface that, when exposed to light, generates reactive species capable of decomposing organic substances. This process helps in maintaining a clean surface by breaking down organic materials that adhere to it. The distinction between hydrophilic and hydrophobic surfaces is based on how water interacts with them. On hydrophilic surfaces (high surface energy), water spreads out and forms a low contact angle, often less than 90° . Conversely, on hydrophobic surfaces (low surface energy), water droplets remain round and exhibit a high contact angle. If a water droplet tends to stick to itself rather than spread across a surface, the surface is classified as hydrophobic, with a contact angle greater than 90° . A water drop that completely wets the surface has a contact angle of 0° , while a drop that interacts minimally with the surface has a contact angle of 180° . Typically, stone surfaces coated for protection show an increased contact angle compared to untreated stone. The smallest angle at which a water droplet will roll off a flat surface is referred to as the roll-off angle. Photocatalysis, on the other hand, involves accelerating a chemical reaction through direct light exposure or by reducing the activation energy of the catalyst via radiation. This process often requires solid-state catalysts, and efficient photocatalysis is frequently achieved with wide-band-gap semiconductors. These materials help in catalyzing redox reactions driven by light, making the process

both effective and cost-efficient. Although numerous studies have been conducted on nanoengineered concrete and nanocoatings, research into their practical applications and large-scale implementation remains in its early stages.

5.2. Biocides treatment

Biocidal treatments are widely employed in monument conservation to mitigate microbial deterioration. The effectiveness of such treatments can be enhanced by combining a biocide with a water-repellent agent, as demonstrated by Wang et al. (2021), who found this combination to be the most effective in reducing microbial damage to monuments. Among the microorganisms involved in biodeterioration, actinomycetes, especially species from the genus *Streptomyces*, are well-known for their roles in degrading organic materials such as lignocellulosic biomass, starch and chitin in sediments. However, *Streptomyces* species are also notable for producing a range of antimicrobial peptides, which serve as alternatives to commercial antibiotics. Despite their well-documented ability to suppress fungal and bacterial growth, their potential in preserving stone monuments remains relatively underexplored. This gap in research is especially pronounced in the context of Egypt's monument conservation efforts, where microbial colonization poses a significant challenge.

5.3. Biocrusts protection

Traditionally, heritage conservation practices have recommended the removal of natural vegetation from heritage structures due to concerns about damage from biological weathering, secreted biochemicals and root activity (Chen et al., 2021). However, research over the past three decades challenges this view. Studies have shown that certain short-rooted and drought-resistant herbs can actually serve as natural buffers against erosion rather than being destructive agents. One emerging approach to cultural heritage preservation involves the use of biocrusts—photoautotrophic communities comprising cyanobacteria, mosses, lichens, other microorganisms, and tightly bound soil particles. These biological formations, previously described in this text, provide a novel and environmentally friendly solution for heritage protection. Biocrusts integrate multiple protective functions into a single system, acting as drainage roofs, sacrificial layers, consolidators, and stabilizers (Shan et al., 2022). By minimizing secondary damage such as cracking, salinization, and water accumulation, which are often

associated with conventional preservation techniques, biocrusts contribute to maintaining the authenticity and structural integrity of heritage sites. However, the impact of vegetation on large-scale man-made structures continues to be a topic of debate.

Wang et al. (2023) found that a significant portion of the Great Wall is covered with biocrusts. Biocrusts naturally regulate the hydrological cycle, heat exchange, and gas aeration, shaping the microenvironment of heritage sites and enhances their protective functions. Wang et al. (2023) suggest that rather than removing naturally occurring biocrusts from heritage structures, it may be beneficial to explore methods for promoting their colonization through artificial inoculation and cultivation. This approach could offer a sustainable intervention, offering a promising strategy for preserving cultural heritage in the face of global climate change and ensuring its protection for future generations.

6. Prospects for Biodeterioration research and management

Although significant progress has been made in understanding microbial colonization of monuments, the underlying mechanisms remain only partially understood. Advances in molecular techniques such as high-throughput sequencing, transcriptomics, and metagenomics have illuminated the diversity of microorganisms that colonize heritage structures. These methods are beginning to provide a deeper understanding of the microbial metabolic processes that affect and deteriorate monument stones. Complementary techniques, including proteomic, transcriptomic and genomic tools like DGGE, 16S-rDNA, cDNA, RFLP, and RAPD facilitate the identification, isolation, and cultivation of these microorganisms. With this understanding, effective preventive measures can be developed and implemented to halt the growth of microorganisms and the deterioration of buildings, which require periodic renovation due to environmental factors, both biotic or abiotic (Fig. 4)

Effective conservation of heritage structures requires preventive measures that address both biotic and abiotic factors contributing to deterioration. Advanced research in materials science holds promise for enhancing these efforts. Innovations such as nanomaterials, hybrid coatings and biopolymers hold great potential for improving the protection of heritage structures. Future studies should focus on optimizing these materials for practical applications and assessing their long-term effectiveness.

The demand for sustainable and eco-friendly conservation methods is also driving a paradigm shift in heritage preservation. Research into natural protective measures, such as biocrusts and bio-based

coatings, can contribute to more sustainable practices in heritage conservation. The development of green technologies that minimize environmental impact while preserving heritage materials are a key area of focus. Enhanced diagnostic and monitoring technologies are equally vital for advancing biodeterioration management. Remote sensing, advanced imaging, and real-time monitoring systems can provide more accurate data and facilitate timely interventions. Addressing biodeterioration effectively requires interdisciplinary collaboration among materials scientists, microbiologists, conservationists, and environmental scientists.

Collaborative research efforts can lead to more comprehensive solutions and innovative approaches to heritage conservation. Increasing public awareness and education about the importance of heritage preservation and the impact of biodeterioration can foster greater support for conservation efforts.

Public engagement and awareness are equally important in supporting conservation efforts. Educational programmes that emphasize the significance of cultural heritage and the effects of biodeterioration can foster community involvement in preservation activities. By promoting best practices and encouraging local participation, conservation efforts can gain broader social and financial support.

Developing and implementing policies and regulations that support the use of advanced materials and conservation techniques is essential. Ensuring that conservation practices align with heritage preservation goals and sustainability standards will contribute to long-term success.

In summary, while challenges in managing biodeterioration persist, ongoing research and technological advancements offer promising prospects for improving the preservation of heritage structures. Emphasizing sustainable practices, and integrating advanced technologies, thereby fostering interdisciplinary collaboration will be crucial for addressing the complexities of biodeterioration and ensuring the longevity of heritage structures, safeguarding their cultural and historical value for future generations.

7. Conclusions

Biodeterioration is influenced by a complex interplay of physical, chemical, biological, and environmental factors. The combined effects of moisture, temperature fluctuations, humidity, pollution, and biological activity (microbial and fungal) contribute to the degradation of materials. Understanding these factors is essential for developing effective conservation and preservation strategies.

Microorganisms such as bacteria, fungi, algae, and lichens play significant roles in biodeterioration. They can cause physical damage through biofilm formation, bio-pitting, and the secretion of corrosive substances. Microbial activity often accelerates material degradation, leading to the loss of structural integrity and aesthetic value. Recent advancements in materials science have introduced innovative methods for protecting heritage structures. Nanoparticles, biopolymers, and hybrid coatings have shown promise in enhancing material resistance to biodeterioration. These technologies offer new avenues for conservation by providing better protection against environmental and biological factors. Natural solutions such as biocrusts and biopolymers offer environmentally friendly alternatives to traditional protective measures. They provide benefits like erosion control, moisture regulation and pollutant absorption. Additionally, biotechnological interventions, including the use of antimicrobial agents and nanoparticles, are emerging as effective methods for safeguarding heritage materials. Despite advancements, challenges remain in scaling up new technologies and understanding their long-term effects. The potential impact of biocides and synthetic coatings on material integrity and aesthetics requires careful consideration. Furthermore, the interaction of new materials with the environment and the need for sustainable practices are critical considerations in conservation efforts. Effectively managing biodeterioration requires ongoing research, interdisciplinary collaboration, and the integration of innovative and sustainable solutions. Balancing technological advancements with environmental considerations will ensure the effective and responsible preservation of cultural heritage for future generations.

CRedit authorship contribution statement

Sangeeta Yadav: Writing – original draft. Diane Purchase: Writing – review & editing.

Data availability

No data was used for the research described in the article.

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