Year: 2014; Volume: 1; Issue: 1
Article ID: MI14 05; Pages: 1-13

BioMed Research

The Open Access Publisher

www.bmrjournals.com

BMR Microbiology

Review Article

Role of Cyanobacteria In Biodeterioration of Historical Monuments -A Review

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Received 12 April 2014; Accepted 22 May 2014; Published 22 May 2014

Abstract

The presence and deteriorating action of microorganisms on monuments and stone works of art have received considerable attention in the last few years. Knowledge of the microbial populations living on stone materials is the starting point for successful conservation treatment and control. This review describes some biodegradation processes originating from activity of microorganisms in black microbial crusts growing on historic monuments. The crusts are mainly composed of gypsum, carbonaceous particles and polycyclic aromatic carbons. They are slowly dissolving gypsum from black crusts represent a continuous source of sulphur for microbial growth. The sulphate bonding capacity of the sheath of cyanobacteria causes a particularly high demand for sulphate, thus making sulphur nutrition a relevant aspect in their physiology. Growing concern for the preservation of cultural heritage has led to a greater interest in the biological attack on these monuments. The importance of cyanobacteria as deteriogens is emphasized and the traditional and more modern molecular methods used to detect microorganisms are discussed.

Key Words: Cyanobacteria, Monuments, Inorganic and organic black crust, Biodeterioration

Introduction

Stone monuments represent an important part of our world's cultural heritage. Natural stones are weathered by physical, chemical and biological factors. One of the most complex problems in monument conservation is to contrast biological deterioration or biodeterioration [1, 2, 3]. Stone monuments, statues and historic buildings are exposed to the effects of physical, chemical and biological deteriorate. This review will focus on the damage caused by micro-organisms. Stone works of art can be colonized by different groups of micro-organisms, including bacteria, cyanobacteria, algae and fungi. Monuments materials exposed to open air deteriorate due to natural causes. Sun, frost, wind, rain, etc. contribute to a gradual process of weathering. Biological activity also plays a role, and its interaction with physico-chemical mechanisms is considered central to the understanding of long term deterioration.

According to several authors, cyanobacteria and chlorophyta (green algae) are considered the pioneering inhabitants in the colonization of stone [4, 5, 6, 7, & 8]. Due to their photoautotrophic nature, these micro-organisms develop easily on a stone surface, giving rise to coloured patinas and incrustations (fig 1) [9]. Identifying the microorganisms involved in biodeteriorations is one of the most important steps in the study of the microbial ecology of stone monuments. It can help us to understand the microbial biodiversity, the phases of colonization and the relationship among populations on the surfaces and between micro-organisms and substrata. Here we review the occurrence of cyanobacteria and green algae indentified on stone monuments, statues and historic buildings in European countries of the Mediterranean Basin, Marble, limestone, travertine, dolomite, sandstone and granite were the six litho types considered in this work, corresponding to the litho types mainly used in construction of buildings and monuments in this region.

Recently industrial and urban activities have modified the composition of the atmosphere, resulting in a more aggressive environment t, accelerating the decay of materials. Sulfur dioxide is one of the major gaseous components of polluted atmospheres urban areas. Because oxidation of sulfur dioxide results in the formation of suphuric acid, there is a strong correlation between the sulphatation of lime stones and the atmospheric concentration of sulfur dioxide. Sulphuric acid attacks limes tones resulting in the formation of gypsum. During the crystallization of gypsum, airborne organic pollutants, carbonaceous particles, dust, pollen, etc. are accumulated at the surface of buildings and trapped in the mineral matrix. This results in the formation of a hard, grey to black crust [10]. Obviously, this crust enriches the substratum and anthropogenic compounds may influence, to a great extent, the colonization and growth pattern of microorganisms in stones located in polluted environments which compared with the growth of microorganisms in similar stones placed in rural environments.

Although it has been reported that a great variety of microorganisms colonize stones in urban environments, and their possible role in the biodeterioration of stones is discussed [11]. The interactions between anthropogenic compounds and microorganisms have barely been investigated.

Cyanobacteria on monuments

Table 1 lists the monuments, statues and historic buildings reported in the literature covered in the review. Most of these works of art were built in lime stone (41%) followed by marble and painted wall (each 19%) and cement surfaces (12%). Granite (9.6), Travertine and dolomite (each 3.2%) were less represented litho types (Fig 2).

Table 2 lists the cyanobacteria detected on monuments and works of art in the tropical and temperate regions, together with the substratum. The data on cyanobacteria occurring on stone monuments and works of art is rather wide. Many taxa of cyanobacteria were reported. Among the taxa, *Lyngbya* was the dominance occurrence followed by *Oscillatoria*, *Phormidium,Chroococcus,Microcystis, Aphanocapsa, Gloeocapsa and Plectonema* were reported in many workers [12,13, 14, 15].

According to the literature reviewed in this study lime stone and marble were colonized with high percentage(41 and 19).Granite and travertine were a litho types present in least percentage .However ,[1] observed granite with a very low porosityand pH is an unfavorable substratum for cyanobacteria. In fact, the colonization of stones is closely correlated with porosity, roughness, hygroscopicity and capillary water absorption, which strongly influence water availability for microorganisms [16,17 & 18].

Cyanobacteria can live in rock fissures and cracks and in cavities occurring in porous transparent rocks such as sandstones and marble, but not in dense dark volcanic rocks. Chasmo- and endolithic cyanobacteria was present in all samples examined from the marbles of the Parthenon and Propylaea Acropolis in Athens, Greece [19].Cryptoendolithic cyanobacteria such as Chroococcidiopsis live beneath rock surfaces together with cryptoendolithic lichens, fungi and bacteria. Chroococcidiopsis can survive extreme cold, heat and arid conditions and it may be the single autotrophic organism most tolerant to environmental extremes [20].

Most of the cyanobacteria mentioned in this review (e.g. *Gloeocapsa, , Phormidium, Chroococcus, Plectonema, Scytonema, Lyngbya* and *Microcol*eus) have a gelatinous sheath that acts as a reservoir of water, where it is bound through strong molecular forces, allowing these cyanobacteria to colonize stone even when dry conditions prevail [4]. The sheath can also play an important role in adhesion to the substratum (Fig.3). Sometimes sheaths may be pigmented. This is particularly evident in some cyanobacterial genera such as *Gloeocapsa* or *Scytonema* that have thick sheaths with intense colours, being the expression of different ecological stages and environmental adaptations. Cyanobacteria can take on a yellow-brown colour under low-nitrogen conditions due to a reduction in chlorophyll and phycocyanin and an increase in carotenoids. In other cases, it has been shown that pigmentation changes in response to environmental factors including light intensity, light quality, nutrient availability, temperature and the age of cells. [21], in a study on monuments located on the Appia Antica road (Rome), observed that grey-black patinas were widespread on marble and travertine stone works exposed to sun irradiation, and that green patinas were more frequent on tufaceous materials and mortar in shaded areas. These coloured patinas cause aesthetic damage, giving an unsightly appearance of neglect to buildings, statues and monuments

Role of cyanobacteria in biodeterioration

Cyanobacteria colonize a wide variety of terrestrial habitats, including rocks, hot and cold desert crusts, as well as modern and ancient buildings. The role of cyanobacteria in the deterioration of surfaces of historic buildings has been the subject of several recent studies [22, 23, 24] They are generally adapted to resist adverse cinditions because of their thick outer envelopes and the presence of protective pigments [25,26,27] suggested that chasmo endolithic cyanobacteria (those that colonize and grow within fissures) contribute to the decay of calcareous stones by exerting pressure from within the stone as a result of water up take, expansion of cell mass, and the precipitation of carbonates and oxalates around the cells. The resulting opening of the fissure allows the entry of dust, pollen, grains, and small animals such as mites, and the increasing internal pressure on the superficial layer of the structure leads to its detachment (spalling).

The increase in local pH levels in phototrophic biofilms throws some doubts on the acid degradation suggested to be produced by algae and cyanobacteria [28], even though [29] showed that cyanothece carries out mixed acid fermentation and could, therefore, induce acid degradation of stone. However, there is little evidence that cyanobacteria produce acids [30] suggested that it is the associated heterotrophic bacteria that lead to acid decay of calcareous materials in the presence of cyanobacteria, as also shown by [31]. Fungal components of biofilms may act in a similar manner [32,33]. [34] suggested that heterotrophic bacteria rather thanphototrophs, may be primary colonisers of stone buildings, but further evidence for the algae and cyanobacteria in this role comes from [35]. They demonstrated that fungal and bacterial growth was inhibited by the treatment of external walls with an algicidal substance (a copper-containing compound), even though the concentration of copper used was too low for bacteriostatic and fungistatic activity. This indicated that the inhibition of algal colonization reduced the growth of fungi and heterotrophic bacteria and supports the accepted colonization sequence. On chemically polluted or painted buildings however, nutrients for nonphototrophs are already present and heterotrophic bacteria could, indeed, be the primary colonizers [36].

Identification methods of Cyanobacteria in Biofilms on Monuments

Traditional identification and classification techniques for cyanobacteria have been primarily based on their morphological characteristics. However, morphology can change with environmental conditions [37]. Photosynthetic pigment content, lipid composition, differentiated cell structures, and other characteristics can also alter because of variable expression of cyanobacterial genes in culture [38,39]. The limitations of these current methods of isolation and identification have favored the development of molecular biology techniques for aquatic and soil cyanobacteria. Enterobacterial repetitive intergenic consensus (ERIC) and repetitive extragenic palindromic (REP) sequences have been used as primers for fingerprinting axenic cyanobacterial cultures [40]; short tandemly repeated re;etitiveseuences (STRR) and highly iterated palindromic sequences (HIP 1) can distinguish symbiotic *Nostoc* and *Anabaena* in cycads [41] and characterize cyanobacteria such as *Synechococcus sp* and some toxin-producing cyanobacteria [42,43]

The methods currently used to detect cyanobacteria are based on the growth of these microorganisms on specific growth media. The traditional isolation techniques were developed in the area of aquatic microbiology, extended to terrestrial habitats, and involve enrichment in liquid media, followed by isolation by means of micromanipulation or culturing on sold medium [44,45]. However, such methods can result in the detection of artificially low numbers, due to the presence of inhibitory and predatory organisms, such as fungi, bacteria, and protozoa. It is apparent that many cyanobacteria species which grow in dry environments are lost in culture because of the activity of fungi, and it has been recognized that many kinds of microorganisms present in the environment are not detected by common culture techniques [46]. The diversity of strains detected in a population can be reduced by selective culture conditions [47, 48] reported the artificially low phototrophic biodiversity after the use of these traditional culture and enrichment techniques and suggested that molecular biology is the answer. However, in order to identify cyanobacterial species based on their nucleic acid sequences, such 16s rDNA, pure cultures are necessary. Many biofilms on historic buildings are dominated by coccoid cyanobacteria [49], [50], which are difficult to isolate in unicyanobacterial cultures. The viable but non culturable species must also be considered. Since cyanobacteria are found in association with other species, such as fungi, algae, and heterotrophic eubacteria (including actinomycetes) in the biofilm, their isolation is laborious, requiring extreme care and successive replication of colonies, cells, and filaments. Some cyanobacteria produce exopolysaccharide sheaths or capsules, to which other eubacteria attach, making the isolation of these species in axenic culture even more difficult [51].

Several articles [52, 53, 54, 55, 56&57] have cited the use of the 16S rRNAgene to provide insights into the phylogenetic relationships of cyanobacterial genera within the orders proposed by [58,59]. However, [57] concluded that 16SrRNA sequence data was not sufficient for establishing species identity and thus not appropriate for studies at the subgenus level. Restriction digests of the more variable 16S-23S ITS region have been used to examine variability and phylogenetic relationships within cyanobacterial orders [60], among genera of heterocystous cyanobacteria [61], and among strains of a single filamentous genus [62]. [63] were the first research group to use direct sequencing of the ITS region to study subgeneric phylogenetic relationships in the genus *Microcystis*. They found that the phylogeny based on ITS data did not correlate perfectly with established Microcystismorphospecies in terms of phycocerythrin production, although there was a relationship with microcystin production.

Inorganic and organic composition of black crusts

Although there is a consensus on the composition of black crusts in terms of gypsum and carbonaceous particles, investigation of chemical nature of the (individual) organic compounds entrapped in the crusts has barely been carried out [64] As a general characteristic, the black crusts from monuments sampled in different locations andCountries resent a diversity of organic compounds entrapped in the mineral matrices. This diversity is determined by the different nature of the aerosols and particulates present in trhe atmosphere of each location. However, black crusts obtained fromdifferent building materials in the same monument had similar composition[65]. identified two types of particles in black crusts: spherical shape, irregular rough surface, and high porosity (assigned to oil-fired combustion plants), and spherical shape with smooth surface (deriving from coal-fired combustion plants). Both types of particles have diameters of about 10µm and contain carbon, silicon, sulphur, aluminium, and calcium as major constituents.

The extractable lipid material of black crust samples consisted primarily of aliphatic hydrocarbons and fatty acids (as methyl esters), represented by homologous series of n-alkanes ranging from $C_8$to C_{32}, and n-fatty acids from $C_4$to C_{30} (Table 3). Furthermore, diterpenoids, triterpanes, steranes, polycyclic aromatic hydrocarbons, and dialkyl phthalates were identified.

Polycyclic aromatic hydrocarbons are the result of combustion of biomass, coal and lil, and have been identified, among other sources, in smoke particles from plant burning [66] and diesel engine soot [67]. Polycyclic aromatic hydrocarbon mixtures encountered in black crust are complex because of the presence of alkyl-substituted compounds, as well as the numerous isomeric parent compounds. Generally, compounds from two to six aromatic rings are widely distributed in black crusts from different European monuments. In addition, ketones, sulphur and nitrogen- substituted aromatic hydrocarbons were identified.

It appears that exposed building materials act as non-selective surfaces, passively entrapping all deposited airborne particulate matter and organic compounds. Accordingly, the black crusts coating the surfaces of building materials located in polluted environments are constituted by a suite of all kinds of organic compounds present inn aerosols and particulate matter. The study of molecular markers present in black crusts indicated that oil and coal combustion are the principal sources of pollutants.

Fig.1. Monuments of cultural heritage showing microbial biofilm

Big Temple

Gangai konda Sozhapuram Temple

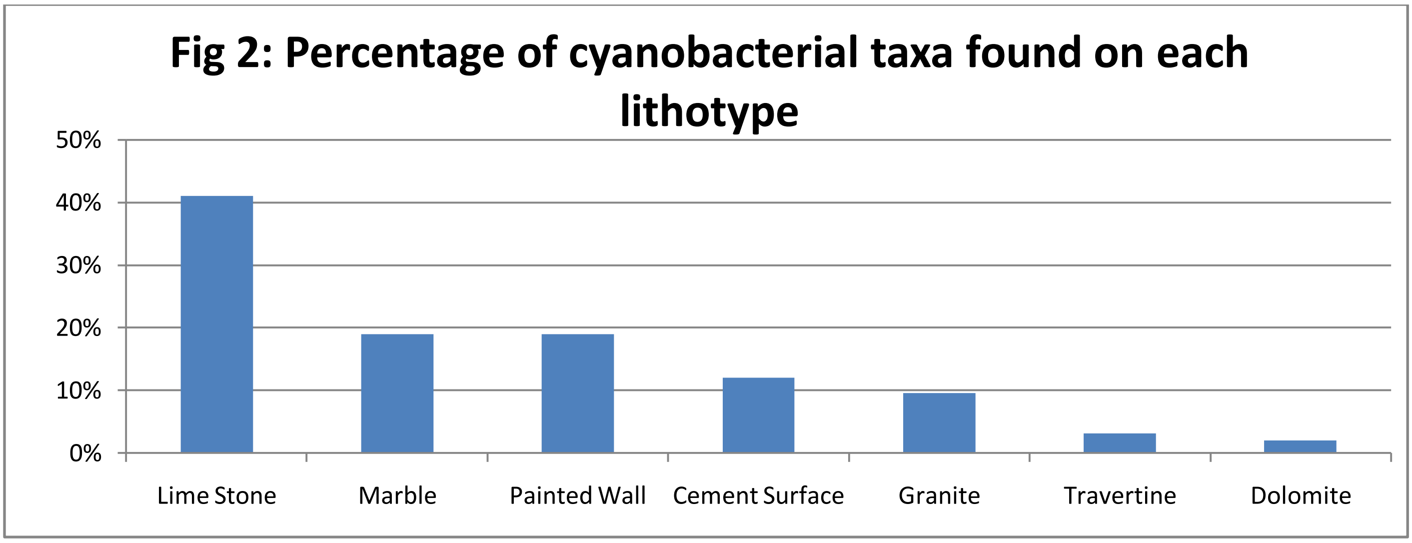
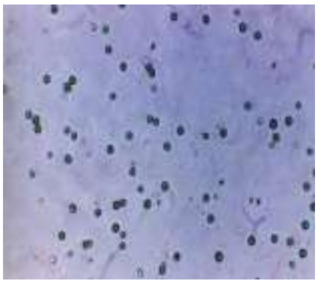
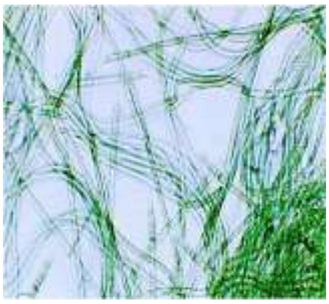


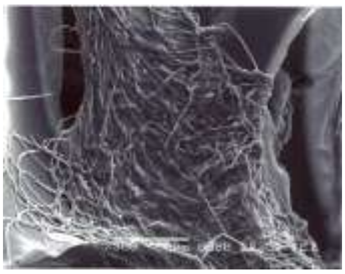
Fig 2: Percentage of cyanobacterial taxa found on each lithotype

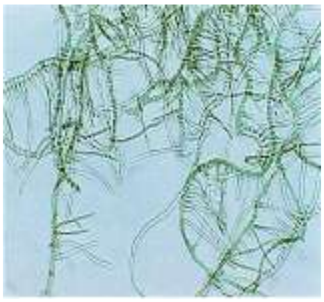
Fig 3: Light microscopic and SEM view of cyanobacteria


***Aphanocapsa pulchra* (Kutz.) Rabenh**


SEM view showing *Aphanocapsa*


***Oscillatoria brevis* (Kutz.) Gomont X400**


SEM view showing *Oscillatoria*


***Westiellopsis prolific* Janet X500**


SEM view showing *Westiellopsis*

Table 1 Investigated monuments, statues and historic buildings in different countries and their bibliographic references

S.NO	Monuments	Reference
1.	Base of urban walls (Europe)	[68]
2.	Building facades (France)	[69]
3.	Building facades and Monuments (India)	[70]
4.	Cathedral of Granada (Spain)	[71]
5.	Calcareous historic buildings (Denmark)	[72]
6.	Cave of southeastern Spain (Spain)	[73]
7.	Churches walls in Porto Alegre (Brazil)	[74, 75]
8.	Cultural Monuments (Ukraine)	[76]
9.	Dwelling on Stone Monuments (Italy)	[77]
10	Exteriors of buildings (Europe)	[78]
11.	Edified granite walls (Spain)	[79]
12.	Hawaiian Islands (Italy)	[80]
13.	Historical temple and Monuments (India)	[81]
14.	La citadels, Blaye (France)	[82]
15.	La Mola quarry,Novelda (Spain)	[83]
16.	Lime stone buildings (Mexico)	[84]
17.	Mayan archaeological sites (Yucatan peninsula) (Mexico)	[85]
18.	Mediterranean Basin (Italy)	[86]
19.	Monastery, Coimbra (Portugal)	[87]
20.	Monuments and buildings (Spain)	[88]
21.	Orologio Tower, Martano (Italy)	[89]
22.	Painted buildings (Latin America)	[90]
23.	Rondane Mountains (Antartica)	[91]
24.	Some archeological sites and Monuments (India)	[92]
25.	Stone work of foundation Granada (Spain)	[80]
26.	Temple and Monuments (India)	[16]
27.	Temple and Monuments (Nepal and India)	[14]
28.	Two Cathedrals Spain	[4]
29.	Urban buildings (India)	[92]

Table 2. Cyanobacteria reported on stone monuments, statues and historic monuments in tropical and temperate regions of the globe

S.No	Cyanobacterium	Substratum	Monuments No (Table1)
1.	*Apanocapsa sp*	Lime- stone	3, 13
2.	*Aphanothece sp*	Lime -stone, Painting Wall	3
3.	*Asterocapsa divina*	Cement surfaces	3, 23
4.	*Asterocapsa sp*	Cement surfaces	19, 24
5.	*Calothrix marchica*	Marble	22
6.	*Chroococcus minor*	Lime stone, Travertine	13, 25, 27
7.	*Coleodesmium sp*	Granite	23
8.	*Gloeocapsa atrata*	Marble	3, 24
9.	*G.nigrescens*	Paint wall surfaces	14, 22
10.	*Haplosiphon sp*	Lime stone, dolomite	2,6,13
11.	*Leptolyngbya antarctica*	Granite, lime stone	3, 29
12.	*Lyngbya dendrobia*	Marble, granite, roof surfaces	6,10,11
13.	*L. spiralis*	Roof surfaces	13, 24, 29
14.	*L. polysiphonia*	Cement surfaces, paint wall	25
15.	*Microcystis sp*	water leakage wall, cement surfaces	8, 13
16.	*Myxosarcina sp*	Lime stone, Travertine	3, 17
17.	*Nostoc muscorum*	Lime stone	6,19,25,28
18.	*N. linkia*	Cement surfaces	3
19	*Oscillatoria chalybea*	Lime stone, Marble	6, 20, 24
20.	*O.okeni*	Lime stone, painted wall	25
21.	*O.ornate*	Granite	1, 19
22.	*Oscillatoria sp*	Marble	6,20,21,22
23.	*Phormidium autumnale*	Cement wall	10, 13, 20
24.	*Ph. ambiguum*	Painted wall	3, 13, 24
25.	*Ph. jandinianum*	Lime stone, dolomite	13, 25, 27
26.	*Ph. priestleyi*	soil crust	23
27.	*Plectonema sp*	Lime stone, sand stone	7, 9, 22
28.	*Spirulina subsala*	Paint wall, roof surfaces	13
29.	*Synechococcus sp*	Sand stone	8, 12, 13
30.	*Scytonema sp*	Lime stone, Marble	1, 12, 17
31.	*Westiellopsis sp*	Lime stone, Building wall	4, 11, 16

Table 3 Major classes of compounds identified in black crusts

Compounds	Range[a]	Compounds	Range
Alkanes	C_8-C_{32}	Diterpenoid hydrocarbons	C_{18}-C_{20}
Alkenes	C_8-C_{32}	Triterpenoid hydrocarbons	C_{27}-C_{35}
Alkylbenzenes	C_1-C_{20}	Tricyclic terpane hydrocarbons	C_{23}-C_{29}
Fatty acids	C_4-C_{30}	Steranes and diasteranes	C_{27}-C_{29}
α,ῳ-Dicarboxylic acids	C_4-C_{18}	Polycyclic aromatic hydrocarbons	C_{10}-C_{18}

[a] Range denotes number of carbon atoms in the compounds.

Conclusion

The role of cyanobacteria in the degradation of cultural heritage cannot be neglected. Since they are phototrophs and require no more than light, water, and mineral ions to grow, these microorganisms, along with algae, readily colonize the external surfaces of historic monuments and develop a biofilm, which, in turn, alters the appearance of the building and serves as a substrate for the growth of other deteriogens. Both these and the cyanobacteria themselves can cause aesthetic, chemical, and physical decay. The detection and identification of this group of organisms is extremely important for the future study of the deteriogenic process and the development of control methods. Currently, there are a few research groups worldwide which are studying the role of cyanobacteria in the degradation of historic monuments [68]. Without doubt, this will lead to the development of rapid and reliable molecular techniques for the identification of this problematic group of microorganisms

Acknowledgement

The authors are thankful to the management of A.V.V.M. Sri Pushpam College (Autonomous), Poondi, for providing them necessary facilities and support to carry out this work.

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