2. REVIEW OF LITERATURE

Biodeterioration on monuments occur due to the growth of lower plants like cyanobacteria, micro green algae, bryophytes and lichens. Over a period of time, the growth of vascular plants leads to further deterioration on the monuments. The current study focuses on the growth of lower plants and the cyanobacteria group growing on monuments and investigates the causes of the deterioration of the monument structure by them. The study of this process of biodeterioration initiated by the pioneering cyanobacteria and then followed by other groups like lichens and bryophytes have not been documented concurrently. Most biodeterioration studies had pertaining to individual groups such as biodeterioration by cyanobacteria and microalgae by Ortega-Calvo *et al*., (1991), Crispim and Gaylarde, (2005), Ortega-Morales, (2006), Macedo *et al*., (2009) and Ramirez M. *et al*., (2010); biodeterioration by bryophytes by Gil and Sáiz-Jiménez, (1992) and Altieri and Ricci, (1997) and biodeterioration by lichens by Chen *et al*., (2000), Seaward, (2001) and Clair and Seaward, (2004) among studies outside India. At the national level also past biodeterioration studies have generally been reported for individual plant group. In India, Rossi F. *et al*., (2012), Keshari and Adhikary, (2013), (2014); Pandey (2013), Bhavani *et al*., (2013) and Pradhan (2018) worked on biodeterioration by cyanobacteria. Biodeterioration studies on different monuments in India by lichens have been reported by researchers like, Ayub, (2005), Bajpai and Upreti, (2014), Shukla *et al*., (2014), Joshi *et al*., (2015), Choudhury *et al*., (2016) and Nayaka *et al*., (2017). Bhatnagar *et al*., (2016), Verma *et al*., (2014) and Palani *et al*., (2017) recorded bryophytes from the archeological sites and different monuments of India. Very few researchers have focused on the diversity of bio deteriorative flora or lithophytes comprising all plant groups like algae, cyanobacteria, bryophytes, lichens, pteridophytes and angiosperms. A few researchers have however focused on four of the five groups mentioned above. These include Duraisamy, (2012) who studied the bio deteriorative flora (angiosperm, bryophytes, lichens and pteridophytes) on the culture heritage in Thiruvarur district, Tamil Nadu and Shrishail and Prashantkumar, (2019) who enumerated the lithophytes (Cyanobacteria, Bryophytes, Pteridophytes and Angiosperm) of Gulbarga fort, Karnataka, India.

At regional level, no such inclusive study has been carried out. In Gujarat, a large number of researchers have worked and are still working on several aspects of these different groups of organisms but their emphasis is not on monument degradation.

The current study depended heavily on the researches of past workers on different aspects of biodeterioration by different organisms. The variety of methods employed by these researchers have played an important role in carrying out and meeting the objectives of the current study. This chapter reviews the literature available on biodeterioration by the different groups like cyanobacteria, bryophytes and lichens which are the primary focus of the current study.

2.1 Cyanobacteria

Cyanobacteria are pioneering organisms and have been extensively studies the world over. The literature available for different aspects of cyanobacteria on biodeterioration studies have been enumerated in Table 2.1 below. The important and relevant aspects of these studies have been elaborated after the table.

Table 2.1 – Important literature covering the relevant topics of the current cyanobacteria study

Sr. No.	Topic of Investigation	Important References
1.	Culturing	Rippka, (1988)⸙; Castenholz, (1988)⸙; Ortega-Calvo *et al*., (1991)⸙; Rippka, (1997)⸙; Shah *et al*., (2000)*; Parikh and Madamwar, (2006)*; Waterbury, (2006)⸙; Lakshmi and Annamalai, (2008)*; Mishra and Jha, (2009)*; Rossi *et al*., (2012)⸙; Keshari and Adhikary, (2013)#, (2014)#; Guruvaiah *et al*., (2015)*
2.	Identification and enumeration	Ortega-Calvo *et al*., (1991)⸙; Ortega-Morales, (2006)⸙; Macedo *et al*., (2009)⸙; Adhikary and Kovacik, (2010)#; Ramirez M. *et al*., (2010)⸙; Bhavani *et al*., (2013)#; Ortega-Morales *et al*., (2013)⸙; Pradhan, (2018)#; Shrishail and Prashantkumar, (2019) #
3.	Identification by Molecular study	Nübel, (1997)⸙; Tillett and Neilan, (2000)⸙; Keshari and Adhikary, (2013)#, (2014)#; Faldu *et al*., (2014)*; Ozturk *et al*., (2019)⸙; Patel *et al*., (2019)*
4.	Exopolysaccharides study to understand the role of biodeterioration by Cyanobacteria	Shah *et al*., (2000)*; Parikh and Madamwar, (2006)*; Sureshkumar *et al*., (2007)*; Mishra and Jha, (2009)*; Khattar *et al*., (2010)#; Rossi *et al*., (2012)⸙; Delattre *et al*., (2016)⸙

⸙Global, #National, *Regional (Literatures categorized for individual topics)

2.1.1 Global Status

Important studies on the different aspects of cyanobacteria studies on biodeterioration have been enlisted in the table 2.1. The relevant aspects of these studies are being reviewed below.

2.1.1.1 Culturing

Culturing the cyanobacteria and green micro algae have been carried out by several researchers to meet their specific objectives. Important references which have relevance to the current study are elaborated below.

Rippka, (1988) studied the isolation and purification of cyanobacteria from soil and freshwater habitats. She had studied isolation on various different media like ASM, BG 11, Z8, SAG, BBM, AA, KMC, Cg 10, Kn and D with different salts composition in mM concentrations. These media had different purpose for growth of cyanobacteria and had given different results. Some of her important findings were that the BBM media should be avoided for isolation purpose, only be used in conjunction with other media of lower trace metal content. Similarly, media D and Cg 10 had good results for isolation of the thermophilic cyanobacteria. The AA, KMC and Kn media were favorable for experimental purpose due to their good growth rates and high yields but they were not tolerated by all species of cyanobacteria possibly as a result of relatively high concentration of phosphate.

Castenholz, (1988) had studied culturing methods for cyanobacteria in different media like Chu No. 10 (modified media), Gerloff *et al*., BG 11, D medium, Allen and Arnon and Kratz and Myers media. BG 11 media was found to be a universal media for cyanobacteria. BG 11 worked very well for the quantitative plating of several unicellular cyanobacteria for isolation. Conditions necessary for growth and maintenance of cultures were lamp of cool white, daylight or warm white light, temperature 30° C normally and in case of thermophilic only 45° and above temperature was preferred, all tubes and vessels were autoclaved and sterilized properly for experimental proposes, agar usually at 1-1.5% w/v concentration were among the major ones mentioned.

Rippka, (1997) had used the classical bacterial loop and microspade tools for isolation while the streak plate method on agar plate was the preferred method. For purification she had suggested antimicrobial agents to be useful as last resort for successful purification. Test of purity was checked by aliquots examined critically under the microscope using phase contrast objectives and oil immersion.

Ortega-Calvo *et al*., (1991) had established monoculture of cyanobacteria and algae using either BG 11 or BBM solid media. They had noted a total of 39 taxa which were composed of 15 taxa of Cyanobacteria, 20 taxa of Chlorophyta, one taxon of Xanthophyta and 3 taxa of Bacillariophya. *Microcoleus vaginatus* and *Phormidium autumnale* filamentous cyanobacteria and *Klebsormidium flaccidum* of chlorophyte were predominant taxa on the study sites. They had also tried to understand the role of biodeterioration by SEM observations. These observations revealed that maximum biofilms were recorded on flat sites of the building where constant water retention or small crevices were present. These sites allowed the filaments to penetrate and further the deterioration process.

Waterbury, (2006) in the Prokaryotes book has reviewed the methods of isolation, purification and identification of cyanobacteria. In the chapter, nine recipes for cyanobacteria media including BG 11, Z8, D, AO, Aquil, SN, SNAX, RC and YOPP were described. The identification keys for identified cyanobacteria from pure culture as a purity check of cyanobacteria pure culture species were also given.

Ozturk *et al*., (2019) had used BG 11 media for growth of both cyanobacteria and green algae. Using this media, they had achieved growth of three green algae and four cyanobacteria.

2.1.1.2 Traditional method for identification

Several researchers had used traditional method for the identification of cyanobacteria and green algae.

Ortega-Morales, (2006) had studied the diversity of cyanobacteria on a few monuments of Latin America. In this study, the maximum colonizers were recorded from the order Plurocapsales followed by Proteobacteria, Firmicute, Actinobacteria and Bacteroidetes were also found from the Uxmal site.

Macedo *et al*., (2009) had given an overview on the diversity of cyanobacteria and green algae on monuments of the Mediterranean Basin. 45 monuments which were composed of different substratum were covered in the study. The major substratum reported was limestone followed by marble, travertine and dolomite. On these monuments, a total of 96 taxa of cyanobacteria and 76 taxa of green algae (Chlorophyta) were recorded by them. Among the green algae, the genus *Chlorella* was widely distributed and showed its presence on 20 monuments. It was represented by four species on four different substrata. Among cyanobacterial group, the genus *Gloeocapsa* had the maximum no. of species and occurred on 20 monuments which represented all the substrata found in the study. *Phormidium* and *Chroococcus* species were recorded to be colonizing five substrata and showed high species diversity. The paper also stated that the green algae and cyanobacteria colonization on the different substrata was primarily based on the physical characteristics of the stone surface such as porosity, roughness and permeability and secondarily dependent on nature of the substratum.

Ramirez *et al*., (2010) studied cyanobacteria biofilm on Mayan monument in Palenque, Mexico. They reported *Asterocapsa*, *Scytonema*, *Aphanocapsa*, *Gloeocapsa*, *Gloeothece*, *Nostoc* and *Trentepohlia* from the El Palacio (Palenque Archeological site). They mentioned that biofilm contained the same species while the relative amounts of these differed by site. They had discussed the bioreceptivity of stone and the relationship between the biofilms and their substrata.

Ortega-Morales *et al*., (2013) had recorded majorly 77% cyanobacteria with genera *Gloecapsopsis*, *Rhabdoderma* and *Psudoanabaena* dominating on the North Acropolis complex, Tikal (Guatemala). Also, except *Chlorella* eukaryotic algae were absent.

2.1.1.3 Identification by 16S rRNA

After the traditional method of microorganisms' identification, the most extensively used technique used by researchers for cyanobacteria and micro green algae identification is the 16S rRNA gene sequence analysis.

Nubel *et al*., (1997) tested oligonucleotide primers to amplify the 16S rRNA genes from various species of cyanobacteria, other bacteria and archaea. Tillett and Neilan (2000), used the xanthogenate DNA isolation method for the environmental cyanobacteria, other bacteria and archaea. They had reported DNA isolation for ten species of cyanobacteria, five other bacteria and one archaea by this method. Ozturk *et al*., (2019) had reported DNA extraction by DNA isolation kit (Qiagen) and amplification was done for the internal transcribed spacer sequence (ITS) and 16S rRNA regions for cyanobacteria and micro green algae.

2.1.1.4 Exopolysaccharides characterization

Exopolysaccharides study has been reported earlier for different aspects in only a few research and review papers using different techniques.

Rossi F. *et al*., (2012) characterized and described the role of exocellular polysaccharides produced by five cyanobacteria. Among these three were coccoid strains belonging to two genera namely *Gloeocapsopsis* and *Gloeocapsa* while the remaining two were filamentous strains of the genera *Leptolyngbya* and *Plectonema* from biofilm growing on Parasurameswar Temple and Khandagiri caves, Orissa state in India. They detected 11 to 12 neutral and acidic sugars named fucos, rhamnose, galactosamine, arabinose, glucosamine, galactose, glucose, xylose, fructose, ribose, galactouronic acid and glucuronic acid from hydrolysed released polysaccharides substances (RPS) by Ion exchange chromatography. RPS showed high affinity towards bivalent metal cations which caused weakening of the mineral substrata. RPS has mycosporine like amino acids (MAA) and scytonemins pigments protect against exposing radiation on exposed surfaces in tropical areas.

Delattre *et al*., (2016) reviewed the production, extraction and characterization of microalgae and cyanobacteria exopolysaccharides. In this review, they described various studies related to EPS. FTIR spectroscopy was used to know the global composition of exopolysaccharides. The molar level composition of EPS was studied by HPLC/MS, GC/MS and HPAEC/PAD techniques. They had also mentioned initial linkage analysis and full structural analysis of EPS by different methods like FTIR and NMR.

2.1.1.5 Biodeterioration study

Biodeterioration study has been reported in a few past studies where they have very briefly described the biodeterioration occurrence on the monuments.

Crispim and Gaylarde, (2005) had broadly reviewed the cyanobacteria and biodeterioration of culture heritage. Their review emphasized the preservation of the heritage against the cyanobacterial group because they formed the biofilm. The trichomes of biofilms organisms penetrate a few millimeters into the rock and damages the rock surfaces. According to them cyanobacteria had the main role in biodeterioration due to their thick outer envelopes and the presence of protective pigments.

Ortega-Calvo *et al*., (1991) had emphasized on biodeterioration on some buildings of Spain (Salamanca, Seville and Toledo Cathedrals) and Sweden (Lund Cathedral) by cyanobacteria and Algae.

2.1.2 National status

2.1.2.1 Culturing and study of biodeterioration on monuments

In India, cyanobacteria study for biodeterioration had been taken up by several researchers like Pandey, (2013), Keshari and Adhikary, (2013), (2014) and Pradhan, (2018).

Pandey, (2013) had reviewed biodeterioration and survival strategies of rock dwelling cyanobacteria. The review stated that cyanobacteria have desiccation tolerance power owing to which they grow on exposed surfaces. They produce extracellular polymeric substances which play vital role in surface colonization, biofilm formation and stabilization.

Keshari and Adhikary, (2013) focused on biofilms characterization of monuments of Shantiniketan. They had cultured the biofilms in BG 11 medium and after culturing they found three species of *Scytonema*, three species of *Nostoc*, two species of each of the genera *Asterocapsa* and *Calothrix* and one species each of the genera *Gloeocapsa*, *Aphanocapsa*, *Aphanothece*, *Gloeothece*, *Chroocococcus*, *Chroococcidiopsis*, *Tolypothrix*, *Aulospira*, *Cylindrospermum* and *Westiellopsis*. For cyanobacterial detail study, they had analyzed pigments, measurements of photosynthesis, respiration and acetylene reduction activity (ARA). Absorption spectra of cyanobacterial pigments showed a high quantity of scytonemin, MAA and carotenoids. ARA ranging from 108 to 116 nmol C_2H_4 g/dry material/hour. They therefore concluded that, all species of cyanobacteria occurring on the stone monuments were capable of fixing nitrogen and able to survive on the outer surface of the monuments in harsh environment conditions.

Keshari and Adhikary, (2014) emphasized on the diversity of cyanobacteria on stone monuments and building facades of India along with their phylogenetic analysis. They isolated 24 different species falling under 11 genera of cyanobacteria. Using the 16s rRNA gene sequences and maximum parsimony analysis they built a phylogenetic relationship between all cyanobacteria species. Nostocales and Stigonematales from different localities of India formed a genera wise monophyletic clade. Tree result showed that the cyanobacteria in the biofilms on the exterior of stone monuments in tropical climate regime like India were clustered quite differently from the species isolated from stone surface of different climate regime.

Pradhan, (2018), enumerated 54 species of epilithic and cryptoendolithic cyanobacteria from the historic monuments and caves located in various districts of Western Odisha namely Bargarh, Bolangir, Boudh, Deogarh, Jharsuguda, Nuapada, Sambalpur and Sonepur. Among all these, four genera namely *Calothrix*, *Westiellopsis*, *Chrlorogloeopsis* and *Nostoc* were cultured in laboratory under control and treated conditions such as low temperature, high temperature and UV-B light. Based on suggested conditions, tolerance capacity of *Nostoc* species was more compared to other genera.

2.1.2.2 Identification by Traditional method

Several researchers had used the traditional method for identification of the cyanobacteria as their work mostly involved the preparation of list of species form specific selected sites

Bhavani *et al*., (2013) studied diversity of cyanobacteria from temples and monuments of Thanjavur district, Tamilnadu. They had found 57 species from big temple of Thanjavur, 36 species from Sivan temple of Gangaikonda sozhapuram, 40 species from Thanjapurisvarar temple of Karanthai, 44 species from Sivan temple of Senthalai and 38 species from Venkatesaperumal temple of Varagur. *Lyngbya* was the dominant genus identified from the temples and monuments followed by *Oscillatoria* and *Phormidium*.

Shrishail and Prashantkumar, (2019) enumerated the lithophytes of Gulbarga fort, Karnataka, India. They had found 5 species of cyanobacteria, 5 species of bryophytes, 5 species of pteridophytes and 44 species of angiosperms. The cyanobacteria isolated included the filamentous form belonging to the genus *Anabaena*, *Oscillatoria* and *Scytonema*.

2.1.2.3 Identification by 16 S rRNA gene sequences

Identification using gene sequences was done by Khattar *et al*., (2010), Singh *et al*., (2011) and Keshari and Adhikary, (2013).

Using the 16S rRNA gene sequence, one species of cyanobacteria named *Limnothrix redekei* was identified by Khattar *et al*., (2010).

Singh *et al*., (2011) used CTAB method for DNA extraction and for amplification they had used their own designed 16 S rRNA primers. Based on this protocol, they had identified four cyanobacteria at the genus level named *Rivularia* sp., *Anabaena* sp., *Synechocystis* sp. and *Synechococcus* sp. and one reported at species level named *Anabaena variabilis*.

Keshari and Adhikary, (2013) reported DNA isolation by CTAB method and the amplification had been done by using the method described by Nubel, *et al*., (1997), The 16S rRNA primers had led to the identification of three species of cyanobacteria named *Scytonema milleri*, *Scytonema* sp. and *Tolypothrix campylonemoides*.

2.1.2.4 Exopolysaccharides characterization

In India, the EPS of *Limnothrix redekei* was studied by Khattar *et al*., (2010). This study investigated the isolation of exopolysaccharide of the single species *Limnothrix redekei* and its characterization by thin layer chromatography and fourier transformed infrared analysis (FTIR). Chemical analysis of EPS recorded presence of glucose or mannose, ribose, rhamnose and uranic acid.

2.1.3 Gujarat status

In Gujarat, we did not come across any earlier investigation on biodeterioration on the monuments. However, several researchers are working on different aspects of cyanobacteria or micro green algae. In such studies, the initial phase i.e. culturing of the cyanobacteria is similar for most aspects of the cyanobacteria or micro green algae study.

2.1.3.1 Culturing

Some of the researchers like Lakshmi and Annamalai, (2008) and Guruvaiah *et al*., (2015) reported BG 11 media for cyanobacteria growth. Some other researchers, had also reported BG 11 media for cyanobacteria as well as algae growth. (Shah *et al*., 2000; Parikh and Madamwar, 2006; Mishra and Jha, 2009). However, except culturing which was relevant to the present study, all these studies focused on other aspects of cyanobacteria and algal research.

2.2.3.2 Culturing and Identification by 16S rRNA gene sequences

Several past studies at the state level involved the of the microorganisms after their growth in culture media.

Faldu *et al*., (2014) studied cyanobacteria by modern method for identification by 16S rRNA gene sequences. For culturing, they had used BG 11 media for growth of the strains.

Patel *et al*., (2019) investigated the diversity of cyanobacteria from the Rann of Kachchh. They had also used BG 11 media for culturing and 16S rRNA gene sequences for molecular identification.

2.2.3.3 Broad characterization of Exopolysaccharides

Exopolysaccharides were broadly mentioned in a few studies for physical properties and its characterization for aspects other than biodeterioration.

Shah *et al*., (2000) had investigated the exopolysaccharide of *Cyanothece* sp. by nuclear magnetic resonance and infrared spectrum. They also studied the physicochemical properties of the exopolysaccharide.

Parikh and Madamwar, (2006) carried out the partial characterization of EPS from four strains of cyanobacteria named *Cyanothece* sp., *Oscillatoria* sp., *Nostoc* sp. and *Nostoc* *carneum* by infrared spectra in which they reported four sugars moieties viz. mannose, glucose, xylose and ribose.

Sureshkumar *et al*., (2007) reviewed the bacterial exopolysaccharides. In this review, they emphasized that both prokaryotic and eukaryotic organisms produced EPS. According to them, chemically the EPS was a high molecular weight polysaccharides and had heteropolymeric composition. Due to this, exopolysaccharide has various applications in the food, pharmaceutical and other industries.

Mishra and Jha, (2009) reported EPS characterization from microalgae by FTIR and HPLC techniques. Based on these techniques, they reported four monosaccharides such as glucose, galactose, fructose and xylose.

2.2 Bryophytes

Several past studies which focused on the study of biodeterioration by bryophytes, had helped understand the diversity and its process. These studies have helped in fulfilling the objectives of the current study. Some of the important studies are cited below in table 2.2 under specific topics and they have been briefly reviewed later.

Table 2.2 – Important studies have helped accomplish the bryophyte aspect of the current study

Sr. No.	Evaluation of research paper by topic wise	References
1.	Diversity and distribution study of bryophytes on monuments	Ezer *et al*., (2008)[ǂ]; Barukial, (2011)[#]; Duraisamy R. (2012)[#], Verma *et al*., (2014)[#], Shah and Gujar, (2015)*, Elharech *et al*., (2017)[ǂ], Palani *et al*., (2017)[#]
2.	Role of Bryophytes in biodeterioration	Garcia Rowe and Saiz Jimenez, (1991)[ǂ]; Saiz Jimenez *et al*., (1991)[ǂ]; Saiz Jimenez, (1994)[ǂ], Tiano P., (2002)[ǂ], Bhatnagar *et al*., (2016)[#], Dakal and Cameotra, (2012)[ǂ]

[ǂ]Global, [#]National, *Regional (Studies categorized under individual topics)

The biodeterioration of monuments by bryophyte is a much less discussed subject in the present day in field of Bryology. Hence, very few past studies are available addressing the biodeterioration by bryophytes at global level and national level. At regional level, such literature on biodeterioration of monuments was not encountered.

2.2.1 Global status

2.2.1.1 Enumeration the bryophytes on monuments

A review of the few studies that have focused on the enumeration of bryophytes on monuments are given below.

Ezer *et al*., (2008) reported bryophytes on the archeological site of Tilmen Hoyuk, Gaziantep (Turkey). They noted 45 bryophytes taxa of which 38 were mosses and 7 were liverworts that belonged to 20 families. Among these families, members of the four families named Pottiaceae, Grimmiaceae, Brachytheciaceae and Orthotrichaceae were dominant representing 12, 6,4 and 3 taxa respectively. Of the bryophyte species, 15 species were recorded only on basaltic walls, 17 species were recorded from the both surface soil and basaltic wall whereas only 12 species were reported only from the soil surfaces of the study sites. All over, 32 species were recorded from the rock substrate. Majority of the members were reported from the Pottiaceae, Grimmiaceae and Bryaceae.

Elharech *et al*., (2017) studied the bryological flora at the archeological site of Chellah, Morocco. They reported 20 species of bryophytes belonging to 10 families. From that, 4 families named Pottiaceae, Brachytheciaceae, Funariaceae and Bryaceae were dominant with 13 species. The species recorded were *Barbula unguiculata*, *Didymodon fallax*, *Timmiella barbuloides*, *Tortula marginata*, *Tortula muralis*, *Trichostomum crispulum* from the family Pottiaceae, taxa *Rhynchostegium megapolitanum*, *Rhynchostegiella curviseta*, *Scorpiurium circinatum* from the family Brachytheciaceae, *Funariella curviseta* and Entosthodon pulchellus from the Funariaceae family and *Ptychostomum capillare* and Bryum radiculosum from bryaceae family were recorded. Only 6 species of liverworts named *Lunularia cruciate*, *Targionia hypophylla* and *Targionia lorbeeriana*, *Riccia crystallina*, *Fossombronia angulosa* and *Sphaerocarpos michelii* from the families Lunulariaceae, Targioniaceae, Ricciaceae, Fossombroniaceae and Sphaerocarpaceae respectively were reported. Family, Fissidentaceae had only single genus reported named *Fissidens bryoides.*

2.2.1.2 Biodeterioration study

Bryophytes as a biodeteriogen on historical monuments have been studied by a few researchers like Garcia Rowe and Saiz Jimenez, (1991); Saiz Jimenez *et al*., (1994), Tiano P., (2002) and Dakal and Cameotra, (2012). The contribution of these researchers in study of biodeterioration by bryophytes has been discussed below:

Garcia Rowe and Saiz Jimenez, (1991) studied the bryophytes and lichens as agent of deterioration of building materials in Spanish Cathedrals. The Spanish Cathedrals are made up of different materials that included sandstones, limestones, granites, tiles, flagstones and mortars. Communities of mosses grew preferentially on mortars with pronounced disintegrating effects due to penetration of rhizoids. Both lichens and bryophytes lead to mechanical and chemical damage to stonework. That represented a stage in the succession which gave rise to the attack by vascular plants whose roots caused important deterioration in the structure of the buildings, mainly roofings, tiles, gutterings, etc. The difficulty in access to the roofs and the traditional lack of cleaning and maintenance which was common to these great monuments were the major reasons for their deterioration.

Saiz – Jimenez *et al*., (1991) investigated the biodeterioration on polychrome roman mosaics. Roman Mosaics were built by bricks, mortars, stones, ceramic and vitreous materials. Lichens were recorded on almost all type of materials encountered while bryophytes were limited to mortar only. Vascular plants were also recorded in the Archeological site of Italica. They postulated that the manual cleaning and removal of the lichens, mosses and vascular plant method was a doubtful method of preservation as the pieces of the removed lichens and mosses could be enriching the substrate with organic matter and humus which could potentially favor the future colonization by vascular plant attack.

Saiz – Jimenez, (1994) have reviewed the biodeterioration of stone of historical buildings and monuments. The review included bacteria, fungi, cyanobacteria, algae, lichens, bryophytes and vascular plants as biodeteriogens. All these organisms initially caused deterioration by mechanical process followed by chemical process. According to them, the attack of vascular plant represents the last step of biological stone decay.

Tiano, (2002) had discussed the biodegradation of the culture heritage by different organisms and their decay mechanisms. They had described the biodeterioration mechanism by liverworts and mosses. Wherein liverworts and mosses were observed on humus deposited substrate. Principle source of this deposition was considered to be the accumulation of atmospheric particles and some dead microorganisms. On these humus deposition, mosses and liverworts could stay hydrated for longer time. According to the author, the mosses had the capacity to accumulate the Ca^{+2} ions which could be related to its biodeterioration capacity.

Dakal and Cameotra, (2012) reviewed the microbial induced deterioration of architectural heritage. Artists and architects had used high strength, durable and beautiful stones like marble and limestone for the construction of monuments like Taj Mahal, Milan Cathedral, Roman Catacombs and Necropolis in Rome etc. These historic monuments are exposed to the environment which has led to easy access by algae, cyanobacteria, fungi, bryophytes and some other group of organisms. These organisms interacted with the mineral matrix of the stone substrate under varied environment conditions. According to the authors this has resulted in the deterioration of stones by multiple mechanisms of various organisms like cyanobacteria, algae, mosses and liverworts. According to them, this has deteriorated the aesthetic value of the structure by chemical deterioration.

2.2.2 National status

2.2.2.1 Enumeration the bryophytes on the monuments of India

Barukial, (2011) enumerated the bryophytes of Assam. In this investigation, a total 162 taxa of bryophytes falling under 90 genera and 39 families from different habitats in Assam were studied. Different habitats such as river bank, river bed, earth cutting slopes, termite mound, tree trunks, knotholes of tree trunks, rotten wood logs, fruit bodies of wood rotting fungi, birds'nest and old historical monuments were selected. Among all these, five bryophytes species were reported on old historical monuments of Assam. This included, one liverwort (*Porella gracillima*) and four mosses (*Fissidens pulchellus*, *Hydrogonium arcuatum*, *Hydrogonium inflexum*, *Hyophilla involuta*).

Duraisamy, (2012), investigated the biodeteriorative flora on cultural heritages sites in Thiruvarur district, Tamil Nadu. He had recorded total 127 plant species comprising of different plant groups such as angiosperm, bryophytes, lichens and pteridophytes from various cultural heritage sites. In this study, angiosperm plants constituted 89% of the species reported (113- wall plants). 4 % were bryophytes (5 species), 4 % were lichens (5 species) while 3% were pteridophytes (4 species). The bryophytes reported comprised of 4 mosses named *Barbula indica*, *Bryum coronatum*, *Gymnostomiella vernicosa* and *Semibarbula orientalis* and a single species of liverworts named *Cyathodium cavemarum*. Among the 5 lichen species, 3 species were foliose lichen such as *Collema polycarpun*, *Heterodermia diademata* and *Physcia dubia* one was a species of leprose lichen *Chrysothrix candelaris* and one was a fruticose lichen *Cladonia coniocraea*.

Verma *et al*., (2014) reported bryophytes from the famous archeological site Talatal Ghar of Sibsagar, Assam. From this monument, seven bryophytes were reported of which four were liverworts and three were mosses. All the liverworts, *Asterella khasiana*, *Plagiochasma rupestre*, *Marchantia palmata* and *Marchantia subintegra* belonged to the order Marchantiales. The mosses recorded include *Physcomitrium japonicum*, *Funaria hygrometrica* and *Fissiden* sp.

Palani *et al*., (2017) enumerated the mosses of Bodamalai Hills in Eastern Ghats, Tamil Nadu. They had reported 52 species belonging to 38 genera and 21 families in their checklist of mosses. Most of the species reported were rupicolous and very few were lignicolous. Among the mosses, Pottiaceae was dominant family followed by Bryaceae, Stereophyllaceae, Sematophyllaceae and Brachytheciaceae. While some bryophytes were reported to grow on monuments, they were not specified.

2.2.2.2 Biodeterioration

Studies on the specific biodeterioration role or calcium uptake by bryophytes has not been reported from India. A study involving a brief overview of biodeterioration on monuments by different organisms has been given by Bhatnagar *et al*., (2016). This study just made a passing reference on bryophytes involved in biodeterioration. According to them the cyanobacteria caused aesthetic damage to monuments by developing various colored microbial films on their surface. These microbial biofilms absorbed inorganic materials from the substrata. This enhanced the water retention capacity of stone by dissolving the nearby material. This organism’s slimy surface promotes the adherence of other biological propagules like bryophytes, fungi etc. They had also observed that the mosses and liverworts mainly deteriorate the stone aesthetically. According to the authors mosses and liverworts could also cause some degree of biochemical disintegration of the stone surface by extracting minerals cations from the stones.

2.2.3 Gujarat Status

Studies on bryophytes involved in biodeterioration of monuments has not been encountered in the literature survey. However, a few earlier studies have reported the presence of bryophytes on monuments. In a study on the distribution and diversity of bryophytes in Gujarat, Shah and Gujar, (2015) reported the bryophyte diversity from a variety of habitats. They reported, a total of 12 bryophytes comprising of 7 mosses (*Gymnostomiella vernicosa*, *Funaria hygrometrica*, *Semibarbulla orientalis*, *Hydrogonium* *consanguineum*, *Hyophila spatulata*, *H. nymanniana*, *H. involuta*) and 5 liverworts (*Plagiochasma rupestre*, P. *microcephalum*, *P. intermedium*, *Asterella angusta*, *Cyathodium cavernrum*) from rock surfaces and old structures all over the state.

2.3 Lichens

The current Lichen biodeterioration study involved the identification of Lichens and investigating the role they play in the biodeterioration of monuments. The table 2.3 below enumerated the past important literature on these aspects of the study. These have been elaborated later.

Table 2.3 – Essential studies on different topics to achieve the objectives of the current lichen study

Sr. No.	Evaluation of research paper by topic wise	References
1.	Diversity and distribution study of Lichens on monuments	Aptroot and James, (2002)[ǂ], Ayub, (2005)[#], Bajpai and Upreti, (2014)[#]; Bultmann H. *et al*., (2015)[ǂ], Joshi *et al*., (2015)[#], Uppadhyay *et al*., (2016)[#]; Choudhury *et al*., (2016)[#], Nayak *et al*., (2017)[#], Aptroot *et al*., (2017)[ǂ], Behera *et al*., (2020)[#]
2.	Role for biodeterioration by Lichens	Chen *et al*., (2000)[ǂ], Seaward, (2001)[ǂ], Clair and Seaward, (2004)[ǂ],Upreti *et al*., (2009)[#]; Bajpai and Upreti, (2014)[#], Shukla *et al*., (2014)[#], Salvadori and Municchia, (2016)[ǂ]

[ǂ]Global, [#]National, *Regional (Studies categorized for individual topics)

2.3.1 Global status

2.3.1.1 Enumeration of Lichens

Lichen species reported on monuments of Europe and the role they play in degrading the monuments have been reported by several workers (Aptroot and James, 2002; Bultmann H. *et al*., 2015 and Aptroot *et al*., 2017). Some of these studies have been briefly described below.

Lichens grow naturally on all substrates including very nutrient poor ones. As lichens mostly do not take nutrients from the substrate, but mostly from the air and ambient water they are poor competitors and tend to favor open habitats and avoid other vegetation. Lichens species are primarily saxicolous in most part of the world outside the tropics. Aptroot and James, (2002) monitored the lichens on monuments in western Europe. The main focus of their study was the biodiversity of lichens on monuments, evaluating their historical dating and understanding the potential harmful effects of lichens on the monuments. They reported up to

56 lichens per site and a total of 128 lichen species form the selected sites. They also noted that at least 14 species named *Aspicilia cupreogrisea, A. grisea, A. verrucigera, Fuscidea cyathoides, F. praeruptorum, Lecanora frustulosa, Lecidea promixta, Lepraria neglecta, Parmelia disjuncta, Rhizocarpon lecanorinum, Rinodina confragosa, Stereocaulon dactylophyllum, S. evolutum* and *Umbilicaria deusta* were restricted to megalithic monuments of the Netherlands.

Bultmann H. *et al*., (2015) investigated the European vegetation dominated by lichens, bryophytes and algae. The study included a large number of habitats including soils, rocks, fresh and brackish water and ice. A total of 49 taxa validated new species in cryptogams vegetation of the Europe by these researchers. Some Bryophytes and lichens species were recorded from the exposed rock surface. *Racomitrietea heterostichi* and *Schistidietea mollusci* were the bryophytes species and *Rhizocarpetea geographici*, *Clauzadeetea immersae*, *Verrucarietea nigrescentis*, *Collematetea cristati*, *Leprarietea chlorinae* and *Roccelletea phycopsis* lichen species enumerated by them.

Aptroot *et al*., (2017) monitored lichens on the megalithic monuments in Netherland for 22 years and found that between 1988 and 2010 the number of lichen species per monument increased, especially those that grow normally as epiphytes and nitrophytes. All these changes in lichen species composition largely reflected through changes in acid deposition, eutrophication and climate. They also mentioned that some changes on species composition can be attributed to life span. For example, several species of lichens have a long life span, but most pioneer species such as *Trapelia coarctata*, acidophytic epiphytes or acid soil crust species such as *Placyntheilla* and *Trapeliopsis* and a few calciphilous species such as *Caloplaca* and *Verrucaria* found on monuments usually had a short life span.

2.3.1.2 Biodeterioration study

Chen *et al*., (2000) worked on the weathering of rocks induced by lichen colonization. According to them the effects of lichens on their mineral substrates could be attributed to both physical and chemical process. The physical processes included mechanical disruption of the rocks caused by hyphal penetration, expansion and contraction of lichens thallus, swelling action of the organic and inorganics salts due to lichen activity. Other than these, excretion of various organic acids particularly oxalic acids, which could dissolve the minerals and chelate metallic cations significantly impacting chemical weathering. As a results, many rock forming minerals exhibit extensive surface corrosion. According to the authors the variety of rocks colonized by lichens in nature have the precipitation of poorly ordered iron oxides and amorphous alumina-silica gels, crystalline metal oxalates and secondary clay minerals.

Salvadori and Municchia, (2016) reviewed the role of fungi and lichens in biodeterioration of stones monuments. The effects caused by many epilithic lichen species in the deterioration of different types of stone has been extensively investigated and demonstrated by them. They put forward an interesting hypothesis involving the secretion of siderophores-like compounds. These compounds are iron chelating molecule. Their results suggested that the siderophore-like compound represented the chemical agents responsible for biodeterioration of both silicate and carbonate rocks through pitting and etching. They also provided another perspective that lichens can provide bio protection for stone surfaces by acting as a barrier against weathering, retaining moisture, increasing waterproofing, reducing thermal stress and erosion and absorbing pollutants. According to them the evaluation of their role in biodeterioration vs. bio protection cannot be generalised and it can vary according to the behaviour of different species as well as environmental conditions. They also highlighted that in order to guarantee the best decision for stone conservation, cleaning operations should not be based on generalised approach, but should rather be based on a careful evaluation of different aspects concerning biodeterioration and bio protection.

2.3.2 National status

2.3.2.1 Enumeration of Lichens

Several researchers were reported the lichens from different monuments across India (Ayub, 2005, Joshi *et al*., 2015, Uppadhyay *et al*., 2016, Choudhury *et al*., 2016, Nayak *et al*., 2017, Behra *et al*., 2020). A brief review of their study is given below:

Ayub, (2005) investigated the lichen flora of some major historical monuments and buildings of Uttar Pradesh. He had found 14 species belonging to 6 genera and 6 families of lichens growing on some major historical monuments and buildings of Agra, Allahabad, Faizabad, Kanpur, Lucknow and Varanasi. Out of the fourteen species only *Lecanora coriensis* and *Arthopyrenia calcicola* were the crustose form of lichens while the remaining 12 species were squamulose lichens. *Peltula patellata* was commonly inhabiting most of the sites except Allahabad and Faizabad. *Endocarpon rosettum*, *E. subrosettum* and *Phylliscum indicum* were the most commonly occurring species on almost all the monuments surveyed.

Joshi *et al*., (2015) studied the lichens on Jageshwar monuments, Almora, Uttarakhand. They identified a total of 18 lichen genera belonging to 13 families colonizing these monuments. According to them the most important lichens inhabiting the monuments were *Caloplaca*, *Phaeophyscia*, *Lecanora*, *Punctelia* and *Lepraria*. In addition to the lichens species, some eukaryotic green algae and bryophytes were also found to be forming greenish spots and coloration on monument surfaces.

Uppadhyay *et al*., (2016) studied the diversity and distribution of lichens from the monuments in and around Gwalior. They had reported 28 lichen species belonging to 16 genera and 9 families. The members of the lichen family Physciaceae, Teloschistaceae and Verrucariaceae were found to be dominant on monuments being represented by 5 species each. Among the different growth forms, the crustose form had exhibited luxuriant growth, followed by squamulose and foliose on various monuments. According to the authors, lichen colonization based on substrate preference was apparent by occurrence of a maximum diversity of lichens represented by 27 species on sandstone, followed by concrete, igneous granite, calcareous and clay which were represented by 7, 6, 5 and 2 species respectively. The rock porosity was calculated to measure water holding capacity and the correlation between rock porosity and lichen growth were studied by them. The results revealed that the squamulose form of lichen *Endocarpon rosettum* and *Endocarpon subrosettum* with thick medullary zone, was found grown on rocks having maximum water holding capacity of 43% each followed by *Phylliscum indicum* and *Endocarpon nanum* growing on rocks with 23% and 16.5% water holding capacities respectively.

Choudhury *et al*., (2016) had done preliminary study on lichens of ancient historical ruins of Bamuni hills, Tezpur, Assam. They had enumerated 16 lichen species consisting 10 genera from the eight families. Family Physciaceae was the dominant family having 6 species. Based on the different growth form crustose lichens were dominant followed by foliose and squamulose lichens with species 8, 7 and 1 respectively. Production of secondary metabolites having chelating properties by enumerated lichens was also reported by them. They concluded that lichen species directly affected the biogeochemical weathering of minerals and rock substratum of the monument.

Nayaka *et al*., (2017) studied the lichens growth on sun temple of Konark in Odisha. They had documented a total of 15 species belonging 14 genera and 11 families growing on the sites surveyed. The predominant species observed were crustose lichens followed by foliose and squamulose lichen species.

Behera *et al*., (2020) studied the lichens on the monuments of Odisha which were made up of khondalite, laterite and sandstone which are porous in nature. A total of 31 species 16 genera and 12 families were enumerated. Microlichens were dominant represented by 24 species. They reported that the growth of foliose lichens such as *Dirinaria* and *Pyxine* were luxuriant and tightly attached to the substratum. They also noted that all the reported lichens produced secondary metabolites that participate in chelation except *Peltula* and *Phylliscum*. Based on these it was concluded that Sun temple (Konark), Raja Rani temple (Rameshwar) and Satrughneswar temples (Bhuneshwar) were monuments at a high risk of deterioration. They also highlighted the potential bio protective role of lichens as an external sheath specifically in monuments made up of sandstone.

2.3.2.2 Biodeterioration study

Biodeterioration on different monuments of India have been studied by Upreti *et al*., (2009), Bajpai and Upreti, (2014), Shukla *et al*., (2014) and others. Their investigations are briefly reviewed below:

Upreti *et al*., (2009) had highlighted the need of lichen study in biodeterioration. They had listed different causes towards deterioration of the Indian monuments due to lichen flora. According to them, initial biophysical stone degradation occurred from the penetration of the attachment devices of the thallus into the pores, pre-existing cracks and fissures in the stone. Over a period of time these cracks and fissures could subsequently widen due to an increase in the mass of the thallus during growth. This could result in loosening the minerals in the form of granules and then later turns to chemical weathering. They had also mentioned that porous and calcareous rocks were more susceptible to physical penetration by lichens. Foliose and crustose lichens were found to be most harmful because of their nature of attachment.

Bajpai and Upreti, (2014) had written a book entitled “Lichens on Indian Monuments Biodeterioration and Biomonitoring” in which they investigated different monuments of India from the selected regions such as Karnataka, Madhya Pradesh, Maharashtra, Orissa and Uttar Pradesh. They investigated more than 1000 lichen specimens found growing on the monuments or buildings. This study resulted in a systematic account of 112 species growing over some selected Indian monuments. They also suggested the techniques to measure the deterioration of monuments, factor affecting deterioration, biomonitoring using active as well as passive monitoring.

The book by Shukla *et al*., (2014) dealt with different topics related to lichen study. These included unique characteristics features of lichens which facilitate their survival in extreme climates. According to them the lichens synthesis of unique secondary metabolites is known to protect lichens against the increasing environmental stress. They also discussed in detail about the role of lichens in monitoring pollution and a significant problem of recent times.