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Bryophyte colonization on the monuments of Champaner Pavagadh – UNESCO World Heritage Site and its association with geological substrates

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ABSTRACT
Bryophyte colonization on selected monuments of Champaner Pavagadh UNESCO World Heritage site was investigated. A total of seventeen bryophytes including nine liverworts, six mosses and two hornworts, causing degradation of the monuments, were recorded. The geological substrate was studied by a thin section of substratum and Powder X-Ray Diffraction analysis. Microscopic images of the thin section of geological substrate of the samples showed the presence of quartz, ferruginous materials, calcareous materials, orthoclase and plagioclase feldspar minerals. The XRD analysis spectra revealed the presence of different minerals such as aluminosilicate, calcite, muscovite, hematite, magnetite, orthoclase and plagioclase feldspar. The geological investigation showed that selected monuments are composed of the most calcareous materials. Hence, calcium uptake by *Asterella wallichiana* (Lehm. & Lindenb.) Grolle and *Hyophila involuta* (Hook.) A.Jaeger from the substratum was studied using a flame photometer. Maximum calcium uptake (i.e., 52 µg/mg) was obtained for plant samples of *Asterella wallichiana* compared to *Hyophila involuta* (i.e., 36 µg/mg). Bryophyte colonization on monuments was initiated by humus formation, followed by the protonema stage, mature gametophyte and rhizoids penetration into crevices as well as porous surfaces, forming cracks and degrading structures. This deterioration mechanism due to bryophyte colonization is explained by pictorial representation.

KEY WORDS
Biodeterioration,
calcium uptake,
liverworts,
mosses,
minerals.

RÉSUMÉ
Colonisation de bryophytes sur les monuments de Champaner Pavagadh – site du patrimoine mondial de l'UNESCO et son association avec les substrats géologiques.
La colonisation des bryophytes sur des monuments sélectionnés du site de Champaner Pavagadh (classé au patrimoine mondial de l'UNESCO) a été étudiée. Au total, dix-sept bryophytes, dont neuf hépatiques, six mousses, et deux anthocérotes, causant la dégradation des monuments, ont été recensées. Le substrat géologique a été étudié par une coupe fine du substrat et une analyse par diffraction des rayons X sur poudre. Les images microscopiques de la section fine du substrat géologique des échantillons ont montré la présence de quartz, de matériaux ferrugineux, de matériaux calcaires, de minéraux de feldspath orthoclase et plagioclase. Les spectres d'analyse XRD ont révélé la présence de différents minéraux tels que l'aluminosilicate, la calcite, la muscovite, l'hématite, la magnétite, les feldspath orthoclase et plagioclase. L'étude géologique montre clairement que les monuments sélectionnés présentent les matériaux les plus calcaires. Par conséquent, l'absorption de calcium par *Asterella wallichiana* (Lehm. & Lindenb.) Grolle et *Hyophila involuta* (Hook.) A.Jaeger à partir du substrat a été étudiée à l'aide d'un photomètre à flamme. L'absorption maximale de calcium (52 µg/mg) a été

MOTS CLÉS
Biodétérioration,
absorption du calcium,
hépatiques,
mousses,
minéraux.

obtenue pour les échantillons de plantes d'*Asterella wallichiana* en comparaison de *Hyophila involuta* (36 µg/mg). La colonisation des bryophytes sur les monuments a été initiée par la formation d'humus, suivie par le stade protonéma, le gamétophyte mature et la pénétration des rhizoïdes dans les crevasses ainsi que dans les surfaces poreuses, formant des fissures et dégradant les structures. Ce mécanisme de détérioration par la colonisation des bryophytes est expliqué par une représentation picturale.

INTRODUCTION

Bryophytes are non-lignified plants found in moist shady places like on tree trunks, rocks, ancient buildings or monuments (Buck & Goffinet 2000). The bryophyte colonization on monuments brings undesirable changes in their physical, chemical, and mechanical integrity. This process, known as biodeterioration, results in the deterioration and damage to their aesthetic value (Allsopp *et al.* 2004; Dakal & Cameotra 2012). The structure of the monuments is built with different geological substrates, such as sandstone, basalt, rhyolite, granite, mortar and bricks. All these materials have different mineral compositions determining which organisms grow and proliferate on the surface. The sources of nutrients for bryophytes are precipitation, dust and substrate (Glime 2017). Bryophytes absorb certain nutrients from the substrate for their growth, leading to substrate degradation. On porous surfaces, rhizoids penetrate into the pores and fissures of the substratum, resulting in crack formation in the surrounding area of the substratum. Such mechanical and chemical potency of the bryophytes causes damage and deterioration to the walls' structure.

India is well known for its culture and heritage sites. It has a total of 38 UNESCO World Heritage Sites (Anonymous 2020). But all these heritage monuments are threatened by adverse environmental conditions, such as a typically warm and humid environment that is ideal for the growth of biological organisms. To preserve them from biodeterioration, it is necessary to study the diversity of bryophytes, their relationship with geological substrate and their mechanism of biodeterioration. But to date, the process of biodeterioration by bryophytes and their role in the process have not been documented at national level. Moreover, across the world, there is very little documentation on the relationship between the geological substrate and bryophytes on monuments. The current paper focuses on bryophyte colonization on specific monuments, their association with geological substrates, and the absorption of calcium (from intercellular regions) that helps them to grow, in order to understand the mechanism of biodeterioration.

MATERIAL AND METHODS

Study sites

Gujarat is rich in heritage sites and monuments. Champaner Pavagadh is a famous heritage site listed among UNESCO World Heritage Sites since 2004 (Sinha *et al.* 2004). Hence, this complex is very important. In the past, it was a regional capital city built by Mehmud Begda in the 15th century (Sinha *et al.* 2004). Champaner Pavagadh complex is constituted of 20 monuments of Champaner and 15 monuments of Pavagadh under the protection of the Archaeological Survey of India (ASI). Among them, three monuments, namely Makai Kothar (22°27'54.40"N, 73°31'20.30"E), Navlakha Kothar (22°28'2.19"N, 73°30'40.79"E) and premises of Jain Temple (22°28'00.8"N, 73°30'54.7"E), located at the hill of Pavagadh, were selected for this study. Three monuments, namely Saher ki Masjid, Mandvi and Amir Manzil (not protected by ASI but under supervision of ASI), located at the foothill of Pavagadh in Champaner town (22°29'0.94"N, 73°31'53.60"E) were also selected (Fig. 1).

Observation, collection and identification of samples

The study was carried out from August 2017 to October 2019. Field sites were visited several times in 2017 (August and October), 2018 (April, August and October), and 2019 (July, September and October) for sample collection and to understand the process. The collection of bryophyte samples was conducted with a few modifications to the methodology given by Glime (2017). A knife or scalpel was used to remove the specimen from the substratum. Samples were preferably collected at the reproductive stage (with sporophyte). Then, they were kept in small air-blown zipper bags or brown paper bags with necessary field information labelled, such as date of collection, location of site, substratum, name of sample, and group of sample (liverwort, hornwort or moss). Other relevant information (direction of light, associated flora, etc.) was recorded in the field diary. In total 25 bryophyte samples were collected for the investigation and herbarium preparation. Collected samples were air-dried in the lab for herbarium preparation following Glime (2017). Fresh samples were observed under the Leica SD6 Stereo-binocular microscope. For the identification of species, morphology and micro-morphological characteristics were studied, and cell dimensions were measured. Photography of observed characters was carried out using a 3.2 MP Tucsen camera. Bryophytes were identified based on micro-morphological characters using dichotomous keys and characters from the flora or monographs such as Gangulee (1969, 1974), Chaudhary & Deora (1993), Bapna & Kachroo (2000), Chaudhary *et al.* (2006, 2008) and Aziz & Vohra (2008). Confirmation of some doubtful samples was done with the help of experts at the Bryology Laboratory of the CSIR – National Botanical Research Institute, Lucknow. The current status of the species

Fig. 1. — Glimpses of sites showing biological colonization and deterioration of the monuments of Champaner Pavagadh.

was checked using Söderström *et al.* 2016 and Anonymous 2014. The samples were deposited in the herbarium at the Maharaja Sayajirao University of Baroda (BARO), Vadodara.

Geological substrate investigation

Collection of the geological substratum samples from the selected monuments was not possible as they were under the protection of ASI, Vadodara circle. Hence, the collection was carried out from the surrounding area of three monuments, Makai Kothar, Navlakha Kothar and the premises of Jain Temple, where some remains had fallen. In the other three monuments, namely Saher ki Masjid, Mandvi and Amir Manzil, where remains could not be found, the study was not possible. Six collected rock samples were studied by a thin section of the substrate and Powder X-ray Diffraction (XRD) analysis. Thin section slides were observed under phase contrast and polarized light microscope (Leica DM EP), and images were captured by 2.5 MP Leica MC 120 HD camera. For XRD analysis, the powder of each sample was prepared using steel mortar and pestle. The powder was sieved by 200 ASTM (75 µ) mesh size as a very fine powder which is amorphous in nature and was required for further analysis. This was analysed by X-ray diffraction position at 2° θ with a range gradually increased from 0° to 80°. After completion of the run, spectra of various peaks representing the data were obtained. Peaks were identified by JCPDS (Joint Committee on Powder Diffraction Standards) software. This software contained a database of standard peaks which were used as references for the identification of obtained peaks in the XRD analysis.

Role of bryophyte as colonization on the monuments

For the investigation, 40 mg (dry weight) 12-18 mm moss shoots (*Hyophila involuta* (Hook.) A.Jaeger) and 40 mg (dry weight) thallus of liverworts (*Asterella wallichiana* (Lehm. & Lindenb.) Grolle) were taken. The samples were cleaned and washed in a Petri plate with distilled water. Plant samples were taken in culture tubes and 20 ml of deionized water was added. Each tube was shaken for a few minutes and the serial wash step was repeated thrice. After serial wash, 20 ml of 25 mmol $SrCl_2$ was added to each tube having a sample (Bates 1992; Altieri & Ricci 1997). These tubes were kept at two different temperatures: 20°C and 37°C for one hour. The tubes were centrifuged at 2000 rpm for three minutes (Altieri & Ricci 1997). The exchangeable ionic fraction (supernatant) was taken for estimation of the calcium concentration by a flame photometer (Systronics flame photometer 130). For calibration of the flame photometer, 10 ppm to 150 ppm standard solutions were prepared from 1000 ppm stock solution. Both stock and standard solutions were prepared in deionized water. First, the flame was observed using deionized

Fig. 2. — Colonizing liverworts on the selected monuments of Champaner Pavagadh: **A**, *Asterella wallichiana* (Lehm. & Lindenb.) Grolle; **B**, *Riccia billardieri* Mont. & Nees; **C**, *R. discolor* Lehm. & Lindenb.; **D**, *R. gangetica* Ahmad.; **E**, *R. grollei* Udar.; **F**, *Cyathodium cavernarum* Kunze ex Lehm.; **G**, *Plagiochasma appendiculatum* Lehm. & Lindenb. and *P. microcephalum* (Steph.) Steph. Scale bars: 10 mm.

water as control and later flame was observed under calcium specific filter. Then, we ran the standard solutions from 10 ppm to 150 ppm. All responses against each standard concentration were recorded. We ran a total of six samples of ion-exchangeable solutions in three replicates each of *Hyophila involuta* and *Asterella wallichiana*. The responses were noted and their concentration was checked from a standard graph of calibration.

Mechanism of biodeterioration due to bryophyte colonization

Each step was observed four times a year, from the pioneer microorganisms to the juvenile stage of bryophytes causing degradation of the monument structure by enlarging cracks and deteriorating the exposed surface of the structure. Pictorial representation was done to clarify the mechanism of biodeterioration by bryophyte colonization.

RESULTS

The present study revealed the presence of a total of 17 species of bryophytes inhabiting the monuments of Champaner Pavagadh. Major bryophyte colonization was observed on the calcareous material. Hence, the role

Fig. 3. — Colonizing mosses and hornworts on the selected monuments of Champaner Pavagadh: **A**, *Hyophila involuta* (Hook.) Jaeg.; **B**, *Hydrogonium arcuatum* (Griff.) Wijk. & Marg.; **C**, *Semibarbula orientalis* (Web.) Wijk & Marg.; **D**, *Fissidens flaccidus* Mitt.; **E**, *Anomobryum auratum* (Mitt.) Jaeg.; **F**, *Gymnostomiella vernicosa* (Hook.) Fleisch.; **G**, *Anthoceros bharadwajii* Udar & A.K.Asthana; **H**, *A. subtillis* St. Scale bars: 10 mm.

of calcium absorption by bryophytes in the degradation of the surface was investigated. The degradation of the surface was deteriorating the structure as explained by pictorial representation.

Diversity of specific bryophytes on the specific monuments

A total of 17 species of bryophytes including nine liverworts, six mosses and two hornworts were recorded from the selected monuments of the Champaner Pavagadh. Their habitat are shown in Figures 2 and 3. Bryophyte species distribution on specific monuments is shown in Table 1.

Quantitatively, maximum bryophyte colonization was perceived on the Navlakha Kothar (40%), whereas minimum colonization on the Saher ki Masjid (8%). To a lesser extent bryophyte colonization on the antiquities of Jain Temple, Makai Kothar, Mandvi, and Amir Manzil (Fig. 4). This might be due to the variations in microclimatic conditions and anthropogenic pressure from tourists destinations. Among all the studied monuments, Navlakha Kothar has the greatest colonization of mosses, inhabiting and forming carpets on the wall of the monuments. Because this monument is located at a higher altitude than the other monuments studied, the humidity conditions are more favourable to the development of mosses.

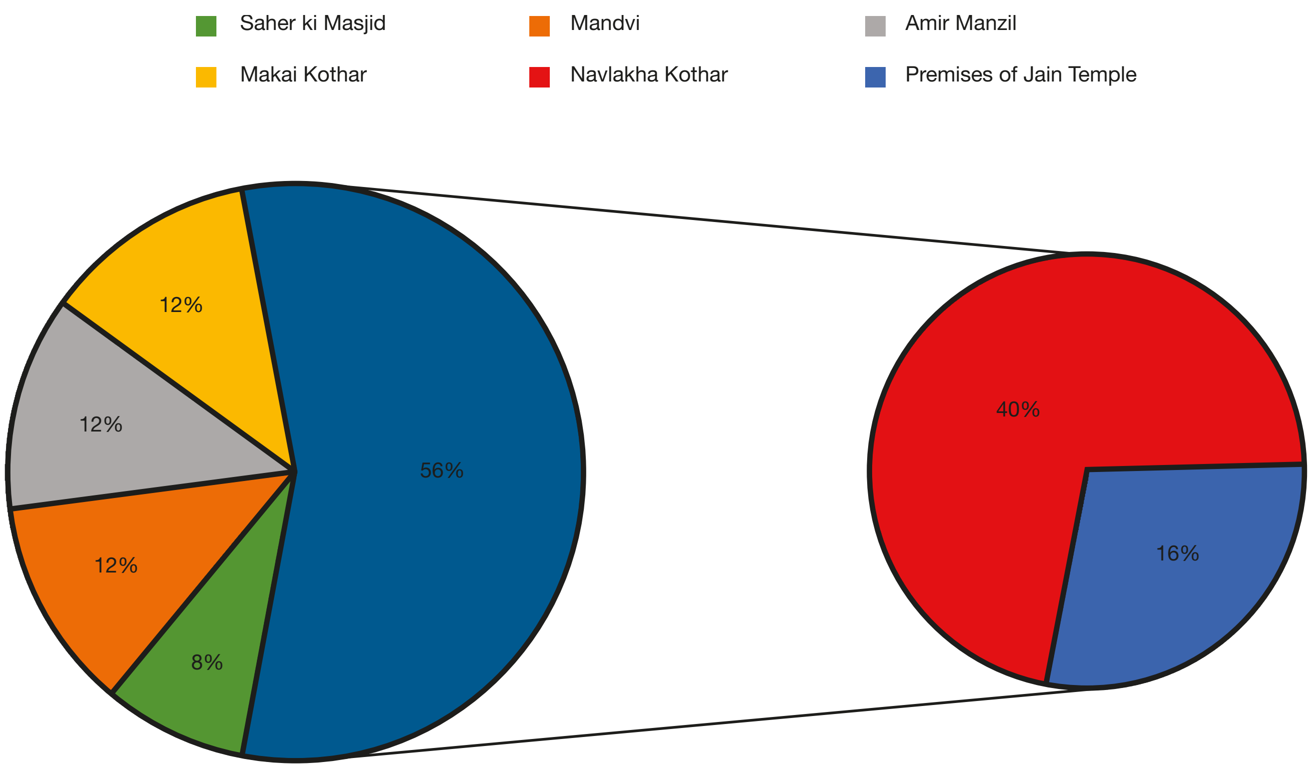

Fig. 4. — Quantitative representation of species colonization on Champaner Pavagadh monuments.

Geological substrate investigation

The substrate study mainly revealed the presence of primary and secondary silica, ferruginous materials, calcareous material and feldspar minerals.

Thin section of rock samples

Thin section microscopic photographs represent various materials of different sites of Champaner Pavagadh (Fig. 5).

Thin section microscopic images show a major groundmass of primary and secondary silica as well as glassy minerals. Glassy minerals are from pyroclastic rock. Pyroclastic rock is an igneous rock which is formed through a volcanic eruption. Volcanic clastic is deposited predominantly as volcanic particles. These glassy minerals have a composition of siliceous materials. Surrounding this siliceous material, quartz was also observed. Images B, D, G and H have fragments of Plagioclase minerals with siliceous groundmass. Plagioclase mineral is within the feldspar group. It is produced by weathering of igneous and metamorphic rock. The chemical composition of plagioclase is calcium or sodium elements containing siliceous materials (silicates). It appears light in colour, glassy, transparent to translucent with striations. In a few images, orange to brown patches of iron oxide was observed, known as ferruginous. Image E shows crystals having colourless or light colour (white or grey) orthoclase. Orthoclase belongs to the feldspar group. The chemical composition of orthoclase is potassium with silicate minerals. Hence, orthoclase is also known as K-feldspar or Microcline. Image I shows muscovite, a mineral-rich in potassium, iron and magnesium elements. It belongs to the Mica family.

XRD analysis of geological substrate

Powder XRD analysis revealed the mineral composition in the geological substrate. Figure 6 depicts the spectra of XRD analysis of the different substrate samples of Champaner Pavagadh.

X-ray diffraction at 2° angle observed various peaks at different angles from the range 0° to 80° shown in Figure 6. Among them some peaks appeared at 6.7°, 8.75°, 9.04°, 12.12°, and 24.31° angles corresponding to planes of lower Miller indices such as (0 1 0), (0 0 1), (1 0 0), (1 0 0), (0 0 1), respectively, which represent the characteristics of mineral from Mica categories like muscovite. The diffraction peaks at 9.38°, 12.90° and 17.2° were due to diffraction from (0 1 0), (1 1 0) and (1 1 1) planes, respectively. This indicates the presence of Aluminosilicate minerals which may have the chemical formula $Ca_2Al_4Si_8O_{24}$ (JCPDS card number PDF # 861548). The 2° θ peaks at 29.37°, 31.39°, 35.93°and 39.37° were due to diffraction from (1 0 4), (0 0 6), (1 1 0) and (1 1 3) planes, respectively. These peaks have characteristic diffraction due to calcite minerals whose chemical formula is $CaCO_3$ (JCPDS card number PDF # 721937). The diffraction peaks at 28.62°, 34.73°, 41.51°, 51.60° and 62.26° were due to diffraction from (1 0 2), (1 1 0), (0 0 4), (2 0 3) and (3 0 0) planes, respectively, due to potassium aluminosilicate which may have chemical formula $KAlSiO_4$ (JCPDS card number #851413). Potassium aluminosilicate is K-feldspar. The peaks at higher angles like 31.5°, 36.23°, 44.5°, 54.25°, 60.5° and 65.25° are due to diffraction from (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0), respectively. These peaks are due to iron oxide-containing minerals ,such as Hematite (Fe_2O_3)

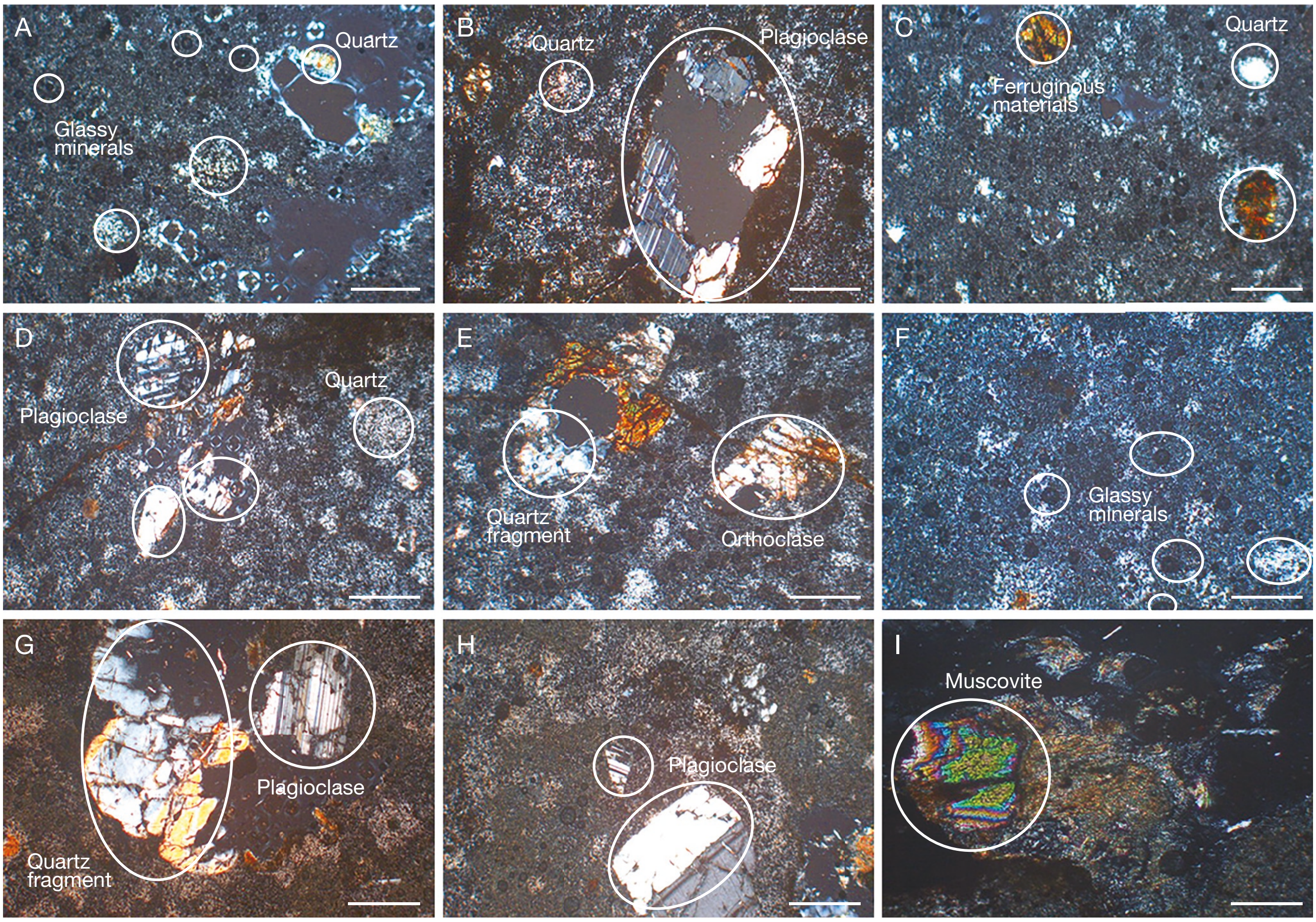

Fig. 5. — Microscopic images of geological substrate thin sections of different sites: **A**-**C**, Makai Kothar; **D**-**F**, antiquity from the surroundings of Jain Temple; **G**-**I**, Navlakha Kothar. Scale bars: 500 µm.

and Magnetite (Fe_3O_4). The major peaks at 20.86°, 26.64°, 42.45°, 50.15°, 50.63°, 59.97°, 68.15° and 68.32° were due to diffraction from (1 0 0), (0 1 1), (2 0 0), (1 1 2), (0 0 3), (2 1 1), (2 0 3) and (0 3 1) planes respectively. All these peaks were due to feldspar minerals. Overall, major samples had feldspar mineral and calcite. Silicate was the secondary component. Aluminosilicate was also the major component of feldspar.

Based on a thin section of the rock samples and powder X-ray diffraction analysis, a qualitative assessment of the mineral composition of the different monuments of the Champaner Pavagadh was done and has been indicated in Table 2. The qualitative analysis revealed that the minerals plagioclase feldspar and silicates were dominant on these monuments. This was followed by Hematite and Magnetite for the Makai Kothar, and to a smaller extent for the Navlakha Kothar. Orthoclase feldspar was very low as compared to other minerals, while aluminosilicate and muscovite were almost equal among the selected monuments.

Study on the role of bryophyte colonization on the monuments

Bryophyte colonization on the monuments is a kind of ecological succession. It starts with pioneer microorganism, cyanobacteria, and some green algae forming a biofilm. Over time, this area gradually becomes covered with bryophytes, and then allied vascular plants. Bryophyte colonization might be dependent to the substrate of the monuments because they take nutrients from the substrate for their growth. Monuments of Champaner Pavagadh have mostly calcareous material. Hence, the current study focused on calcium uptake from the substratum by the bryophytes. In bryophytes, uptake chemicals are easily trapped outside the plasma membrane and in between the cells (Bates 1992). This region is broadly known as the apoplast region where materials can diffuse freely. Understanding of calcium uptake of *Hyophila involuta* and *Asterella wallichiana* from geological substrata was achieved through flame photometry and their response values were calculated from the calibrated (standard) graph shown in Figure 7. When the solution tube was kept at 20°C no response value was shown on the digital screen but when the solution tube was kept at 37°C the response value appeared on the digital screen. The concentration of calcium from the obtained responses of each sample was calculated using the equation mentioned on the calibration graph.

Based on the calculation, the average value of calcium uptake by *Hyophila involuta* was 72.88 ppm, and 105.54 ppm by

Table 1. — Bryophyte colonization on different monuments of Champaner Pavagadh. Monuments: **1**, Saher ki Masjid; **2**, Mandvi; **3**, Amir Manzil; **4**, Makai Kothar; **5**, Navlakha Kothar; **6**, premises of Jain Temple.

		Monuments					
Serial number	**Name of the species**	**1**	**2**	**3**	**4**	**5**	**6**
Mosses							
1	*Anomobryum auratum* (Mitt.) Jaeg.	–	–	–	–	+	–
2	*Fissidens flaccidus* Mitt.	–	–	+	–	+	–
3	*Hydrogonium arcuatum* (Griff.) Wijk. & Marg.	+	–	–	–	–	–
4	*Hyophila involuta* (Hook.) Jaeg.	+	+	+	+	+	–
5	*Semibarbula orientalis* (Web.) Wijk & Marg.	–	–	–	–	+	–
6	*Gymnostomiella vernicosa* (Hook.) Fleisch.	–	–	–	–	+	–
Liverworts							
7	*Asterella wallichiana* (Lehm. & Lindenb.) Grolle	–	–	–	+	+	–
8	*Cyathodium cavernarum* Kunze	–	–	–	–	+	–
9	*Lejeunea aloba* Sande Lac.	–	–	–	–	+	–
10	*Riccia gangetica* Ahmad ex L.Söderstr., A.Hagborg & von Konrat	–	–	–	–	–	+
11	*Riccia discolor* Lehm. & Lindenb.	–	+	–	–	–	–
12	*Riccia grollei* Udar.	–	–	+	–	–	–
13	*Riccia billardieri* Mont. & Nees.	–	+	–	–	–	+
14	*Plagiochasma microcephalum* (Steph.) Steph.	–	–	–	–	+	–
15	*Plagiochasma appendiculatum* Lehm. & Lindenb.	–	–	–	+	+	–
Hornworts							
16	*Anthoceros bharadwajii* Udar & A.K.Asthana	–	–	–	–	–	+
17	*Anthoceros subtilis* Steph.	–	–	–	–	–	+

Table 2. — Qualitative assessment of the minerals on the different monument sites of Champaner Pavagadh.

Serial number	**Minerals**	**Makai Kothar**	**Antiquity from the Jain Temple**	**Navlakha Kothar**
1	Aluminosilicate	++	++	++
2	Calcite	++	+	++
3	Orthoclase	+	+	+
4	Plagioclase	+++	+++	+++
5	Muscovite	++	++	++
6	Hematite and Magnetite	+++	+	++
7	Silicates (Quartz)	+++	+++	+++

Asterella wallichiana. Hence, 1.45 mg and 2.11 mg calcium concentration in 20 ml solution was prepared from 40 mg plant samples of *Hyophila involuta* and *Asterella wallichiana*, respectively. Calcium uptake was a maximum of 0.052 mg per mg plant samples of *Asterella wallichiana* (liverwort) vs 0.036 mg per mg plant samples of *Hyophila involuta* (moss).

Mechanism of deterioration due to bryophyte colonization

Monuments of the Champaner Pavagadh were invaded by liverworts and mosses. Deterioration of the monuments due to bryophyte colonization was initiated by humus formation. On this humus, spores of the biological organisms adhered and took nutrients from the substrate. In favourable conditions, spores germinate and form the juvenile stage of mosses and liverworts – protonema and following mature gametophyte. Over a while, bryophytes covered the majority of the area of the structure. Gametophytes possess root-like structures called rhizoids that help them to adhere to the substratum. The rhizoids penetrate deep into the crevices and porous surface of the substratum and create pressure (Glime 2017). As a result of this growth, there are mechanical damages to the monuments that enlarge cracks and crevices with time. Figure 8 provides a general illustration of this understanding through a pictorial representation.

DISCUSSION

Diversity of bryophytes on the monuments

A total of 17 species of bryophytes were found from the selected study locations. Of these, species of the order Marchantiales followed by Pottiales were dominant. Within the Marchantiales order, the maximum occupancy by any species was *Riccia* L. followed by *Plagiochasma* Lehm. & Lindenb., *Asterella* P.Beauv. and *Cyathodium* Kunze ex Lehm. Verma *et al.* (2014) also reported members of Marchantiales such as *Plagiochasma*, *Marchantia* L. and *Asterella* on the monuments of Talatal Ghar, Assam. In the current study, *Lejeunea aloba* Sande Lac. of the Porellales order was also reported. It was observed on the wall of the monuments and it is unique for this particular species as it is an epiphytic species typically growing on the trunk of a tree. Members of the Pottiales named *Semibarbula* Herzog ex Hilp., *Hyophila* Brid., *Hydrogonium* (Müll.Hal.) A.Jaeger and *Gymnostomiella* M.Fleisch. followed by Dicranales genus named *Fissidens* Hedw., and the order Bryales genus *Anomo-*

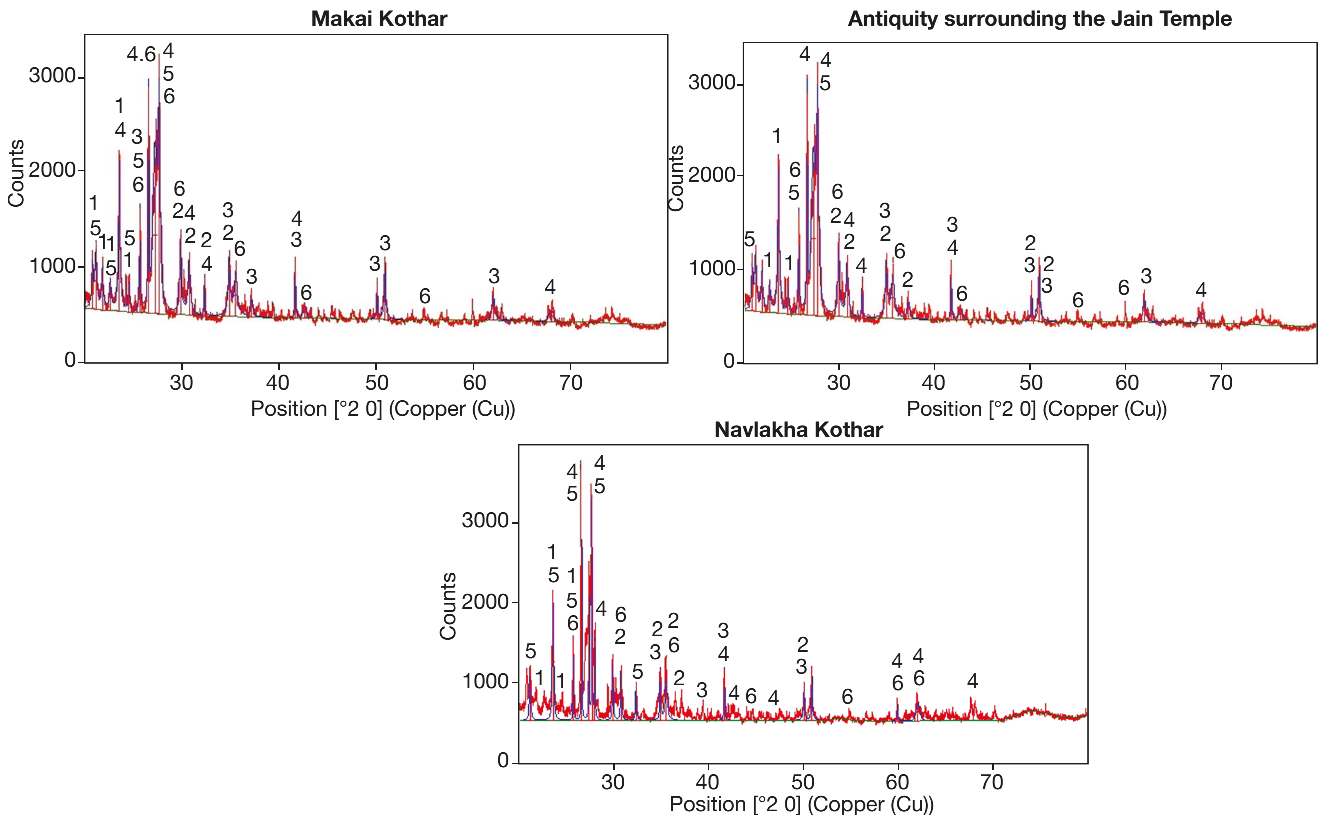

Fig. 6. — Spectra of the PXRD of the geological substrate samples from the different sites of Champaner Pavagadh. Peaks number: **1**, aluminosilicate; **2**, calcite; **3**, orthoclase; **4**, plagioclase; **5**, muscovite; **6**, hematite and magnetite.

bryum Schimp. were found on the monuments of Champaner Pavagadh. Similarly, *Fissidens* sp. and *Fissidens bryoides* Hedw. of the order Dicranales were reported earlier from the monuments of Talatal Ghar, Assam and the archaeological site of Chellah, Morocco, respectively (Verma *et al.* 2014; Elharech *et al.* 2017). Species of the order Pottiales followed by Bryales have been reported to take over the biofilm crust mosaics on mortar surfaces in Spain (Gil & Sáiz-Jiménez 1992). In the current study members of Funariales were not observed. But an earlier study reported *Funaria* Hedw. and *Physcomitrium* (Brid.) Brid. from the Talatal Ghar, Assam, and *Funariella curviseta* (Schwägr.) Sérgio and *Entosthodon pulchellus* (H.Philib.) Brugués from the archaeological site of Chellah, Morocco (Verma *et al.* 2014; Elharech *et al.* 2017). A species from the order Bryales (*Anomobryum auratum* (Mitt.) A.Jaeger) was found in the study locations. Similarly, species of Bryales (*Ptychostomum capillare* (Hedw.) Holyoak & N.Pedersen and *Bryum radiculosum* Brid.) were reported from the archaeological site of Chellah, Morocco (Elharech *et al.* 2017). As per the earlier studies, mosses were dominant followed by liverworts on the archaeological sites or monuments (Verma *et al.* 2014; Elharech *et al.* 2017). Moreover, three species of mosses (*Tortula muralis* Hedw., *Didymodon vinealis* (Brid.) R.H.Zander and *Didymodon luridus* Hornsch. ex Spreng.) were also reported from the weathered sandstone of Carrascosa del Campo church, Spain (Gómez-Alarcón *et al.* 1995). In the current study, liverworts were predominant over mosses in terms of population, but in terms of growth, mosses were found to profusely growing.

Role of bryophyte colonization on the monuments

Colonization on monuments implies an ecological relationship between the substratum and the colonizing organisms. It is dependent on the physical-chemical properties of the building materials such as surface roughness, porosity and mineral composition considered essential for assessing their bio-receptivity (García-Rowe & Sáiz-Jiménez 1991; Hallingbäck & Hodgetts 2000; Miller *et al.* 2012; Verma *et al.* 2014). Bryophyte colonization on rock surfaces is largely influenced by the availability of water on the surface and the physical properties of the rock, such as its pore structure and ability to absorb and retain moisture.

Mosses can accumulate Ca^{+2} ions that could be related to colonization on monuments or any structure causing deterioration (Keller & Frederickson 1952; Tiano 2002). The rhizoidal hyphae are the source of other products of their metabolism such as proteins, which can act as chelating agents responsible to solubilize calcium cations capture in rocks. (Morillas *et al.* 2019). There have been very few studies regarding calcium ions exchangeable by mosses on rocks or mortar. In the current study, the calcium cation exchange analysis was done in the dominant species *Hyophila involuta* (moss) and *Asterella wallichiana* (liverwort) on the selected monuments built with different mortars. This calcium cation exchange analysis is a

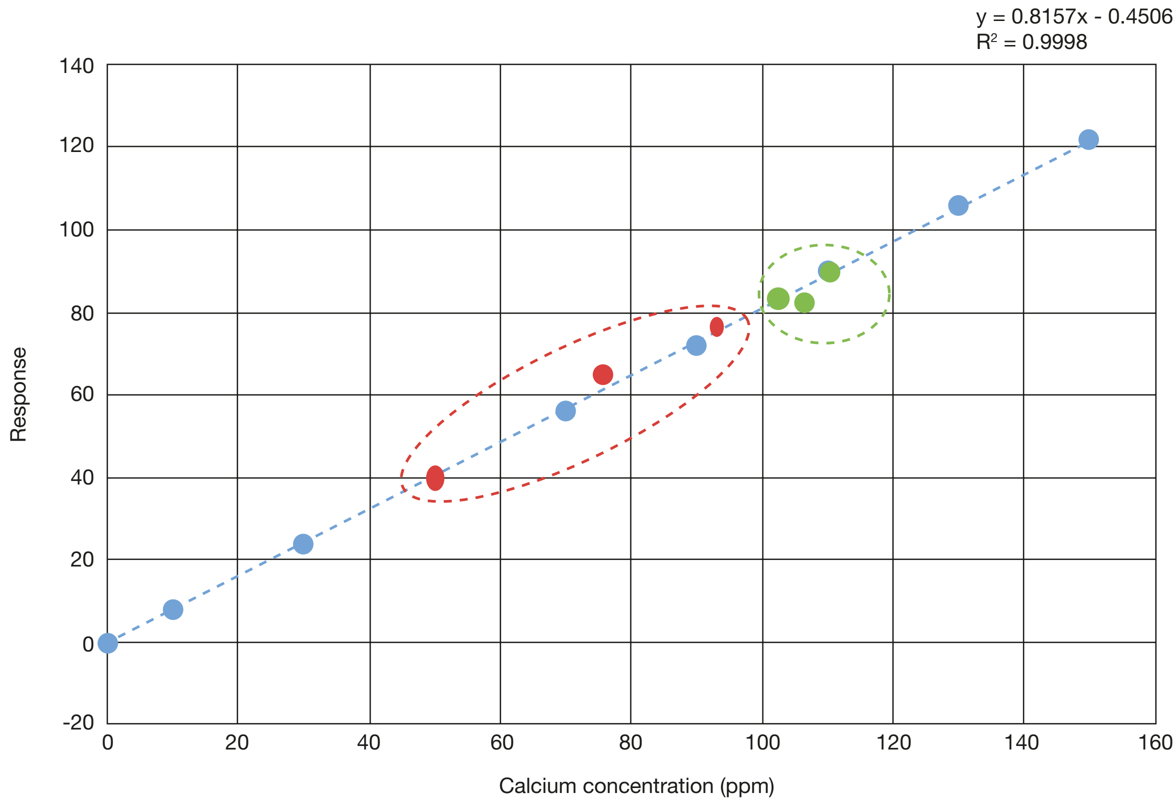

FIG. 7. — Estimation of Calcium concentration (ppm) of *Hyophila involuta* (Hook.) Jaeg. and *Asterella wallichiana* (Lehm. & Lindenb.) Grolle: **red circles**, clusters showing *Hyophila involuta*; **green circles**, clusters showing *Asterella wallichiana*.

first-time approach for liverworts. Earlier this analysis was done on the moss *Pleurozium schreberi* (Willd. ex Brid.) Mitt. growing on acidic soil in Windsar forest, United Kingdom, and *Grimmia pulvinata* (Hedw.) Sm. growing on structures made up of marble and travertine (Bates 1992; Altieri & Ricci 1997). In the current study, calcium uptake was 52 µg/mg for plant samples of *Asterella wallichiana* and 36 µg/mg for plant samples of *Hyophila involuta*. Bates (1992) reported Ca^{+2} exchangeable cations of 55 µmol/g moss *Pleurozium schreberi*, and Altieri & Ricci (1997) mentioned calcium uptake of 6 mg/g plant and 3 mg/g plant of the species *Grimmia pulvinata* growing on marble and travertine, respectively. It was noticed that maximum calcium was absorbed by the liverwort *Asterella wallichiana* as compared to the moss *Hyophila involuta*.

Lime and sand mortar facilitate the transport of water and salts, enriching the mortar with nutrients. Most of the mineral matter of proto-soil was quartz and calcium carbonate. This is why bryophytes began to colonise these monuments. In the present study, the bryophytes colonized the substrate containing orthoclase, plagioclase, calcite, quartz, mica, magnetite and hematite minerals. Likewise, Jackson (2015) analysed the XRD of rock samples inhabited by bryophytes and lichens in a boreal forest and found that rocks included the presence of potassium feldspar, plagioclase, quartz, calcite and mica. In the current study, this type of mineral substratum is home to moss species *Hyophila involuta*, *Semibarbula orientalis* (F.Weber) Wijk & Margad., *Hydrogonium arcuatum* (Griff.) Wijk & Margad. and *Fissidens flaccidus* Mitt., and to the liverwort *Asterella wallichiana*. Whereas *Bryum caespiticium* Hedw., *Tortula brevissima* Schiffn. and *Didymodon luridus*, most abundant species, were reported on mortar mosaics (Gil & Sáiz-Jiménez 1992).

MECHANISM OF BIODETERIORATION

The biodeterioration process varies according to the type of stone and its position in the monument. The relative effects of each of the organisms fluctuate according to the topo-climatic environmental conditions, stone type, its state of preservation and location of the monument (De los Ríos *et al.* 2004). Bryophytes are not the primary colonizers on monuments because they are preceded by algae and cyanobacteria (Dakal & Cameotra 2012; Cozzolino *et al.* 2022). The surfaces of buildings were porous and rough thanks to which they retained moisture. Firstly, microorganisms and organic materials are transported to the building by wind and water, and adhere to the substrate (Cozzolino *et al.* 2022). These conditions facilitate the formation of cyanobacterial biofilm. Earlier studies have shown that microbial colonization occurs more on rough surfaces and increases with the increase in the roughness (Characklis & Marshall 1990; Donlan 2002). On this microbial colonization dust and other organic matter settles and forms humus. Propagules of other biological organisms like mosses or liverworts

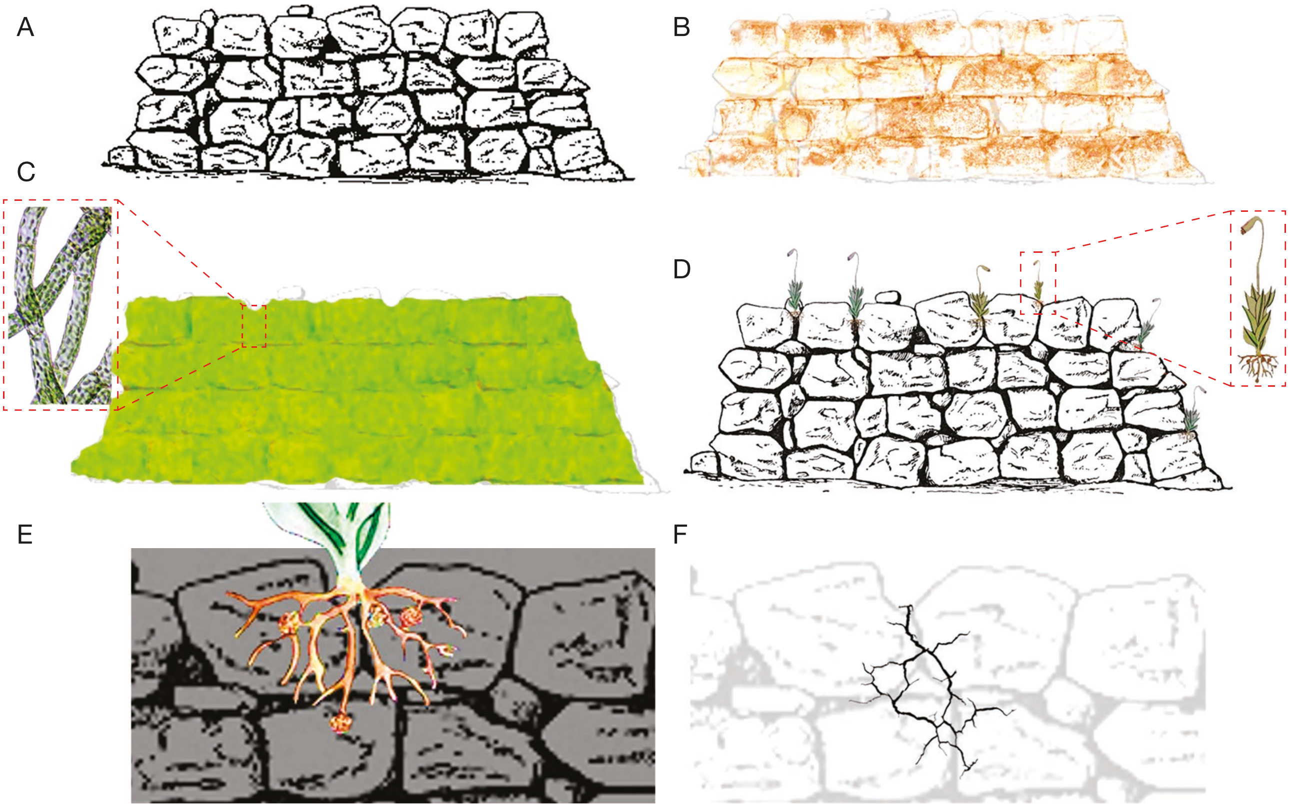

Fig. 8. — Pictorial representation of the steps involved in colonization and deterioration of the substrate by bryophytes: **A**, wall structure with rough surface; **B**, settled dust and humus to capture spores; **C**, covered by protonema; **D**, growth of mature gametophyte; **E**, penetration of rhizoids; **F**, enlargement of cracks deteriorating the wall structure.

adhere to the humus and occupy the area. Over time, the community of moss and liverwort species covers maximum areas. Monuments of the Champaner Pavagadh were colonized by liverworts and mosses. In the case of liverworts, the thalloid liverworts commonly occur on the monuments because they possess the root-like structures called rhizoids that help them to adhere to the substratum (Verma *et al.* 2014). Apart from liverworts, mosses may directly promote the degradation of a stone surface, which may be of considerable significance. The penetration described probably facilitates the entry of water into the granules of the rock and this may lead to the separation of flakes of stone from the surface (Hughes 1982). Shirzadian & Uniyal (2008) revealed that carboxylic acids secreted by mosses gradually corrode structure material and chemical conversion of chelate ions from the surface of minerals gives rise to degradation. In the current study, monuments were colonized by acrocarpic mosses like *Anomobryum auratum*, *Hyophila involuta*, *Hydrogonium*, etc. Acrocarpic mosses had two types of growth form, namely cushion or carpet, profusely covering the surface of the monuments. These growth forms are considered to be the best and most efficient forms as they densely cover the substratum (Caneva *et al.* 2008). Some of these moss species like *Gymnostomiella vernicosa* (Hook. ex Harv.) M.Fleisch., *Semibarbula orientalis*, *Anomobryum auratum*, *Hydrogonium*, etc. have the advantage of asexual reproduction (gemmae or fragmentation) during favourable seasons. Consequently, gemmae produced new protonema filaments and gave rise to other individuals. During the favourable season sexual reproduction occurred in mosses, especially *Hyophila involuta*. The sporophytes of these species burst, releasing the spores, and those spores settled on the substratum. The sexual reproduction of the mosses is more detrimental to the monuments (Caneva *et al.* 2008) because, during the process of reproduction, it produces protonemata. These protonemata cause discolouration of the monuments. Protonema is the elongated thread-like structure formed after the germination of spores (Glime 2017). Rough surfaces, mortar and sandstone have the potential to hold such a large community because of their porous and permeable nature (García-Rowe & Sáiz-Jiménez 1991). Moreover, all the monuments studied in the present investigation fall into the above-mentioned categories. This whole process was repeated year after year and formed colonization of different or the same species. In dry conditions, the organism can survive without any problem, dehydrating its thalli, and activating itself again in presence of low humidity. The presence of rhizoids in the rock sample is notorious and is always present under the lithic material layer, supporting the penetration action (Caneva *et al.* 2015; Morillas *et al.* 2019). During the fissures or crack formation on stones, they were able to cause erosions by decreasing the cohesion between stones and increasing the size of the cracks. The development of living populations raises soil formation by changing the stone structure and facilitates the adaptation of new types of plants (Hosseini & Caneva 2021). Over a long period, this whole process of community colonization leads to the deterioration of monuments both physically as well as aesthetically (García-Rowe & Sáiz-Jiménez 1991; Cappitelli *et al.* 2020).

CONCLUSIONS

We have concluded that the monuments' surface plays a vital role in bryophyte colonization. Indeed, the biological propagules start to fix and absorb nutrients from the substratum and its surroundings, leading to the formation of bryophytes community. The present study shows that bryophyte colonization on monuments depends on mortar material with different mineral compositions such as feldspar, calcareous material, silicate, mica, etc. Selected monuments were rich in calcareous material, hence calcium cation exchange was studied. The maximum calcium was taken up by liverworts. This result correlates with other studies in which liverworts colonize monuments. This investigation provides helpful guidance to design effective conservation strategies, including water resistance and pore sealing. In this way, biological propagules will not adhere there.

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Conflict of Interest
Authors have no conflicts of interest.

Authors' contributions
DM and DS conceptualized and agreed on the study. DM carried out the analysis of this research and drafted the manuscript. DS oversaw the study and gave valuable suggestions.

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