Biodeterioration of marble in an underwater environment

Beatriz Cámara[a1*], Mónica Álvarez de Buergo[a], Manuel Bethencourt[b], Tomás Fernández-Montblanc[b], Mauro F. La Russa[c], Michela Ricca[c], Rafael Fort[a]

(a) Dept. of Geomaterials, Instituto de Geociencias (IGEO, CSIC-UCM), José Antonio Novais 12, 28040 Madrid, Spain. *Corresponding author: b.camara@igeo.ucm-csic.es; alvarezm@geo.ucm.es; rafael.fort@csic.es.

(b) Dept. of Material Science, Metallurgical Engineering and Inorganic Chemistry, Campus de Excelencia Internacional del Mar (CEI·MAR), Universidad de Cádiz, Avda. República Saharaui s/n, Puerto Real, 11510, Cadiz, Spain. manuel.bethencourt@uca.es; tomas.fernandez@uca.es.

(c) Dept. of Biology, Ecology and Earth Sciences (DiBEST), Università della Calabria, Via Pietro Bucci 87036, Arcavacata di Rende, Cosenza, Italy. mlarussa@unical.it; michela.ricca@unical.it.

Abstract

This study examines the deterioration of geomaterials used throughout history that today may be found lying on the ocean floor. Submerged archaeological sites including cargoes from shipwrecks or ancient city ruins have been a topic of interest from a perspective of *in situ* musealization, as a way of making underwater cultural heritage accessible to the public. In an experimental study conducted at an underwater archaeological site in the Bay of Cádiz (SW Spain), we subjected two types of marble (Carrara and Macael) to three conditions to which submerged archaeological objects are often exposed: full exposure to the water column, natural processes of burial and unearthing, or permanent burial. After an 18-month study period, the factor found to mostly affect these materials was their biological colonization. This factor was assessed by estimating total surface biocover and the rate of surface biocolonization, and also through the identification of skeletons and associated alteration forms by light

[1]Present address: Centro Nacional de Biotecnología (CNB,CSIC), Dept. Systems Biology, Darwin 3, 28049 Madrid, Spain. bcamara@cnb.csic.es

microscopy, and scanning electron microscopy (SEM). Biofouling and bioerosion were the main causes of biodeterioration and dependent on the position of the marble specimens in the seawater. The response of both materials was similar, though dolomite crystals in the Carrara marble acted as a protective barrier against actively penetrating microorganisms. These investigations have allowed the study of tracers left by epilithic encrusting organisms and endolithic bioeroders on marbles intentionally exposed to seawater, providing new insights to the understanding of the biodeterioration processes occurring in cultural heritage stones, with significant implications when they are part of underwater archaeological remains.

Keywords: bioerosion, biofouling, Carrara, decay, Macael, submerged archeology

1. Introduction

With changing environmental conditions, stone materials undergo transformation in a new scenario. This transformation, or decay process, starts at the surface in direct contact with the environment. Weathering of stone materials, besides causing surface aesthetic changes, will also affect their functional characteristics (bulk properties). Decay is mainly determined by the type of material and the prevailing conditions of the new environment (Warscheid and Braams 2000; Doehne and Price 2010). Materials deteriorate differently when exposed to subaerial or underwater conditions, since distinct environmental factors are involved in each case. In a marine environment, the decay of archaeological or engineering materials is still poorly understood, as several agents interact simultaneously and different types of deterioration processes take place (Pearson 1987; Fernández-Montblanc et al. 2014; Winton 2015). Among all factors, we should highlight the important role played by hydrodynamic variables, hydrochemical features, sedimentary dynamics and biological communities. Several processes including physical (abrasion), chemical (corrosion) and biological occur as a consequence of interaction of the different factors with the stone. One of the main causes of the decay of stone materials in an underwater environment is biodeterioration (Crisci et al. 2010; Davidde et al. 2010; La Russa et al. 2013, 2015) in the form of biofouling and bioerosion phenomena. In the biofouling phenomenon, defined as the undesirable accumulation of microorganisms and

organisms on surfaces of submerged materials, are involved bacteria, yeast and diatoms (microfouling), macroalgae, barnacles, tube worms, bryozoans, molluscs and mussels (macrofouling) are involved (Faÿ et al. 2011). The establishment of these fouling communities occurs in the sequential phases of biochemical conditioning of the surface, adhesion of bacteria, yeast and diatoms, unicellular eukaryotic colonization and pluricellular eukaryotic fouling (Wahl 1989; Faÿ et al. 2011). The second type of biodeterioration, bioerosion, not immediately visible to the naked eye, is defined as the removal of stone material through the actions, mechanical and/or chemical, of endolithic microorganisms (cyanobacteria, algae, heterotrophic bacteria, fungi, foraminifera as microbierosion) and organisms (clionaid sponges, polychaetes and lithophagine bivalves as macrobioerosion) (Davidde et al. 2010; Tribollet et al. 2011). Microbioeroders actively penetrate the rock interior (euendoliths) and leave their fingerprints via biochemical dissolution of the materials (Golubic et al. 1981; Davidde et al. 2010; Tribollet et al. 2011; Ricci et al. 2013) involving mechanisms of calcium pumps, respiratory carbonic acid and/or enzymes (Tribollet et al. 2011 and references therein). However, the most damaging actions have been attributed to macrobioeroders, capable of excavating cavities and tunnels and causing irreversible loss of material in archaeological artefacts (Davidde et al. 2010; La Russa et al. 2013).

Biodeterioration will differ depending on a variety of environmental conditions in seawater (chemical composition of the water column and its sediments, light regime, nutrient availability, waves and currents) (Bethencourt et al. 2014). Besides being conditioned by these factors, biodeterioration will also vary widely in relation to the intrinsic features of the materials (mineralogical composition and textural features e.g., porosity or crystal distribution) (Pearson 1987; Aloise et al. 2013).

According to its good aesthetic and physical properties, white marble has been one of the most widely used materials throughout history for both structural (e.g., buildings) and decoration or artistic purposes (e.g., sculptures) (Gorgoni et al. 1998; Ricca et al. 2015). To preserve this extraordinarily rich cultural heritage, interest grows in trying to understand how marble decays in each environment, and especially in marine environments, which so far have been the least well characterized. This is because marble has been much exposed to this environment as some ancient cities have been submerged due to natural phenomena such as bradyseism (Ricci et al. 2009; Aloise et al. 2013; La Russa et al. 2015), and marble has often also appeared as

transported cargo in ship wrecks (Carlson and Aylward 2010; Beltrame et al. 2012; Russell 2013). In effect, the discovery of remains of shipwrecks and their stone cargo lying on the seabed has provided valuable information on transport routes and embarkation points, on the marble trade, and on quarries and monument construction (Beltrame et al. 2012).

Within this context, it needs to be determined to what extent these underwater biodecay processes could affect the durability of stone materials, especially when these are underwater archaeological remains. According to the main principles of the UNESCO Convention on the Protection of the Underwater Cultural Heritage 2001, underwater cultural heritage is defined as all traces of human existence having a cultural, historical or archaeological character that have been partially or totally submerged, periodically or continuously, for at least 100 years (Maarleveld et al. 2013). In this scenario, *in situ* prevention and protection measures were described as priority options for the conservation of underwater cultural heritage. These measures are considered to protect an archaeological site fully or in part in the marine environment. In addition, any protection or prevention measure taken should be monitored, as the protection conferred or deterioration rates underwater will vary in time with changes in the environmental marine conditions of the archaeological sites (Gregory et al. 2012; Fernández-Montblanc et al. 2014; Winton 2015).

The purpose of this study was to determine the deterioration of two types of marbles when exposed to seawater by estimating surface biocover (overall and of major groups of epilithic encrusting organisms) and rate of surface biocolonization. In addition, polarized light (PLM) and electron microscopy (SEM) were used to identify encrusting organisms by their calcareous skeletons and to investigate biodecay forms (epilithic and endolithic) derived from the interaction of biological components with the substrate in an attempt to understand biodeterioration processes occurring in the marine environment.

2. Study area

The archaeological site selected for our underwater exposure experiment was Bajo del Chapitel in the Bay of Cádiz (southwest coast of the Iberian Peninsula) (listed as Assets of Cultural Interest and recognized as Archaeological Zone in the General Catalogue of the Andalusian Historical Heritage). Currently, this archaeological site is

being monitored in the framework of the ARQUEOMONITOR project. The archaeological remains of the S.M.I. (*Sa Majesté Impériale/ His Imperial Majesty*, in this specific case, in honour of Napoleon). Bucentaure and Roman/Punic battleships (Fig. 1A-B) (Bethencourt et al. 2014) were found scattered on a rocky-sandy seabed at a depth of 11 m. The S.M.I. Bucentaure of the French-Spanish fleet was sunk in the Battle of Trafalgar (1805) during a violent storm that struck the coast of Cádiz.



Fig. 1. A. and B. Location map (Google Earth) showing the archaeological site of Bajo del Chapitel (Bucentaure site) in the Bay of Cádiz (southwest coast of Spain).

The archaeological site Bucentaure shows seasonal water temperature oscillations from 13-14ºC in February-March to 24ºC in September, small variations in salinity (36.0-36.2 psu, practical salinity unit), well oxygenated water (8 mg/L), slight seasonal and depth pH variations (~ 8.2) and redox potential fluctuations of the water column (50-200 mV). Sediments at the Bucentaure site can be classified as moderately-sorted coarse quartz sand (D_{50} =1.095 mm). The site also features a high current velocity of 0.35 m s^{-1} (Bethencourt et al. 2014) and wave heights < 1 m for 50% of the year. During extreme storm events, offshore wave heights greater than 4 m from the WSW have been recorded (Fernández-Montblanc et al. 2016).

3. Experimental

3.1. Materials

The materials used for the experiment were Italian Carrara and Spanish Macael marbles. There are records of the uninterrupted use of both these materials in cultural heritage from Roman times to the present (Malesani et al. 2003; Sáez-Pérez and Rodríguez-Gordillo 2008). In addition, their presence has been reported in several underwater archaeological sites (Davidde et al. 2010; Aloise et al. 2013; Russell 2013; La Russa et al. 2013, 2015).

Macael is an Upper Triassic marble belonging to the sequence of carbonate rocks of the Las Casas geological formation. This formation is part of the Nevado-Filabride Complex (IGME 1975) found in the Betic Internal Zone of the Iberian Peninsula. It is among the most commonly stone material used in Spain's cultural heritage, and one of the most exported Spanish building stones. Macael marble was used in the Lions Fountain of the Alhambra (Granada, Spain) (Sáez-Pérez and Rodríguez-Gordillo 2008) and has been intensively quarried in the Macael area of Almería (Spain) since ancient times. Its outstanding technical and artistic qualities make it comparable to the world famous Italian marble, Carrara, which was also tested here. The use of Macael marble dates back to the 7th century (BC) by the Phoenicians (sarcophagi) and to the 1st-2nd centuries of the Roman Empire (columns, pavements, tomb-stones). However, this marble was more exploited in the Muslim period (10th-15th centuries) continuing into the present (Bello et al. 1992; Navarro et al. 2013).

Carrara marble (Hettangian marble) occurs in the Upper Triassic unit of the Apuan metamorphic sequence (Apuan Alps region, Northern Apennines, Italy) (Attanasio et al. 2000). This marble has been intensely quarried in the basins of Torano, Miseglia and Colonnata of the Carrara district (Attanasio et al. 2000), and it has been widely used for buildings and sculptures (Molli et al. 2000; Tegethoff 2001). Its use dates back to the 6th century BC by the Etruscans, and in much larger quantities from the mid-1st century BC by the Romans. At the end of the 2nd century AD, it had been practically abandoned but mining of Carrara marble recommenced in the early 12th century, mainly to supply Renaissance sculptors (Beltrame et al. 2012).

3.2. Underwater exposure test

To assess deterioration of the stone materials, a field test was carried out at the Bucentaure site (Bajo del Chapitel). Pieces of both marbles (10 x 5 x 3 cm, the sides of the stone prisms to be exposed, being smoothed by polishing) were submerged along with fragments of the modern materials included in the ARQUEOMONITOR project (copper, brass, cast iron, carbon, steel, pine and oak). Thirty six samples (of each marble type) were anchored to 9 plastic panels and submerged for an 18-month period (January 2012 - April 2013). A further 14 specimens of each marble type were kept in the laboratory as controls.

Three different conditions were set up at the archaeological site: i) exposure to the water column on a metal support at an angle of 65 ° in reference to the sea surface, at a depth of 10 m (referred to the chart datum or Lowest Astronomical Tidal) or 1 m over the bottom (condition I, Fig. 2A); ii) resting on the sea bottom, at a 11 m depth, subjected to natural processes of burial and unearthing (condition II, Fig. 2B); and iii) buried at 30-40 cm and covered with a dark hessian fabric (to help their localization, enable water exchange and prevent unearthing) (condition III, Fig. 2C). The bad weather conditions of the sea during the underwater exposure test, such as extreme storm events, provoked significant losses of the marble specimens. Consequently, after the period of immersion, biological colonization and associated alteration forms were assessed in the recovered specimens (12 of each marble type). The environmental characteristics of each condition were published by Fernández-Montblanc et al. (2014b).

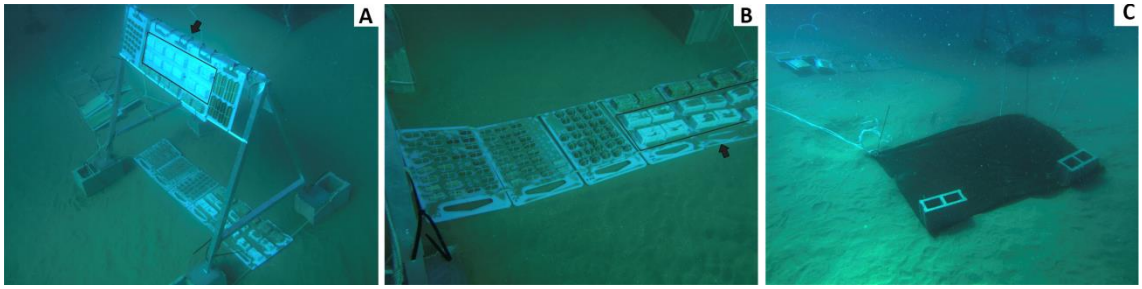


Fig. 2. Exposure conditions of the underwater test (ARQUEOMONITOR project). A. Samples in plastic panels exposed to the water column using a metal support (condition I, boxed area). B. Samples resting on the sea bottom (condition II, boxed area). C. Samples buried under the seabed (condition III). Note that marble samples were submerged along with other modern materials (metal, wood) included in the ARQUEOMONITOR project.

3.3. Techniques and analysis

3.3.1. Material characterization

3.3.1.1. Polarized light microscopy (PLM)

Thin section analysis was done using a petrographic or polarized light microscope (PLM) in transmitted light (Axioskope 40, Zeiss Axiolab) equipped with a digital camera. For material characterization, 2 thin sections of control Carrara and Macael samples and 8 thin sections of each marble type exposed to the 3 conditions were prepared. Sample preparation was conducted by the Sample Preparation Service of the Dept. of Petrology and Geochemistry of the Universidad Complutense de Madrid. Briefly, due to the fragility of biocolonization, samples were pre-consolidated with an epoxy resin by vacuum impregnation. Next, they were polished and fixed to a glass slide, and sections cut again to 2 mm in thickness and further reduced on a thin section grinder to a minimum thickness of 50-100 μm. Finally, the thin sections were polished with silicon carbide (for PLM observation), and alumina (for SEM observations, see 3.3.2.2 section) of different grain size to obtain a section thickness as low as 30 μm (Humphries 1992). Calcite was distinguished from dolomite by Alizarin red S staining (Evamy 1963). The thin sections were petrographically described using Tucker's descriptive worksheets (Tucker 2001), according to average grain size (AGS) and maximum grain size (MGS, the largest calcite grain found within thin sections of the same marble type, Moens et al. 1988).

3.3.1.2. X-ray powder diffraction (XRD)

The mineralogical composition of the Carrara and Macael marble was determined by X-ray powder diffraction analysis (XRD) in powdered samples (grinded to a particle size ≤ 40 μm), using a copper anode Philips PW1752 diffractometer fitted with a graphite monochromator at the IGEO (CSIC-UCM) Microscopy and Mineralogy Technical Unit. The operating conditions were 40 kV and 30 mÅ running on PC-APD (Q1) (automated powder diffraction) software. The measurement range was 2–68 θ, recording rate 2˝/min, and mode continuous. DIFFRAC^{plus} EVA analytical software (Bruker AXS, Karlsruhe, Germany) was used to identify mineral phases.

3.3.1.3. X-ray fluorescence (XRF)

The chemical composition of the Carrara and Macael marble was determined by X-ray fluorescence spectroscopy (XRF) using a Philips PANalytical Axios spectrometer with a 4 kW wavelength dispersive (WD) at the X-ray Diffraction Research Support Centre (Universidad Complutense de Madrid). One control sample of each marble type (8 g each) was prepared in pressed powder aluminium pellets of 40 mm diameter (F=25 kN for 20 s). Major elements were semi-quantitatively analyzed using IQ+ software, and trace elements were quantitatively determined with the ProTrace package, both from PANalytical.

3.3.1.4. Optical Surface Roughness (OSR)

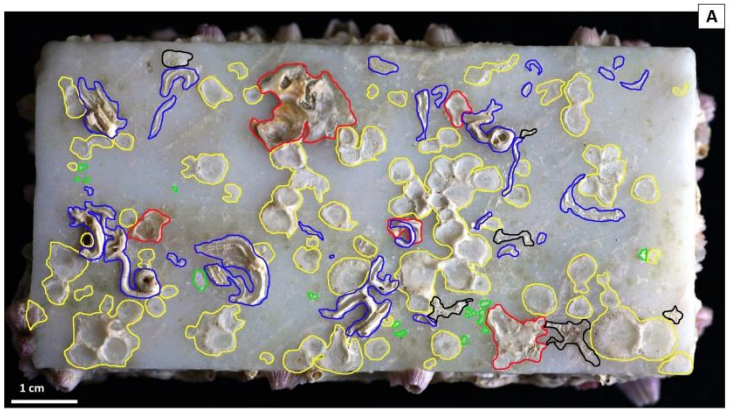
Due to the fact that a great surface roughness in materials render them prone to microbial colonization, according to Miller et al. (2012a), the stone side of the prismatic specimen to be exposed was polished, to homogenize initial surface conditions. A non destructive and portable optical surface roughness meter (OSR, TRACEiT®, Innowep) was used to measure the value of Rz (mean roughness depth, standard ISO 4287:1984), in both marbles. Two measurements were taken in the exposed surface of each of the initial 72 marble specimens, and the average and standard deviation was calculated.

3.3.2 Biological colonization and associated deterioration forms

3.3.2.1. Statistical analysis of epilithic colonization cover

Total invertebrate and coralline epilithic colonization in relation to the total surface area of the exposed marble was estimated in the samples recovered from the underwater environment. Images from marble samples were obtained by a CANON EOS 5d camera, with a light incidence angle of 45º. For general views, Canon 50 mm, f 1.4 lens were used, while for detailed images, Tamron 90 mm, f 2.8, Macro 1:1 lens, were utilized. Images were RAW captured, following with Abobe Camera Raw developing processes and finishing with Photoshop CS6 software. These images were then analyzed using the ROI Manager tool (Analyze Menu -> Tools) of the ImageJ 1.50c software (Rasband WS 1997-2012, National Institute of Health), which enables to select and label manually the areas colonized by each type of epilithic organisms, as

can be observed in Fig. 3A. Cover values for the two marble types were compared by two-way analyses of variance (ANOVA), including exposure condition and type of marble as possible factors influencing biocolonization. Tukey's HSD post hoc tests were used to identify significant differences among the 3 exposure conditions (I, II and III). Prior to analysis, the normality assumption was tested and variables were squared-root transformed to meet ANOVA assumptions. All statistical tests were performed using the package STATISTICA 8.0 (Tulsa, OK, USA) and graphs were obtained using SigmaPlot 11.0 (Systat Software, Inc). The biocolonization rate was determined as cm^2 of surface area colonized by encrusting organisms over the 18 months of the underwater test. Overall colonization cover was also calculated using the conventional Braun-Blanquet cover-abundance scale (Braun-Blanquet 1932; Wikum and Shanholtzer 1978). This scale ranges from the highest value of 5 - for a cover of more than 75% of the surveyed surface – to the lowest value (defined by +) for a sporadic cover of less than 1% (Fig. 3B).



B

BRAUN-BLANQUET SCALE	RANGE OF COVER (%)
5	75-100
4	75-50
3	25-50
2	5-25
1	< 5
+	< 1

Fig. 3. A. Example of digital image analysis (ImageJ software) of colonization cover on a Carrara marble specimen according to the major groups of organisms (barnacles: yellow; tube worms: blue; molluscs: red; bryozoa: black; green: unknown deposits). B. Braun-Blanquet scale and corresponding % cover.

3.3.2.2. Microscopic characterization (SM, PLM, SEM)

Nine marble fragments of each type (1.5 cm wide x 3 cm long x 1.5 cm high) were examined to identify invertebrate and coralline colonizers and characterize the nature of their skeletons by stereomicroscopy SM with an EMZ-5D instrument (MEIJI EM), and SEM using an FEI Quanta 200F Philips (at 15 kV and high vacuum). For SEM

analysis, marble fragments were first carbon coated. Additionally, 8 thin sections of each marble type were also observed by PLM and environmental SEM (ESEM) to determine the extent of decay at the organo-mineral interface. For ESEM observations, a FEI QUANTA 200 SEM equipped with a four-diode semiconductor back-scattered electron (BSE) detector and coupled to an energy dispersive X-ray spectroscopy (EDS) microanalytical system was used at the Electron Microscopy Unit of the Museo Nacional de Ciencias Naturales (CSIC) in Madrid. Operating conditions were 20-27 kV acceleration potential and low vacuum. No prior sample preparation (metal sputtering) was required.

4. Results and Discussion

Table 1 summarizes some of the characteristics analyzed in both stones.

Both Carrara and Macael marbles are composed of calcite ($CaCO_3$) and differ mainly in calcite crystal size and shape. Our observations revealed a larger calcite crystal size for Macael than Carrara in terms of AGS (average grain size) and MGS (maximum grain size) (Table 1). According to the MGS categories proposed by Gorgoni et al. (1998), our data indicated a medium to coarse-grained marble for Macael and a fine-grained marble for Carrara (Table 1).

Table 1. Summary of the characteristics of both marbles (Carrara and Macael) determined in this study

Marble	Macael	Carrara
Colour	White with greyish bands	White
Petrographic features (PLM)		
Texture	Heterogeneous granoblastic	Homogeneous granoblastic
Fabric	Mosaic	Mosaic, polygonal
Mean AGS[1]	1.08±0.50mm	0.18±0.07mm
MGS categories[2]	Medium-coarse-grained (2.8±0.5mm)	Fine grained (0.7±0.2mm)
Crystal boundaries	Curved, slightly sutured and straight	Curved to straight
Other	Rhombohedral cleavages, 120° triple junctions, slight preferential crystal orientation	Rhombohedral cleavages, 120° triple junctions, no preferential crystal orientation
Mineralogy (XRD)	Calcite	Calcite (+dolomite)
Roughness (Rz)	Rz = 12.1 ± 2.2 µm	Rz = 10.6 ± 2.0 µm

[1] AGS: average grain size
[2] MGS categories: maximum grain size (Moens et al. 1988) categories according to Gorgoni et al. 1998

The obtained roughness values (Rz) were for Macael marble 12.1 ± 2.2 µm, while, for Carrara, although similar in range, were slightly lower (less rough or smoother), 10.6 ± 2.0 µm, which is directly related to the crystal size of each marble, larger in Macael than in Carrara (Table 1).

Petrographically, Macael is characterized by a heterogeneous granoblastic texture of mosaic fabric (Fig. 4A). This marble is white and sometimes shows a pattern of greyish bands. Calcite crystal boundaries are curved, slightly sutured and straight in some points where they form 120º triple junctions (Fig. 4B). Rhombohedral cleavages appear within calcite crystals. There is a slight preferential orientation of calcite crystals (Fig. 4 A-B). Carrara marble is also white with a homogeneous granoblastic texture of mosaic fabric, although sometimes it adopts a polygonal fabric. Grains show well-defined and curved-to-straight boundaries and frequent 120º triple junctions, typical of marbles (Fig. 4C). Rhombohedral cleavages appear in the interior of calcite crystals. No preferential orientation of calcite crystals can be observed. Veins of dolomite crystals could be clearly detected in thin sections stained with Alizarin red S. No pores were observed at this scale in either marble.

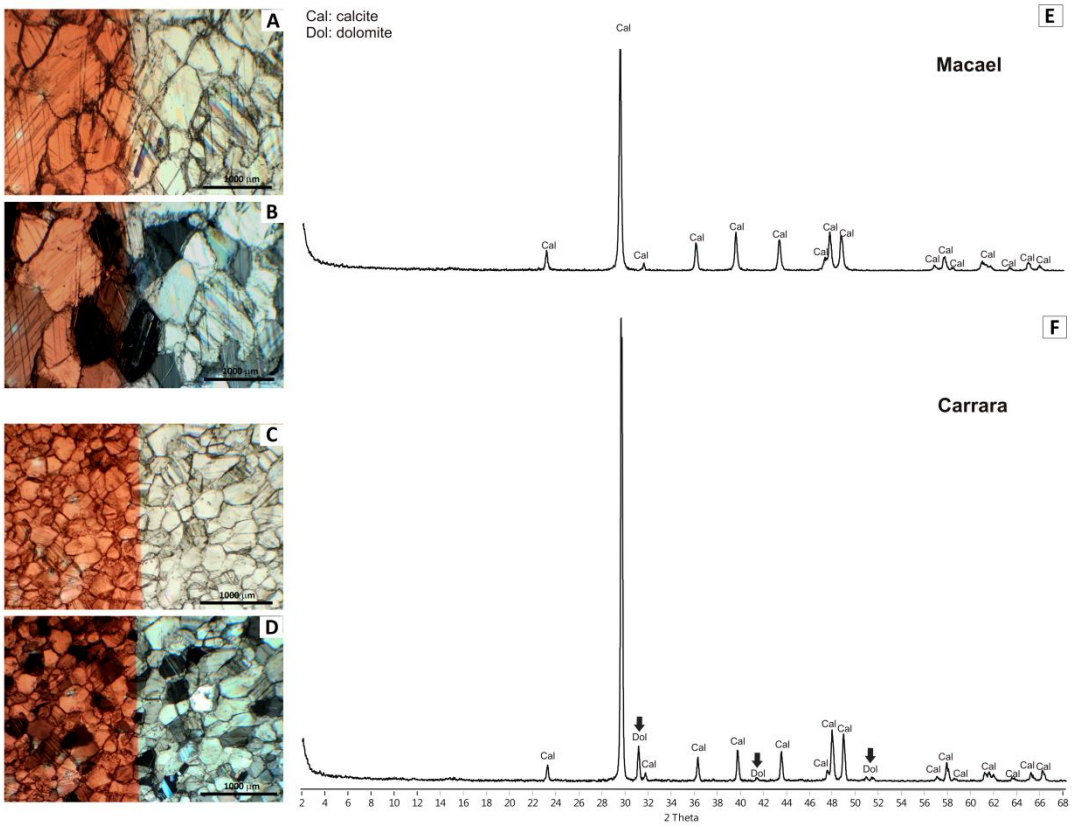


Fig. 4. A-D. Micrographs (PLM) of Macael (A and B) and Carrara (C and D) marbles; A and C are plane polarized light images, while B and D are cross polarized light ones. Calcite crystals stained red with Alizarin red S (staining was applied only on the left half side of the thin section). E-F. X-ray diffraction patterns (XRD) of Macael (E) and Carrara (F) marble showing the presence of the main reflections corresponding to calcite (Cal). In the latter, arrows indicate reflection peaks (at 30.96, 41.14 and 51.12 theta) corresponding to dolomite (Dol).

The presence of calcite as the main mineralogical component of Carrara and Macael marbles was confirmed by XRD analysis (Fig. 4E-F). Only Carrara showed peaks corresponding to the reflection of dolomite (Dol, $CaMg(CO_3)_2$) (arrows in Fig. 4F). Our XRF results (Table 2) revealed Ca as the main element in the Carrara and Macael marbles. However, some minor differences were also noted including a slightly higher Mg content and lower Sr content for Carrara.

Table 2. XRF data (normalized to 100%) obtained for the control Carrara and Macael marble samples

Marble	Carrara	Macael
CaO*	97.76	98.42
MgO*	1.95	1.18
SiO_2*	0.10	0.16
Al_2O_3*	0.05	0.03
Fe_2O_3*	0.04	0.10
SO_3*	0.02	0.05
NaO*	0.02	-
K_2O*	0.01	-
Cl*	-	0.01
Sc$^{\Delta}$	26	29
Mn$^{\Delta}$	29	30
Co$^{\Delta}$	-	1
Ni$^{\Delta}$	1	-
Zn$^{\Delta}$	4	4
As$^{\Delta}$	5	5
Rb$^{\Delta}$	1	1
Sr$^{\Delta}$	142	181
Y$^{\Delta}$	1	1
Nb$^{\Delta}$	-	1
Ag$^{\Delta}$	9	8
Cd$^{\Delta}$	12	11
Cs$^{\Delta}$	2	5
Ce$^{\Delta}$	35	38
Nd$^{\Delta}$	6	9
Ti$^{\Delta}$	5	5
Pb$^{\Delta}$	5	6
Bi$^{\Delta}$	1	-
Th$^{\Delta}$	3	2
U$^{\Delta}$	3	2

* major elements %
$^{\Delta}$trace elements ppm

After 18 months of underwater exposure, the pieces of Carrara and Macael marbles recovered from the Bucentaure site clearly differed accordingly to the exposure conditions (I, II and III) in terms of biological colonization in the largest exposed surface (Figs. 5). Figs. 5A, B and E, are representative of Carrara marble samples exposed to the three different conditions, and Figs. B, D and F, correspond to Macael marble samples. It can be clearly observed - in the largest exposed marble surfaces - that samples from the exposure condition I show (Figs. 5A-B), at first glance, a greater surface colonized by different groups of organisms of encrusting nature including barnacles, tube worms, bryozoans, molluscs and coralline algae, being barnacles and tube worms apparently the most abundant. However, this is not the case for marble samples, both Carrara and Macael, exposed to the conditions II (Figs. 5C-D) and III (Figs. 5E-F), where the biological colonization is occasional, randomly distributed and the number of colonizing organisms is much lower, but still tube worms seem to be predominant.

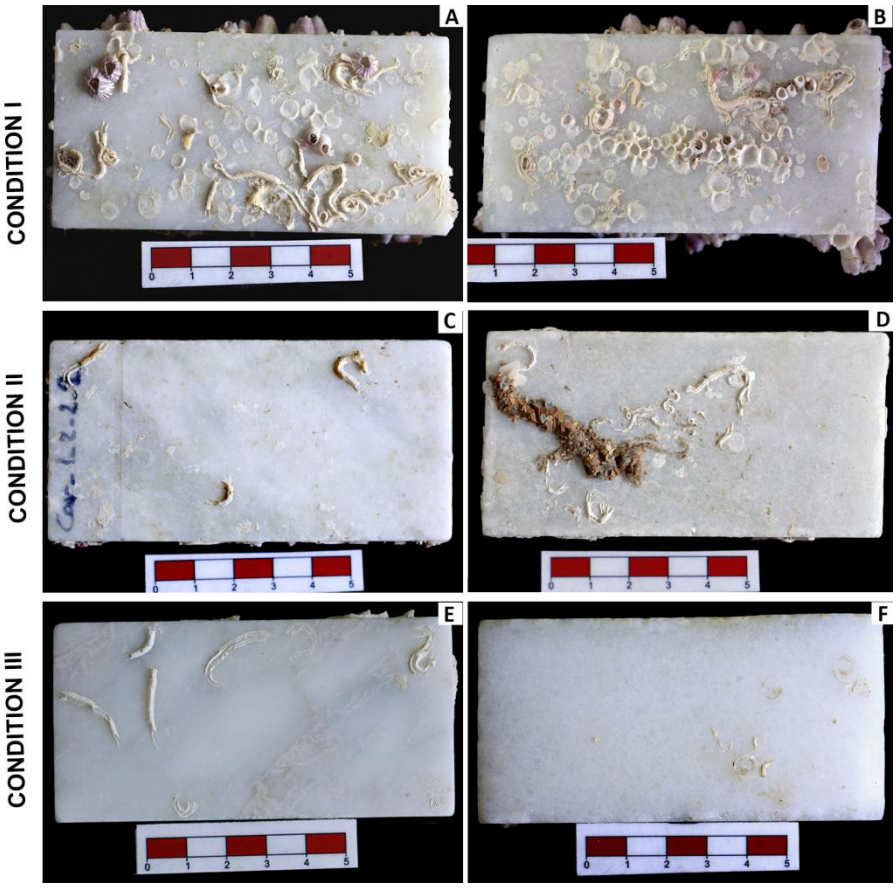


Fig. 5. Macroscopic view of specimens of Carrara (A, C, E) and Macael (B, D, F) marbles recovered from the Bucentaure site after 18 months of underwater test. Note at first glance the remarkable differences of biological colonization cover in the largest exposed surface area of samples among the three exposure conditions tested, especially between condition I and the other two conditions (II and III). Barnacles, tube worms, bryozoans, mollusc and coralline algae are among the most abundant encrusting organisms in the samples studied.

Two-way ANOVA identified a significant effect of the exposure conditions on colonization cover values obtained for both types of marbles (F=57.025; p< 0.0000), but no significant differences resulted between both types of marble (F=0.0026; p< 0.9598). Biocover values for both Macael and Carrara significantly increased from baseline to 32-35% of the total surface in samples subjected to condition I (Tukey's post hoc test: I vs II, p<0.00016; I vs III, p<0.00016) as that producing the greatest effect (Fig. 6A). This main effect of the exposure conditions was also evident in the two-way ANOVA results for the major groups of organisms identified on the surface of the marbles (Figs. 6B-F). The colonization cover of barnacles, tube-building annelid worms and bryozoans was significantly influenced by the type of exposure condition (barnacles: F=324.89; p<0.0000, tube worms: F=10.644; p<0.0010, bryozoans: F=5.8028; p<0.0119), showing the highest values of coverage (%) in the condition I (Fig.6B-D). In addition, the colonization cover of molluscs was affected significantly by both the type of exposure condition (F=5.5722; p<0.0138) and the type of marble (F=5.3676; p<0.0333, Fig. 6E). And finally, the presence of coralline algae was mainly determined by the interaction between the type of exposure conditions and the type of marbles (F=3.8087; p<0.043, Fig. 6F). Tukey's post hoc tests revealed differences in biocover values for condition I that were significant for barnacles but not for the rest of the organisms (letters a and b in Fig. 6B).

The biocolonization rate of both marbles was similar for each of the three conditions after 18 months of underwater exposure (Table 3). For condition I, marble surfaces showed an area of 13-22 cm^2 (of a maximum area of 50 cm^2) covered by deposits and skeletons. Biocolonized surface areas for marble samples resting on the seabed were 1 to 7 cm^2 and for those buried in the sediment were much lower (0.01-4 cm^2). Braun-Blanquet cover values revealed the similar behaviour of both types of marbles (Table 3) and confirmed the results obtained in the statistical analyses. Braun-Blanquet indices were highest at 3 for both marbles exposed to condition I (cover was 32% and 35% for Carrara and Macael, respectively), and lowest at 1 and + for the buried marbles (condition III) which showed colonization cover values of ~3% (Carrara)

and ~1% (Macael). For practically all samples and conditions, barnacles and tubes worm were the most abundant organisms. Bryozoa were also widely detected but not as a dominant group. When present, the abundance of molluscs and coralline algae was scarce. Moreover, molluscs were not detected in samples exposed to condition II and coralline algae were not detected in samples exposed to condition III.

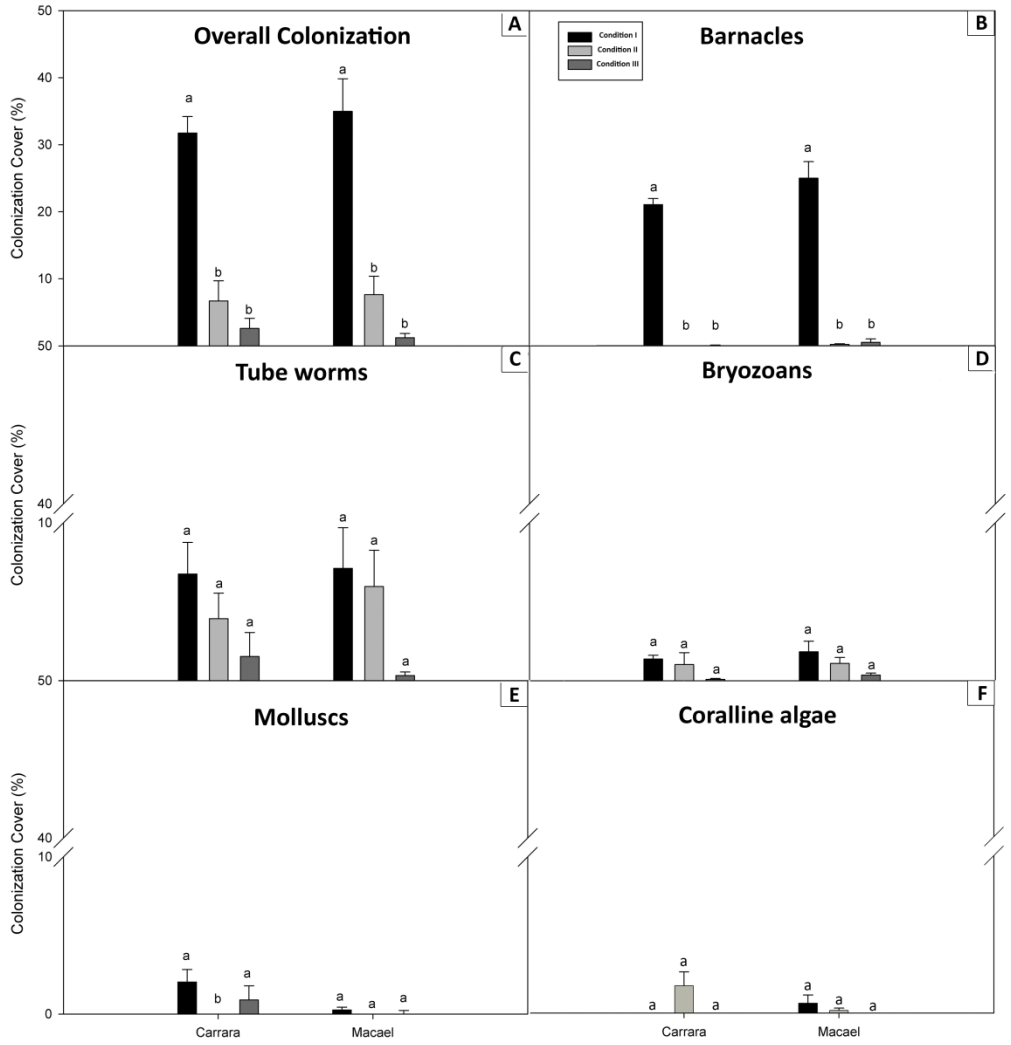


Fig. 6. Colonization cover (%) measured as overall colonization and as colonization by major encrusting organisms in samples of Macael and Carrara marble after their exposure to the underwater environment: A. Overall colonization. B. Barnacle cover. C. Tube worm cover. D. Bryozoan cover. E. Mollusc cover and F. Coralline alga cover. Different letters (a,b) above the columns indicate significant differences among the different types of exposure conditions according to Tukey's HDS test. When both letters (a,b) are present, as in Fig. 6A, it means that the overall colonization cover (%) is significantly different between samples exposed to the water column condition (I, letter a) and samples exposed to the other two conditions (II and III, letter b).

Table 3. Groups of organisms colonizing the surface of Carrara and Macael marble samples after their immersion in seawater. Also shown for each condition and marble type are biocolonization rate, expressed as cm^2 of marble surface colonized during the 18 months of the underwater experiment, and Braun-Blanquet (B-B) abundance indices for overall colonization cover

Marble	Condition	Overall colonization cover (%)	Rate of biocolonization (cm^2/ 18 months)	B-B Index	Groups of organisms				
					Barnacles	Tube worms	Bryozoans	Molluscs	Coralline Algae
Carrara	I	31.7 ± 5	15.7 ± 2	3	√*	√	√•	√	x
	II	6.7 ± 5	3.4 ± 3	2	x	√*	x	x	√•
	III	2.6 ±3	2.6 ± 1	1	√•	√*	√	√	x
Macael	I	35 ± 10	17.5 ±4	3	√*	√	√	√•	√
	II	7.6 ± 5	4 ± 3	2	√	√*	√	x	√•
	III	1.2 ± 1	0.6 ± 0.6	+	√*	√•	√	x	x

*Most abundant organisms
• Least abundant organisms

On both marbles subjected to the 3 conditions, we distinguished two main types of biodeterioration: biofouling and bioerosion. Biofouling has been detected as the accumulation on exposed marble surfaces of deposits and encrustations, mainly of a calcareous nature, whereby the original surface is lost and the appearance of the marble changes completely. The deposits observed in this research, varied in thickness, were mainly remains of encrusting organisms from the epilithic community including barnacle shells (Fig. 7A-D), tube-building annelid worms (Fig. 7E-I), bryozoan skeletons (Fig. 8A-C), and calcareous deposits of coralline algae (Fig. 8D-G) and molluscs (Fig. 8H-J).

Barnacle (Trecostraca, Cirripedia, *Balanus* sp.) shells occupied large areas of the marble surface, especially in samples exposed to condition I. These shells, consisting of a peripheral shell and a base plate (Fig. 7A-B) firmly attached to the marble, adapting their structure to the surface roughness of the stone (Fig. 7C-D). In

some samples, the presence only of calcareous base plates indicated the previous occurrence of these sessile crustaceans (asterisks in Fig. 7A).

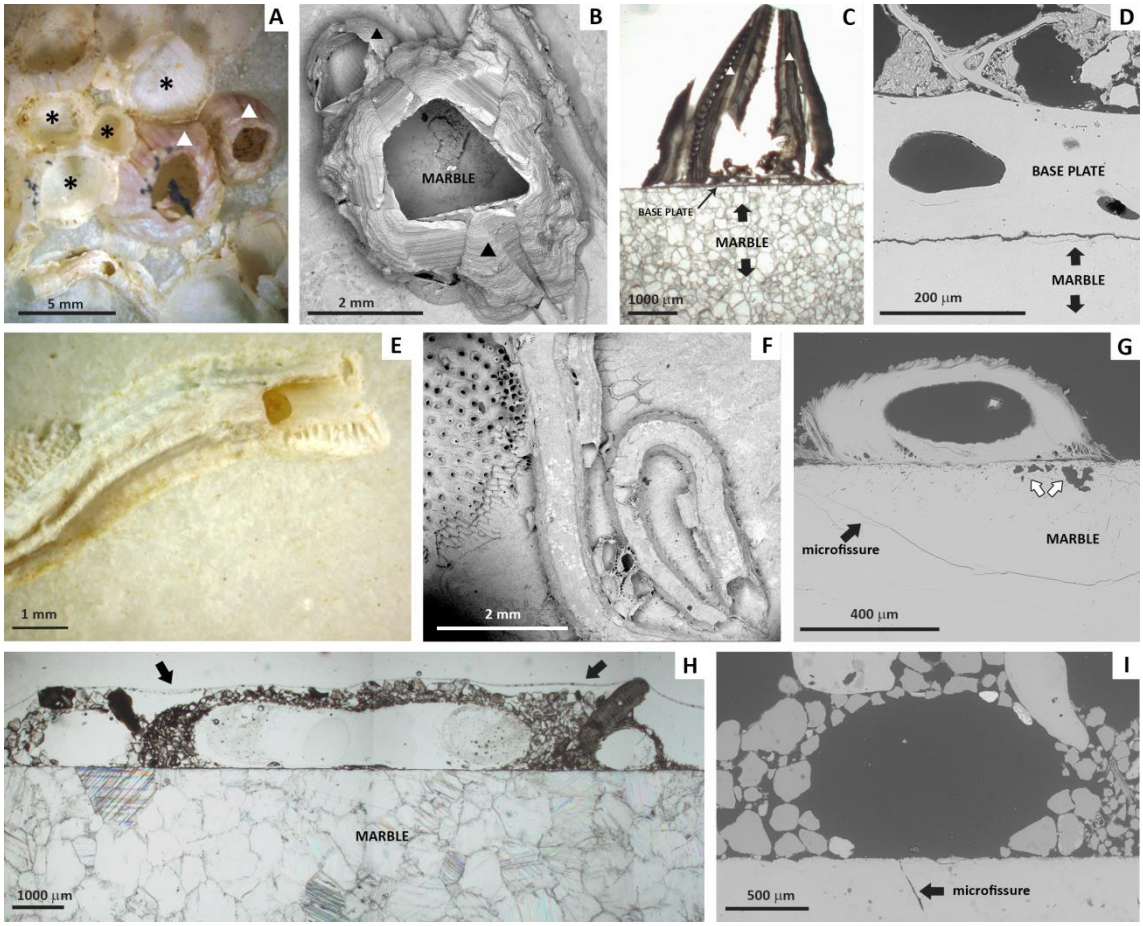


Fig. 7. Superficial deposits of the encrusting organisms barnacles and tube worms detected in samples of Carrara (B, C, D from condition I) and Macael (A, E from condition III; F, H and I from condition I; G from condition II) marbles after the underwater test. A. Stereomicroscope and B. SEM-BSE images of a top view of peripheral shells and base-plates of acorn barnacles. C. PLM of a longitudinal section of an acorn barnacle attached to the marble surface. D. Detailed SEM-BSE image of the interface between the calcareous base-plate of a barnacle and the marble surface. E. Stereomicroscope and F. SEM-BSE images of calcareous tubes of serpulids on a marble surface. G. SEM-BSE image of a cross-section of a calcareous serpulid tube. Microfissures (black arrows) and loss of material (white arrows) in the adjacent stone are also indicated. H-I. PLM and SEM-BSE images of a sand tube belonging to another specie of polychaete worm made of sand clasts and shell fragments (foraminifera, diatoms) agglutinated by an organic matrix (black arrows,H). Note also the presence of microfissures in the marble below (I).

Two types of deposit were attributed to tube-building annelid worms (Fig. 7E-I). Calcareous tubes of serpulids (Annelida, Polychaeta) were observed randomly distributed on samples exposed to the three conditions (Fig. 7E-G). The most abundant species colonizing the marble surfaces was *Spirobranchus triqueter* (Linnaeus, 1758). On the other hand, in less abundance tubes comprised of sand clasts, fragments of shells (foraminifera, diatoms) and debris were also detected (Fig. 7H-I). These tubes were composed mainly of medium-sized sand grains (132 ± 83 μm; 454 - 41 μm, measurements obtained for 85 grains) of quartz and feldspars (mainly potassium-aluminium silicate and zirconium silicate; data obtained by EDS, not shown), which along with biogenic detritus were agglutinated by an organic matrix (arrows in Fig 7H). Microfissures and voids were observed in the adjacent stone marble.

Epilithic bryozoa colonies were observed on most of the samples (Fig. 8A-C). These colonies showed a variety of sizes and irregular shapes, but were mostly ellipsoid-shaped and their size was in the range of 5-10 mm (largest diameter). These deposits were composed of a one-layer sheet of calcareous exoskeletons (zooecium, white arrows in Fig. 8B) of the individuals (zooids, black circles, Fig. 8B) forming part of the colony. They appeared tightly attached to the marble surface, adapting the morphology of their base to the irregularities of the marble surface (Fig. 8C), but sometimes epibiosis phenomena occurred and bryozoans settled on calcareous deposits of other epilithic organisms (e.g. serpulids in Fig.7E-F). Microfissures were detected beneath these deposits (black arrow in Fig. 8C).

Crustose calcareous algae (Rhodophyta, Corallinales) were also found on the surface of some marble samples, showing extensive plate-like growth (Fig. 8D-E). Several layers of cells could be observed to form a thallus with a honeycomb structure (43 ± 5 μm width) (Fig. 8F-G). In this type of structure, calcareous deposits were contained within cell walls. This type of epilithic organism was also observed to be firmly attached to the marble, adapting its morphology to the surface roughness. Microfissures and pitting patterns were also detected in the underneath marble (Fig. 8G).

The base shells of some molluscs (Bivalvia, *Anomia* sp.), when present, covered extensive areas of exposed surfaces, following their irregularities (Fig. 8H-J). Inside these epilithic deposits, it was possible to observe entrapped calcareous skeletons of smaller size, such as those of bryozoans and serpulids (Fig. 8H-I).

Moreover, free-living microbial cells were partially or completely permineralized, and mainly preserved their external cell envelopes in the calcareous matrix (black arrows in Fig. 8J). In some areas of the marbles covered with these calcareous mollusc deposits, it was possible to detect the presence of subparallel and trans-crystalline microfissures (black arrow in Fig 8I) and pitting patterns beneath them (white arrows in Fig. 8I).

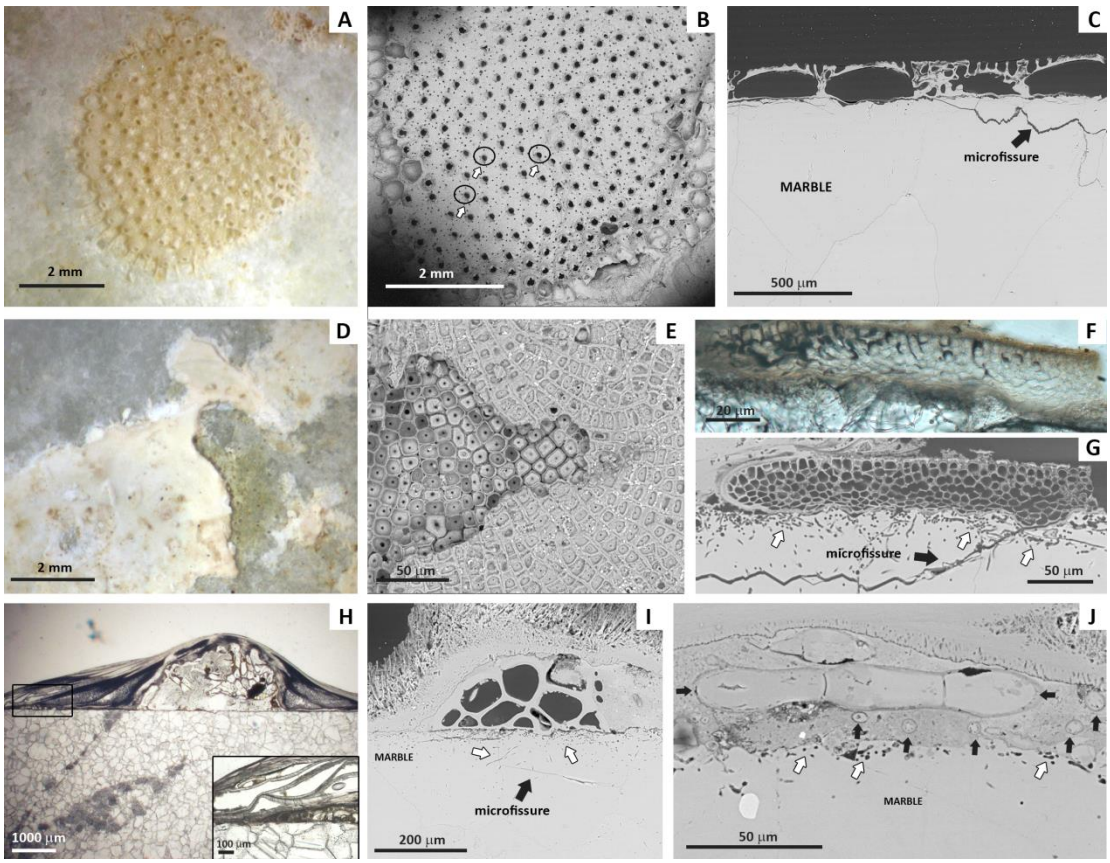


Fig. 8. Superficial calcareous deposits of the encrusting organisms bryozoans, coralline algae and molluscs on Carrara (B, D, E, F and G from condition II; H and I from condition I), and Macael (A from condition II; C from condition III and J from condition I) marbles after the underwater test. A. Stereomicroscope and B. SEM-BSE images of an encrusting bryozoan colony showing a one-layer sheet of the calcareous exoskeletons (zooecium, white arrows) of the individuals (zooids, black circles) comprising the colony. C. SEM-BSE image of the bryozoan colony-marble interface and microfissures (black arrow) beneath the encrusted deposit. D-E. Stereomicroscope and SEM-BSE images of a crustose calcareous alga showing a honeycomb-like thallus. F-G. Cross-section (PLM and SEM-BSE images) of the algal thallus-marble interface. Note how this structure is firmly attached to the stone surface. Microfissures (black arrow) and pitting patterns immediately beneath it (white arrows) are also indicated (G). H. Stereomicroscopd and I-J SEM-BSE images of calcareous deposits associated with the base shell of certain molluscs showing entrapment of other calcareous skeletons of smaller organisms (boxed area in H, and I), microfissures (black arrow in I) and pitting patterns (white arrows in I and J) in the adjacent stone along with free-living microbial cells preserved in the calcareous matrix (black arrows in J).

Biofouling phenomena, besides causing aesthetic damage to the marble as a material used in cultural heritage, could ultimately lead to partial or complete loss of value and functionality of the stone artefact or element. This involves mostly a loss of value of the archaeological object of which they are part (Crisci et al. 2010).

Numerous studies have attributed to calcareous deposits a bioprotective role, as they become new areas to be colonized, preventing the action of more damaging endolithic microorganisms on stone materials (Aloise et al. 2013; La Russa et al. 2013, 2015). However, in the present study, it is not possible to determine this protection, as bioerosion patterns were also detected beneath these deposits (Fig. 8G-I and 9A). Monitoring the biological succession on these marbles during the underwater exposure test would allow to figure out whether the action of some endolithic microorganisms was previous or not to the settlement of epilithic encrusting organisms.

Furthermore, microfissures were also identified in the most superficial areas of the marbles, especially beneath epilithic calcareous deposits. Even though, this deterioration pattern has been enumerated as collateral effect of the presence of calcareous deposits on stone materials (La Russa et al. 2015), being uncertain the origin of these microfissures. The fact that microfissures were detected in control samples, leads to think that some of them were probably derived from the preparation of samples (cutting and resin impregnation steps), but others could have been generated by underwater exposure agents (physical, chemical or biological). In any case, epilithic encrusting organisms and actively-penetrating endolithic microorganism could have been attracted by these microfissures, as they might constitute points of access to the rock interior where the marble turns out to be more susceptible to biological colonization (De los Ríos et al. 2004; Cámara et al. 2008).

Signs of bioerosion consisting of microscopic pitting patterns were similar on both the Carrara and Macael marbles subjected to the three conditions tested, while the extension of the area affected by this type of biodeterioration varied in relation to the exposure conditions (Fig. 8-9). In view of the results obtained, it seems that the impacts of the endolithic community on the marbles studied should not be underestimated. Marble samples whose whole surface was fully exposed to the water column (condition I) showed the greatest area damaged by microbioerosion. This condition likely promoted the growth of damaging endolithic microorganisms providing optimal light access and nutrient availability. On the contrary, in buried samples

(condition III), bioerosion was scarcely observed. Dark environments such as these will probably favour more the growth of light-independent and organic-dependent boring microorganisms.

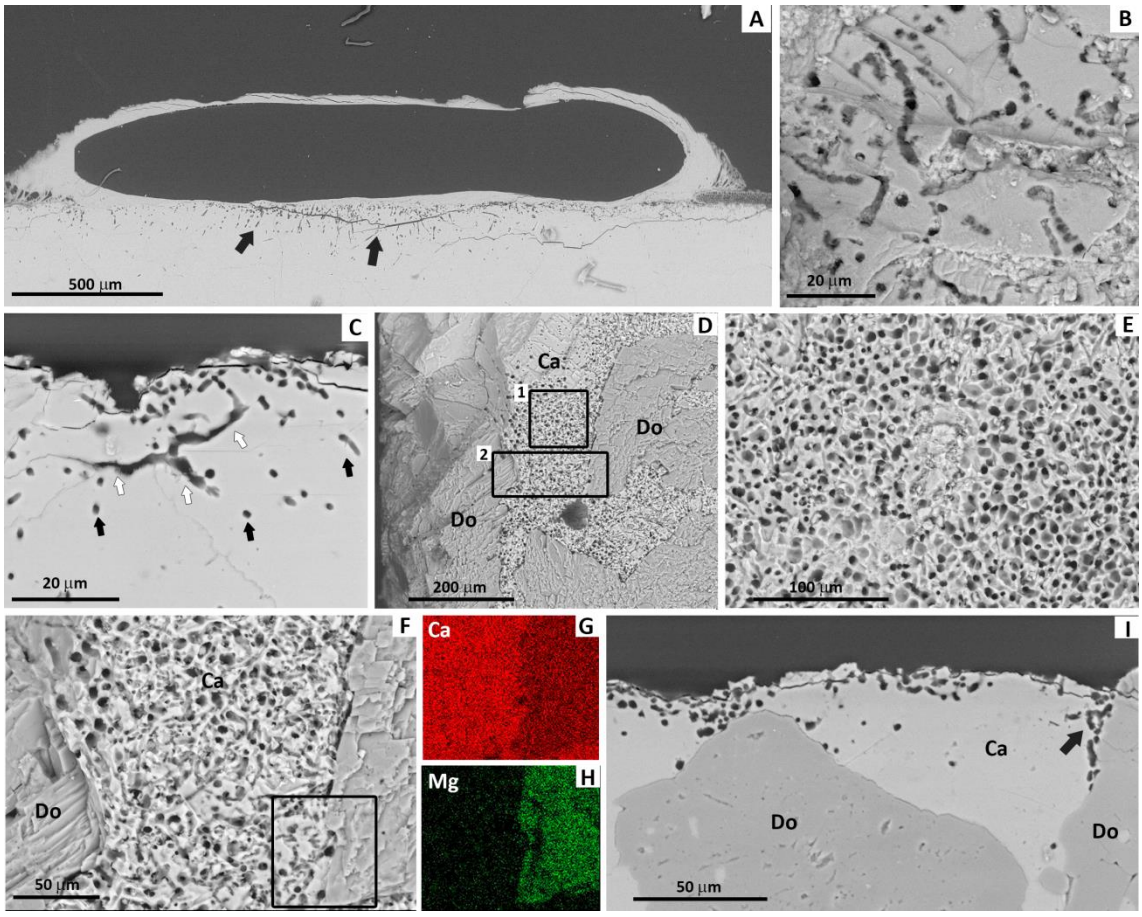


Fig. 9. SEM-BSE images of bioerosion patterns produced in the Carrara (A, B, D, E, F, G, H, I from condition II), and Macael (B from condition II) marble specimens after their underwater exposure. A. Micropitting patterns on the marbles beneath calcareous deposits of serpulids (arrows). B. Superficial view of circular and filament-shaped micropitting patterns in the absence of calcareous deposits. C. Cross-section of the marbles showing a network of microgalleries, some connected to the surface. Note the presence of branched cavities (white arrows) and microboring patterns running across grains (black arrows) attributed to endolithic microorganisms D-H. Surface and (I) cross-section view of microboring patterns which were especially abundant in the calcite crystals comprising the Carrara marble: Ca (calcite crystals) and Do (dolomite crystals). G-I. The red and green images correspond to EDS mapping showing the spatial distribution of Ca and Mg respectively, in the area of the Carrara marble indicated (boxed 2).

Bioerosion was observed in areas just beneath calcareous skeletons of different

epilithic encrusting organisms (coralline algae in Fig. 8G, molluscs in Fig. 8J and serpulids in Fig. 9A), but it also appeared in their absence, being sometimes externally visible on the marble surface by microscopy (Fig. 9B, D-F). Microboring patterns were limited to the most superficial areas of the marble specimens, spreading from the surface to a depth of 1 mm. They were generally characterized by circular and filament-shaped forms (Fig. 9B). Branched or irregular cavities were also commonly detected at different depths throughout the substrate, and probably arose via the coalescence of several microholes (white arrows in Fig. 9C). In cross-section, a network of microgalleries could be observed, most of which were likely connected to the surface. In general, these pitting forms did not show any preferential orientation. However, in some situations, microperforations followed contact lines between crystal grains, with the possible consequence of eventual enlargement of these micropore spaces. Mostly, however, microperforations ran crosswise to the crystal grains (black arrows in Fig. 9C). Considering that each endolithic microorganism could generate specific perforation patterns under identical conditions (Golubic et al. 1975), the morphological features (shape and dimensions) described here could be attributable to the fingerprints left by the microbial activity of green algae, cyanobacteria and/or microfungi (Tribollet et al. 2011; Ricci et al. 2013; Sacco Perasso et al. 2015). As the penetration capacity of photosynthetic microorganisms into the substrate is known to be limited by their light requirements (Perry and Macdonald 2002; Davidde et al. 2010; Tribollet et al. 2011), it is likely that they are responsible of the pitting patterns closest to the marble surfaces. Ricci et al. (2013), reproduced the microborer traces left in archaeological stones for their ichnotaxonomic identification (Radtke and Golubic 2005). Among the most predominant microborers is the green alga *Ostreobium quekettii* (Born & Flahault 1889, Ord. Bryopsidales, Fam. Ostreobiaceae) (Ricci et al. 2013; Sacco Perasso et al. 2015) identified through its fingerprints as *Ichnoreticula elegans* (Radtke and Golubic 2005). Identical fingerprints were observed in our Carrara and Macael marble samples, but we cannot state with certainty that they were produced by these endolithic microorganisms. The precise identification of endolithic microorganisms will be covered in depth in further investigations.

As mentioned before, microbioerosion patterns were similar, in extent, size and shape, in calcite crystals of both Carrara and Macael marbles. However, the dolomite crystals of Carrara did not show any sign of this type of biodeterioration, revealing a

preferential spatial distribution, in that it appeared in abundance in calcite crystals yet were absent in dolomite crystals (Fig. 9D-I). This fact was confirmed both by microscopy and X-ray analysis and was more pronounced in areas of calcite-dolomite crystals interface of the Carrara marble. Although it has been shown in other studies that endolithic microorganisms perforate both types of carbonate grains (Cámara et al. 2011; Casanova et al. 2014), this time-limited experiment has showed a significant difference in rates and their effect. This seems to indicate a greater bioreceptivity or susceptibility of calcite crystals to biological colonization (Guillitte 1995) in this underwater environment, which could be related to the textural features and chemical composition of calcite crystals. These features include their microcrystalline structure, grain size and type of porosity, which has been pointed out to facilitate endolithic colonization (Cámara et al. 2008, 2011; Miller et al. 2012b). In a study examining marine bioerosion phenomena, it was observed that relative proportions of calcite and dolomite crystals in marble could determine the perforation capacity of endolithic microorganisms (Davidde et al. 2010). In effect, the different solubility of calcite and dolomite when exposed to an acid environment has been well established (Driessens and Verbeeck 1990), and could explain the greater susceptibility of calcite than dolomite to micropitting. We propose that the dolomite crystal veins present in Carrara marble could act as a chemical barrier to penetrating microorganisms, especially in the first 18 months of underwater exposure. In a long-term test, the dolomite crystals could also be colonized by euendolithic microorganisms and the differential rate of colonization between calcite and dolomite crystals could be completely hidden. Although, in this study we could not observe differences in bioreceptivity between Carrara and Macael marbles, as they were covered by the exposure condition effect, these probably would derive from the presence of dolomite crystals, but also from the different textural features (grain size).

On the other hand, macrobioerosion patterns attributable to the actions of endolithic sponges and bivalve molluscs have been commonly reported in submerged archaeological artefacts, and described as one of the main causes of stone decay in underwater environments, leading over time to their total or partial destruction and disappearance (Davidde et al. 2010; La Russa et al. 2013). Several studies have pointed out to the settlement of macroborers after one year of immersion (Bromley et al. 1990; Casoli et al. 2014). However, the present Macael and Carrara marbles

showed no signs of macrobioerosion in response to 18 months of exposure to seawater. The reasons for the lack of macroboring in our samples could vary depending on the underwater conditions. Hence, for samples completely exposed to the water column, the main cause could be the short time interval available for endolithic sponges and molluscs to settle. Marble samples resting on the seabed were eventually buried/unearthed and subjected to abrasion by surrounding coarse-grained sediments. Thus, this and the short test duration may have prevented the attachment of these organisms to the marble surface. Finally, in the samples permanently buried in the sediment, marble surfaces were not available for colonization by endolithic sponges (e.g. *Cliona celata*) and certain molluscs (e.g. *Petricola lithophaga*).

Our results indicate that decay through biological colonization was more related to the underwater conditions than to the intrinsic characteristics of the materials themselves (textural and mineralogical features). In condition I, the marble samples were completely exposed to the water column, currents, light and nutrients, and susceptible to epilithic and endolithic biological colonization. This determined that samples showed the greatest percentage of biocover (32-35%) by organisms mostly including barnacles, tube worms, bryozoans, molluscs and coralline algae, as well as the largest area affected by bioerosion, in the form of circular-filament shapes in the first millimeters of marbles. Under condition II, samples placed on the sea bottom were partially buried during some periods of the experiment. This meant that some marble surface zones were periodically covered by sediment, and subjected to light/dark cycles, so they eventually suffered abrasion phenomena, and also biodeterioration but to a lesser degree than the condition I samples. Encrusting organisms such as barnacles, tube worms, bryozoan and coralline algae were detected on the surface, along with micropitting patterns. Finally, the samples buried in sediment, in contact with sand grains and exposed to a dark environment, were more protected from biological colonization. These condition III samples showed the least extent of biodeterioration as superficial deposits, especially from barnacles and tube worms, and boring patterns were scarcer and randomly distributed on the marble surface.

In comparison to the other materials that were submerged simultaneously with marbles (copper, brass, cast iron, carbon, steel, pine and oak) as part of the ARQUEOMONITOR project, exposure conditions were also found to be the main determinants of biological colonization. Thus, materials placed on a hard substrate and

exposed to the water column were more intensely colonized than those placed on the seabed. In addition, biocover percentages varied according to the material tested and revealed that wood was the most colonized of all (Bethencourt et al. 2014, Fernández-Montblanc et al. 2014). In other studies, it was noted that the textural features of the stone materials conditioned the dominant biodeterioration process when these were exposed to a marine environment. La Russa et al. (2015) examined marble fragments recovered from the remains of the ancient city of Baiae (Underwater Archaeological Park of Baia, Naples, Italy) submerged since the 4th century AD due to bradyseism. These authors were able to correlate the intrinsic characteristics of the archaeological marbles, particularly grain size and compactness, with the extent of bioalteration. Although Macael and Carrara marbles used in this study were classified as coarse- and fine-grained marbles respectively, their behaviour in terms of biodeterioration was equivalent and directly linked to the exposure conditions. It should be noted that the archaeological marbles examined by La Russa et al. (2015) were subjected over centuries to underwater conditions so their findings are not comparable to ours.

Similarly, we cannot rule out that in the longer term, the coarse-grained Macael and fine-grained Carrara marbles will not differ in their behaviour according to their textural properties. Crisci et al. (2010) artificially aged natural stone materials, including marbles, occurring in the submarine archaeological site of Crotone (South of Italy) at a depth of 7 m and monitored deterioration forms in two stages (4 months and 1 year). These authors noted greater damage than that observed in our marble samples due to calcareous encrustation and bioerosion induced by endolithic sponges. The prevailing conditions at the archaeological site of Crotone such as depth, light and currents differ greatly from conditions at the Bay of Cádiz, and this could explain the different biodeterioration of materials of similar properties. The greatest expanse of biocover quantified here was about 32-35% of the total surface area, which was approximately a third of the exposed area (13-22 cm^2) of each specimen for condition I. This is a very low rate of colonization in comparison with the results reported by Crisci et al. (2010) for a similar period of immersion. In view of these results, factors such as temperature, the amount of light reaching the seabed, and also the currents and waves in this archaeological area seem not to be conducive to similar biological colonization to that observed at the sites mentioned above.

The value of studies such as the present is that they could help to understand

the degree of biodeterioration of artefacts made of Carrara or Macael marbles in underwater archaeological sites. This information is especially useful for the design of appropriate *in situ* protection and prevention measures against biological colonization without having to compromise the integrity and essence of the cultural heritage. The aim of these actions should be to preserve our cultural heritage in the best possible way in the conditions in which they have persisted for long periods of time. These actions will also make them accessible to the general public through the *in situ* musealization of underwater archaeological sites.

Our results identified the condition of burial in the sea bottom as the least affected by biological colonization, since marbles in this condition revealed the least biocover and scarce micropitting patterns. Other authors have recommended reburial of underwater archaeological objects as an effective preservation method, since it can protect underwater cultural objects against environmental agents of bioerosion, hydrodynamics and abrasion (Davidde et al. 2010; Ricci et al. 2016; Stewart et al. 1995). However, this practice would need to be experimentally tested for each material. Additional prevention measures have been recently developed including new cleaning procedures (Crisci et al. 2010) and nanostructured materials (Ruffolo et al. 2013, 2017) designed to prevent the growth and adhesion of marine organisms, protecting surfaces of underwater archaeological remains.

Lastly, the state of preservation of underwater archaeological objects should be monitored periodically, since underwater conditions can change over the years, and *in situ* prevention and protection measures need to be adapted to each new scenario.

5. Concluding remarks

This multidisciplinary investigation sought to assess biological colonization and associated degradation effects in geomaterials exposed to the marine environment of the Bay of Cádiz for a mid-term of 18 months of submersion.

In this underwater test, we tried to simulate the conditions to which archaeological artefacts found on the seabed are presently subjected. Our results indicate that the biodeterioration (both biofouling and bioerosion) suffered by both Carrara and Macael marbles was strongly influenced by their underwater position. Permanent burial under the physicochemical conditions of the selected site resulted to

be more protective for the marbles. When exposed to open seawater, epilithic benthic organisms and microorganisms apparently preferred to settle on these ground carbonates in addition to those that actively penetrate into them for protection or to obtain the carbon source for their metabolism. In this last condition, the presence of epilithic calcareous deposits of barnacles, tube worms, bryozoans, molluscs and coralline algae, epibiosis phenomena and microbioerosion tracers of endolithic activity indicated an early stage of biological community development (Wahl 1989; Callow & Callow 2002; Casoli et al. 2014). Potential colonizers for the next successional stages may be macroborers including endolithic sponges and bivalve molluscs with a more destructive capacity of the stone materials. The smooth and homogeneous surfaces of the marbles probably may have influenced the settlement and growth of the endolithic and epilithic biological components in the marine environment of the Bay of Cadiz, especially in the initial steps of conditioning of surface and of adhesion of primary colonizers such as bacteria, yeast and diatoms in the fouling sequence. Under the testing conditions, Carrara marble emerged as more resistant at least for 18 months, to the endolithic activity of microorganisms, which was attributable to the presence of dolomite crystals acting as a protective barrier.

The methodology used in the present study proved useful and may be appropriate for the diagnostic analysis of the biodeterioration of underwater cultural objects. Indeed, through a detailed microscopy study of the organo-mineral interface of our samples we were able to determine the real impacts of biological colonization on Carrara and Macael marbles, both as a whole and as individual groups of organisms.

As microboring patterns were not visible to the naked eye, it was only possible to assess the full extent of biodecay by microscopy. In future studies, the organisms and microorganisms should be monitored in time-series to identify, in each stage of biological succession, the associated biodecay and the most damaging biological agents producing them. Furthermore, materials of different mineralogical and textural characteristics need be investigated in the long-term at different archaeological sites, as they could lead to new scenarios of underwater biodeterioration.

Overall, this study has led to a better understanding of the biodeterioration phenomena occurring at micro and macroscopic levels on cultural heritage marbles exposed for a mid-term to the marine environment of the Bay of Cadiz due to the action of epilithic encrusting organisms and endolithic boring microorganisms. Burial of marble

artefacts resulted to be the most protective strategy against the biological action in the natural conditions selected, and thus to be considered as a potential preventive measure for the *in situ* conservation of submerged works of art made of marble.

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