DOCTORAL SCHOOL IN BIOLOGY
Section: Biodiversity and Ecosystem Analysis

XXIII CYCLE

Erosion of carbonatic lithotypes by endolithic organisms under terrestrial conditions: ecological aspects and biodeterioration problems

Erosione di litotipi carbonatici ad opera di organismi endolitici in condizioni subaeree: aspetti ecologici e problematiche di biodeterioramento

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Front cover: pitting deterioration of a natural rock (Photo by V.Lombardozzi).

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To my mother

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PREFACE

This thesis is based on three papers, two published and one submitted to peer-reviewed journals. In the common introduction were briefly addressed the issues related to biodeterioration phenomena, to the peculiar characteristics of endolithic organisms and the overall aims of the thesis. Chapter 2 and chapter 4 correspond to papers in particular:

CHAPTER 2: Lombardozzi V., Castrignanò T., D'Antonio M., Casanova Municchia A., Caneva G. An interactive database for an ecological analysis of stone biopitting. Submitted

.CHAPTER 4:

Caneva G., Lombardozzi V., 2011 – The Erosion of the carbonatic external walls of the "Church of the Virgin" in Martvili (Samegrelo Region, Republic of Georgia) by endolithic organisms. Proceedings of the 2nd Symposium of Georgian Arts and Culture "Georgia on the Crossroads. Cultural Exchange across the Europe and Beyond", Florence November 2-9, 2009.

Caneva G., Nugari M.P., Pietrini A.M., Salvadori O., Tufano M., Lombardozzi V., 2010 - Biodeterioration problems of mural paintings in rock habitat: the prehistoric site of Filiano (Basilicata, Italy). Proceedings of the 4th International Congress on "Science and Technology for the Safeguard of Cultural Heritage in the Mediterranean Basin" (A.Ferrari, E. Sirugo, S. Tardiola, eds), Cairo (Egypt) 6th-8th December 2009.

Chapter 3 deals with the samplings collection and analyses of this reseach project, and with the problematics which have arised in this study.

In the final section, the outcomings of the papers and of the analyses in chapter 3 were utilized for drawing conclusions on the status of knowledge on *pitting* and the organisms related and for discussing further steps to be undertaken in order to make more efficient the protection of Cultural Heritage from biodeterioration.

CHAPTER 1 – INTRODUCTION

CHAPTER 1

Introduction

In the last decades the growing concern for the preservation of Cultural Heritage monuments has led to the understanding that the role of biological matter in their degradation processes, has not to be neglected. Most investigations have stressed the importance of phototrophs in the physical and chemical deterioration of stones. In the Conservation of Cultural Heritage this is called biodeterioration, that is "*any undesiderable change in the properties of a material caused by the vital activities of organisms*". (Hueck, 1965, 1968). The role of these organisms in the deterioration of surfaces of historic buildings has been the subject of several studies but it appear to be not perfectly clear. Their activity in weathering of rocks is considered to occur by means of chemical and physical processes, often deeply connected between them. In many cases, microorganisms are considered one of the main causes of stone deterioration (Warscheid & Braams, 2000). Epilithic and endolithic organisms can potentially contribute to the breakdown of rock crystalline structures. Some investigations in the past decades have suggested that many physical, chemical and biological factors act in both synergistic and antagonistic associations to effect durability of the material (Crispim & Gaylarde, 2005).

Depending on the environmental conditions, bacterial polysaccharides may precipitate calcium carbonate, while some acids may react with calcium from stone to form other minerals. Similarly, oxalic acids can form calcium oxalate on limestone. Solubilization processes, including acidolysis, complexolysis, alkalinolysis corresponding to the formation of acidic, complexing and alkaline metabolic compounds, have been reported (Chen *et al.*, 2000).

Several studies suggest that the chemical processes induced on the stone material by microorganisms are represented by the excretion of corrosive acids especially on limestone and marble, while other studies put forward the hypotesis that precipitation of calcium salts on cyanobacterial cells growing on limestone could favour the migration of calcium from neighbouring sites. Precipitation of poorly ordered iron oxides and amorphous alumino-silica gels, the neoformation of crystalline metal oxalates and secondary clay minarals have been freqeuntly identified in a variety of rocks colonized by lichens (Chen *et al.*, 2000). Some cyanobacteria pose an external layer that binds calcium ions producing local high level of pH and it has been suggested that this repeated mobilization by organisms' cells could lead to degradation of calcareous stone. Moreover also biofilms EPS (extracellular polysaccharide substances) have been observed to increase dissolution rate of calcium carbonate, suggesting that they also may contribute to deteriorating the stone.

In the case of chasmoendolithic cyanobacteria it has been suggested that the contribution to the decay of calcareous stones consists in the exertion pressure from within the stone as a result of water uptake, expansion of cell mass and precipitation of carbonates and oxalates around the cells. The results is the opening of fissures which allow the entry of dust, pollen, grains and small animals and furthermore, the increasing internal pressure on the superficial layer of the structure that may lead to its detachment. (Danin & Caneva, 1990; McNamara & Mitchell, 2005).

Taking a good look it appears clearly that almost all natural carbonate rock surfaces, as well as carbonate building stones are, in various ways, colonized by micro-organisms (Pohl *et al*., 2002). Depending on the way in which these organisms occupy rock surfaces, distinction should be made between epiliths, which live on the surface of the substrate, and endoliths which dwell within the substrate itself. Endolithic organisms, moreover can be divided depending on the way they colonize the inside of the stone in (1) chasmoliths, which adhere to the surfaces of pre-existing fissures and cracks; (2) criptoendoliths, which colonize structural cavities within porous rocks, and (3) euendoliths which actively penetrates into carbonates substrates (Golubic *et al*., 1975) (fig.1).

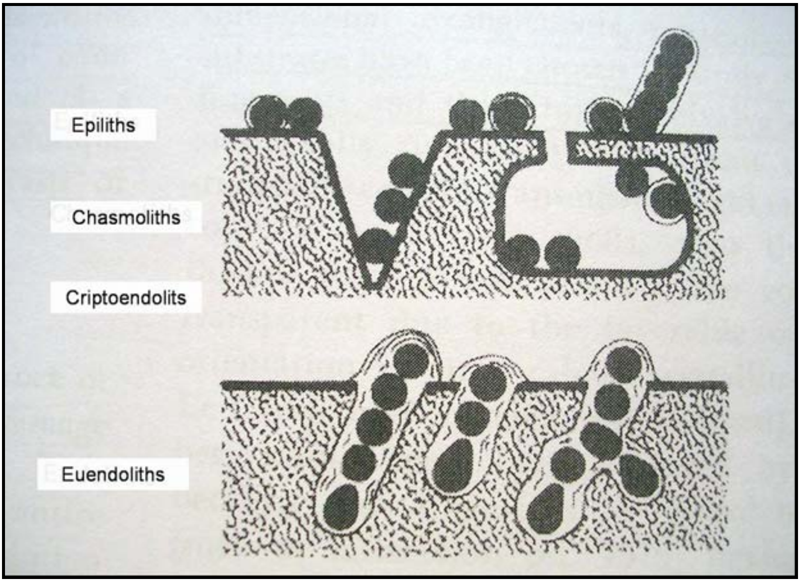

Fig.1 Description of endolithic organisms. (Golubic *et al*., 1975, modified).

The density of colonization may vary as well as the maximum penetration depth and biomass per unit area. Even on surfaces which are strongly marked by chemical corrosion, euendolithic microorganisms have been reported to actively create small cavities where they survive despite the most estreme and adverse conditions. Accordin to Pohl *et al.* 2002, ‘bare rock’ as described frequently in literature, should, at least where carbonate rocks are concerned, be considered rare exception.

Endolithic colonization develops beneath the rock surface and gives rise to complex communities depending on environmental conditions and physico-chemical properties of the material.

The existence of microbial population that dwell on and inside hard mineral substrates, primarily carbonates and phosphates, has been noticed since the 19th century, but the early reports were not clear in distinguish between phototrophic and heterotrophic microboring organisms (Golubic *et al*., 1975).

Endoliths appear to colonize the spaces beneath rock surface which has not been colonized by epilithic communities. It has also suggested that escape into endolithic environment is driven not by hostile surface conditions, but by the decreased competions for nutrines and space. Among the lithophytes, organisms native to the endolithic environment are generally described to be slow-growing, adaptive and tolerant to severe stresses including extreme temperatures, disseccation, oligotrophy and high UV flux (Sigler *et al.*, 2003; Salvadori, 2000).

In the case of photosynthetic microborers they are limited in their extent mainly by the depth of light penetration in the specific substratum where they are found, so that the characteristics of the carbonate rock, physical and chemical, are important factors in favouring or not their development.

Cyanobacteria (blue-green algae) are considered as one of the most important group as pioneer organisms of rock surfaces. Together with them are mostly found eucaryotic algae, fungi and lichens.

Pitting is considered one of the most important deterioration process for what concerns natural and building calcareous rocks under terrestrial conditions and it is characterized by the formation, on the substrate, of pits and holes of variable depth and diamenter. The processes that causes pitting still appear to be controversial. Several factors seem to influence the weathering rate of the substrate by inducing the formation of pits. These, beside the petrographic and physico-chemical parameters which can create differential localized effects, include the morphology and micromorphology of the surface but also the inclination and exposure of the surface and finally the eventual action of stone-carving tools. However a larger consesus has recenlty gained the idea that pitting could be associated with microorganisms activity. Infact, often it is possible to observe that pits are colonized by microbial populations. According to Danin, the respiration processes of an organisms that estabilish itself among rock crystals, accellerates the weathering rate of the substrate near such organisms. This depends on the realease of CO_2 to the water when the rock is wetted, forming the weak acid, carbonic acid (H_2CO_3) which dissolves the rock parts that are in direct contact with the organism. Thus, the coherence of particles to the rest of the rock decreases, and their erodibility by splashing raindrops increases. This, due to the faster weathering processes here than that of the entire surface, would explain the formation of depression in the rock which, otherwise, exposed only to enviromental agents, should be expected to weather at about the same rate all over the surface. In such pits, infact, the transition from the area populated with the supposed pit-inducing organism to the area not populated with it is sharp and the pit walls are steep.

Gehrmann *et al.*, 1992, give a classification of pitting defining micro-, meso- and macropitting:

- Micropits are microscopic holes detectable only via microscopy with diameter on average from 0.5 to 20 µm produced by bacteria and microscopic fungi as single organisms. These pits can join together forming larger micro-pits;
- Mesopits are circular or oval pocket-like impressions in the stone surface, with size that ranges between 20-1000 µm on average and diameter 4-5 times bigger than the depth of ther crater. These appear to be linked with etchings of the fruiting bodies of endolithic lichens as well as of their phycobionts;
- Macropits are deterioration forms with a diameter on average from 1mm to 2cm, and as well as mesopits show a diameter 4-5 times bigger than the depth of ther crater. Their origins hasn't been explained clearly.

However, these definition are far more distant than be considered more than preliminary and as a first step towards a classification of biogenically and abiogenically formed surface changes in building materials. And moreover it is still yet to demonstrate clearly whether the organisms which are found inside the pits actively contribute to their formation or simply they have found in them a more favourable ecological condition. (Caneva *et al.*, 1992; Chacon *et al.*, 2006).

Since their first description endolithic organisms have been observed in a variety of extreme ecosystems including warm and cold deserts and Polar regions. They have been detected also in temperate environments such as the Niagara Escarpment, Canada and in a variety of rock types (Sigler *et al.*, 2003).

Many studies have been performed on endolithic microflora in coastal environment while even though the occurrence of phototrophic microorganisms on stone monuments has been recognised frequently, only few authors have reported the presence of endolithic photosynthetic microorganisms on monuments and often only in general ways not investigating the relationships between the organisms and the nature of the substrate or the ecological environmental conditions.

Besides the number of investigations due to the growing interest in preserving stone monuments from biodeterioration, what is still missing is a comprehensive picture of the knowledge achieved up to now on this subject in order to make possible trying to draw general rules on environmental conditions and mechanisms of colonization.

In fact, it is only through a clear understanding of the detailed endolithic communities structure and composition, of the ecological conditions in the various microhabitats and of the detailed mechanisms and rates of stone decay, that protection from biodeterioration could become more efficient, preventing more than curing the damages.

The aims of this research project have been than to:

- Understand the state of the knowledge on biodeterioration phenomena, up to now;
- Understand the macro- and microclimatic parameters which favour their estabilishment;
- Characterize euendolithic colonization through polyphasic approach;
- Analyses of peculiar case of studies.

CHAPTER 2 – AN INTERACTIVE DATABASE FOR AN ECOLOGICAL ANALYSIS OF STONE BIOPITTING

CHAPTER 2

An interactive database for an ecological analysis of stone biopitting

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Abstract

The literature on stone pitting due to biodeterioration is wide, but the diffusion of this phenomenon and the taxonomy of the organisms supporting it in the different environmental and edaphic conditions are questions not sufficiently described. Therefore an interactive on line database has been built up from the literature concerning the pitting of stone under terrestrial conditions, in order to synthesize the available information and to give a critical view on bibliographic data. Among the about 800 papers selected in the first step, only 24 reported parallel information on the object, the affected material, and the organisms associated to the pitting, necessary to be included in the data bank. These first data are concerning 83 different sites, for a total of 249 samplings, mainly coming from the Mediterranean bioclimatic area, even though the phenomenon is not exclusive to this climate. The most recurrent etiological agents are cyanobacteria, and the associated environmental conditions are dryness, arising from the low porosity of the stone, exposure conditions and from the bioclimate. These factors explain the so high recurrence of marble, the preference for vertical or subvertical surfaces and the high recurrence of Mediterranean and desert climate. The lack of information describing the phenomenon in the whole (stone/exposition/biodeteriogens) has not permitted to use the full potentiality of the interactive data base.

Keywords: stone biodeterioration, pitting, endolithic organisms, cyanobacteria, data base

1.Introduction

Rocks as well as stone buildings materials can be more or less easily colonized by different microorganisms and plants in relation to their characteristics, the surrounding environmental conditions, and the time of outdoor exposition (Ortega-Calvo *et al*., 1995; Warsheid & Brahms, 2000; Pohl & Schneider, 2002; Caneva *et al*,, 2009).

Weathering due to biological agents is however often underestimated when the growth of microorganisms does not form typical coloured patinas and incrustations and it is frequently misunderstood with chemical and physical phenomena. In fact, organisms can colonise and grow on the upper surface of the stone (epilithic) or inside fissures already existing or newly formed by themselves (endolithic), giving rise to exfoliation or pitting phenomena, consisting in the formation of blind holes close together and generally of cylindrical shape (Golubic *et al.,* 1980; Danin, 1992b) (Fig. 1).

The process that causes pitting of stone is controversial due to the difficulty to determine whether the organisms which are found inside the pits have actively contributed to their formation or simply they have found in them more favourable ecological conditions (Salvadori, 2000; Caneva *et al*., 1992).

Whether of biological or abiological origins, pitting causes damages to the stone material that, concerning stone monuments of cultural heritage, could prove to be quite remarkable. Due to this fact, the attention on pitting phenomena and the possible organisms linked to them has increased and consequently several studies concerning its origins, the physiology and ecology of the organisms found inside the pits have been carried out.

Prior to the advent of the internet, biological databases and scientific publications were absolutely separate entities. However, now that scientists use cyberinfrastructure in their daily research, databases and publications continue to be so distinct from each other. There are myriad ways in which the content of an article can be used in a computational manner and the technology and infrastructure to make this happen already exists; this is demonstrated by, among other things, the existence of biological databases and related data mining tools. Indeed, a successful biological database is integration via data mining with other related databases or resources. Yet integration with literature, which is unquestionably the primary medium through which scientists communicate their research, is conspicuously lacking.

Databases help in collecting and analyzing the available data in literature, even if problems arising from the absence of standards on methods of analysis, and the different aims of the various papers often creates difficulties in elaborating data (Caneva *et al*., 1985).

An interactive online database has been already developed for two main purposes:

1) Synthesizing the available information in literature on stone pitting phenomena
2) Giving a critical view on bibliographic data. In particular we wish to find recurrent frequencies of these phenomena, evaluating: a) which are the most favouring environmental conditions, b) which is the most affected kind of stone, c) which are the most common biodeteriogens and d) which are the most significant recurrence among the interacting factors.

Fig.1 a) Detail of SE exposure (drum XI), (replicas of 1862)
b) Different morphology of pits of the Trajan's column

2.Materials and Methods

The design and the development of Biopitting data-base has been based upon a critical survey of the existing literature. In particular, we selected papers responding to the following three requirements: a precise description of the *object* under examination (both stone monuments or stones in natural sites); the description of the *stone* affected by this phenomenon and the report of *pitting* biodeterioration phenomena found and analysed. We also limited, at this moment, the selection to the euendolithic organisms of terrestrial habitat, because they are the most interesting in the biodeterioration of monuments, as described in the literature.

In parallel, we selected the descriptors of the database in order to give a better ecological, biological and technical description of the pitting phenomena and the organisms related: firstly the bibliographical references, then the information about the object, considered as "sampling site" (including constitutive material, geographical location, phisical and environmental parameters). Then we considered the descriptors of each sampling reported, such as the sampling techniques used and the season in which it has been collected, the morphology of the biodeterioration phenomenon (the diameter, depth, shape and chromatic variations of the pits), the rate of weathering and chemical and biochemical analysis applied to it. Finally we searched for taxonomical and physio-ecological information (the type and species of organisms found in the pits, if they are endolithic and are still alive or not and any information about cultural techniques applied) (Fig. 2).

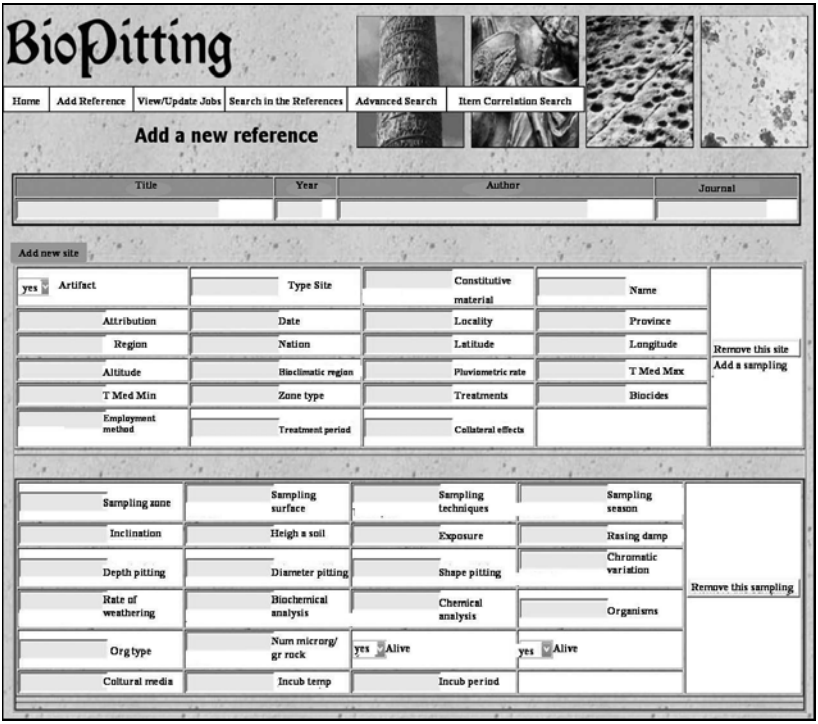

Fig.2 Structure of the database (http://mi.caspur.it/biopitting/biop_home.php)

For every paper selected to be uploaded in the database, it was provided a window in which are reported samples' and samplings' descriptors, which would have been taken by reading accurately each paper.

After filling in the database the chosed papers, we compared some parameters between each other and pointed out some observation concernig their relations, using the possibility given by the database itself to perform queries and download the results in excel tables. Due to the heterogeneity of the information in the database, in order to make some general considerations, we grouped in wider ensembles some of the information reported in it, in particular those about the substrate and the type of organisms. For what concernes the lithotypes we grouped into 4 groups the 18 lithological tipologies that we found in the literature. These are: carbonate rock (which gather together calcitic rock, calcareous litheranite, hard, Lioz and Turonian limestone, Verona red stone and Istrian stone, calcarenite, massive biocalcarenite and dolomia), marble (which includes pentelic and Carrara marbles), granite and concrete.

Moreover we normalize the highly different ways of referring to the organisms found in the pits into only four taxonomic major groups, which are Cyanobacteria, Algae, Lichens and Fungi.

The information concerning the descriptor “Bioclimatic Region”, important under the ecological point of view, have been introduced by us, considering the location of the investigated site.

Biopitting (interactively accessible at http://www.caspur.it/biopitting/) is a relational database available through a dedicated web interface. The database is implemented on a SUSE Linux Enterprise Server (SLES 10) running MySQL 5.1 enterprise. The web interface (written in PHP) runs on a Scientific Linux Server (version 6.1) through Apache server.

3.Results

Among almost 800 papers searched dealing with stone biodeterioration, only 24 have proved to match the three characteristics that we thought of in building this biopitting database. The sum of the investigated sites is however not negligible and data deals with 83 different sites, for a total of 111 sampling zones (i.e. the part of the sampling site were samples have been collected) and a total number of 249 samplings. Among the sampling site, 57 are monuments and 26 are natural sites, as described in Table 1.

Many information were however only scarcely reported, such as stationary parameters, (exposures, inclination), or precise geographical indications such as latitude, longitude, altitude, and climatic data (pluviometric rate and minimal and maximal temperature). When different samplings were described, the information on each sampling seemed to be often incomplete and less detailed. Information about the methodology, techniques and sampling season and also about the sampling zone is scarcely reported.

Moreover, information concerning the use of biocides, type, employment method, treatment period and collateral effects, have never been found.

Monuments	Italy, Rome, Forum Traianum
Greece, Athens,Dyonysos Theatre	Italy, Rome, Forum Traianum, statue
Greece, Corfù, obelisque	Italy, Rome, Pasquino's statue
Israel, Jerusalem, Bet Hakerem	Italy, Rome, St. Stephen Basilica
Israel, Jerusalem, el-Kebkebi Mausoleum	Italy, Rome, Trajan's column
Israel, Jerusalem, Emir Adughdi el	Italy, Trento, Neptune fountain

Kubaki	
Israel, Jerusalem, house's walls (2 sites)	Italy, Venice, Ducal Palace
Israel, Jerusalem, Moslem cemetery (3 sites)	Italy, Sanctuary of Macereto, Visso (Macerata)
Israel, Jerusalem, walls of the old city (2 sites)	Portugal, Lisbon, Jeronimos Monastery
Israel, Maale Efrayim	Portugal, Lisbon, tower of Belem
Israel, Qidron Valley, Grave of Pharaoah's Daughter	Spain, Jaca, Roman Cathedral
Israel, Qidron Valley, Zacharia's grave	Spain, Segovia, Convent of Santa Cruz la Real
Israel, Sede Boqer, northern Negev Highlands	Turkey, Didim, Temple of Apollo
Israel, Tomb of Pharaoh's Daughter	**Natural sites**
Italy, Agrigento, Concordia Temple	Antarctica, McMurdo valley, Commonwealth Glacier
Italy, Catania, balaustrade, S. Francesco d'Assisi	Antarctica, McMurdo valley, Goldman Glacier
Italy, Catania, rail’s arches, Pacini Villa	Antarctica, McMurdo valley, Mont Falconer
Italy, Catania, Bianchi’s Confraternity	Australia, Naumburg Nat. Park, Pinnacles desert
Italy, Catania, Archbishopric	Egypt, desert of Sinai
Italy, Catania, colonnade, Mazzini Square	Greece, Crete cliffs
Italy, Catania, Fac Liberal-arts Faculty/ Unversity	Greece, Crete, wetter areas
Italy, Catania, fountain “the kidnapping of Proserpina”	Hungary, Bukk Mnt
Italy, Catania, fountain, Botanical garden	Israel, Jerusalem, desert
Italy, Catania, main facade, S. Placido	Israel, Jerusalem, hilltop
Italy, Catania, Benedectines Monastery	Israel, Jerusalem, near Hebrew University
Italy, Catania, stock market Palace	Israel, Jordan Valley N of Jericho
Italy, Catania, porch, De Felice Inst	Israel, Lake Kinneret
Italy, Catania, Garibaldi town’s gate	Israel, Mediterranean Israel cliffs

Italy, Catania, Uzeda town’s gate	Israel, Negev Highlands
Italy, Catania, General Post office	Israel, SE Judean Desert
Italy,Catania,rectorate/arcades, University	Israel, Southern Negev
Italy, Catania, S. Camillo	Italy, Carrara, quarry (2 sites)
Italy, Catania, staircase, Greek theater	Italy, Trieste karst (2 sites)
Italy, Catania, SS. Trinità	Namibia, Namib Desert, NE of Wlotzasbaken
Italy, Messina, statue	Namibia, near Swakapmund, mist desert
Italy, Ragusa Cathedral's fountain	Turkey, Belevi, Ephesus, quarry
Italy, Rome, Caestiu's pyramid	
Italy, Rome, capital Santo Eustachio street	

Table 1 Geographical location of the 83 different sites considered in the biopitting database

Most of the sampling sites described in the literature come from the Mediterranean Basin and are distributed in 10 countries for a total of 47 geographical localities (i.e, municipalities).

In particular, 67 sites are included in the bioclimatic Mediterranean region (87% of samples) while only 7 in the Saharian one (4 %), 6 in the Temperate zone of Eurasia (5,4%), and 3 in the Polar one (2%).

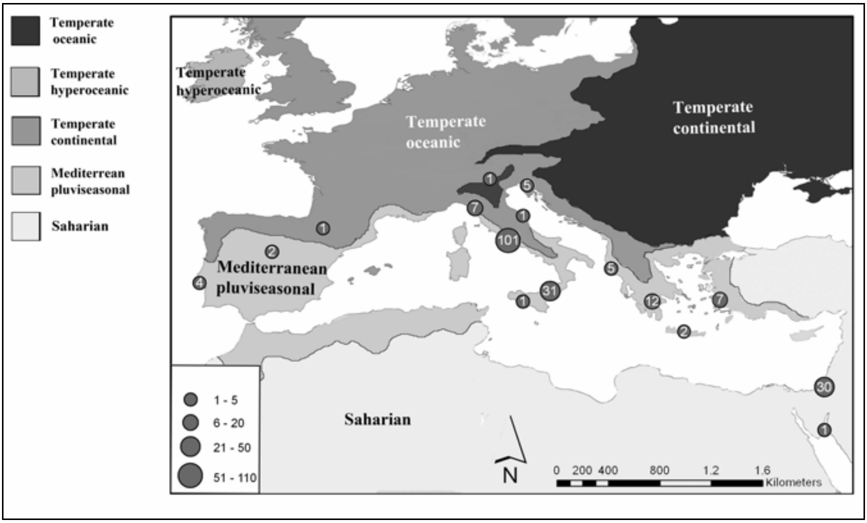

Fig 3. Location of the 83 case studies on stone biopitting used data, corresponding to 47 municipalities (14 in Italy, 18 in Israel, 4 in Greece, 2 in Namibia, Turkey, Spain and Portugal, 1 Australia, Antarctica and Egypt) (Worldwide Bioclimatic Classification System, 1996-2009, S.Rivas-Martinez & S.Rivas-Saenz, Phytosociological Research Center, Spain. http://www.globalbioclimatics.org)

Looking at the selected case studies and precisely in those where data on inclination of the surfaces were reported, the phenomenon was mainly described for vertical ad subvertical surfaces showing a certain preference for the Southern expositions (Fig. 4).

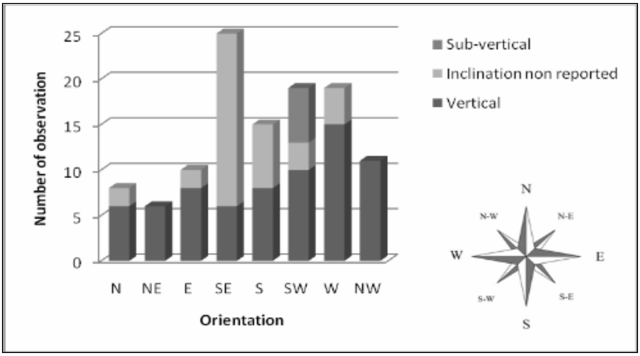

Fig 4. Expositions of the sampling sites

Moreover, according to the information collected in the database, the stone that is reported to be more frequently in association with this kind of stone deterioration, is marble, counting to the 53% of the total number of samplings, followed by carbonate rock with 44%. The remaining 3% is referred to granite and concrete (Fig.5a).

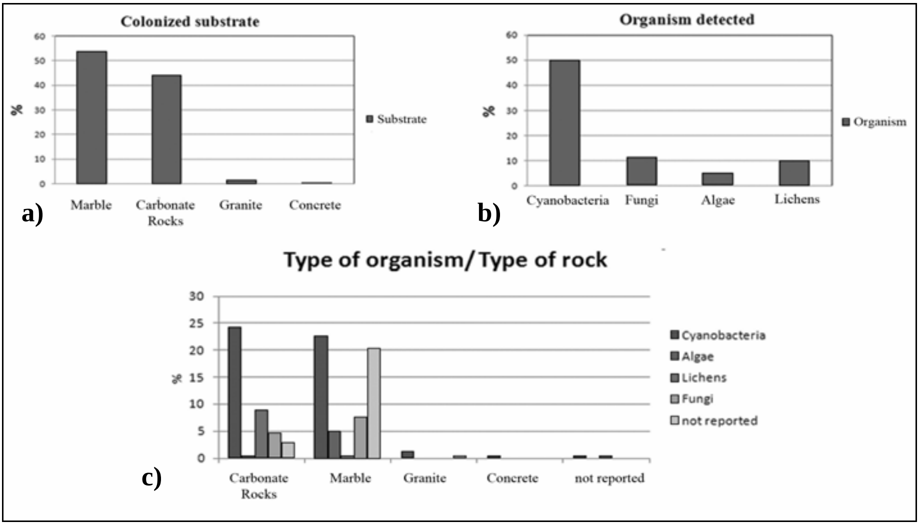

Fig.5 a) Type of substrates affected; b) Frequency of the organisms detected c) Frequency of the organisms on the different substrates

According to the papers uploaded in the database, cyanobacteria, with 50%, are the most commonly described biodeteriogens found in association with pitting, followed by fungi, lichens (respectively 11% and 10%) and algae with 5%. The remaining 23% of the total number of samplings refers to some case of studies in which no precise indication of the type of organisms found in the pits sampled is reported (Fig. 5b).

Looking at the organisms in relation to different kind of stone, on carbonate rocks we found also the most evident recurrence of cyanobacteria (27%) such as for marble, but in lower amount (22 %), probably due the highest number of not reported identification. Lichens in particular are most frequently mentioned on carbonate rocks than on other material (Fig. 5c).

The species collected from pittings, considering the relative numbers of citations and the papers dealing with them, are listed in Table 2. It shows again that cyanobacteria have the highest biodiversity of collected taxa, and many species are mentioned as endolithic (e.g. *Hyella* and *Lichenotelia),* but in other cases, especially when only the genus is detected, it is difficult to interprete if they were epi- or endo-lithic.

Cyanobacteria	**n° Tot presence**	**n° papers**	**n° sampling sites**
Aphanocapsa muscicola (Meneghini) Wille	1	1	1
Aphanocapsa roeseana de Bary	3	1	2
Calothrix marchica Lemmermann	1	1	1
Calothrix marchica Lemmermann var. *crassa* Rao	3	1	1
Chroococcus minor (Kutz) Nageli	1	1	1
Chroococcidiopsis sp.	1	1	1
C*lastidium setigerum* Kirchner	1	1	1
Cyanothece sp.	1	1	1
Dalmatella sp.	1	1	1
Entophysalis deusta (Meneghini) Drouet et Daily	4	1	3
Entophysalis rivularis (Kutzing) Drouet	21	1	20
Entophysalis sp.	1	1	1
Gleocapsa alpina	1	1	1

Gloecapsa biformis Ercegovic	3	1	2
Gleocapsa calcarea Tilden	2	1	2
Gleocapsa sp.	2	2	2
Gloeothece rupestris (Lyngbye) Bornet in Wittrock et Nordstedt	1	1	1
Hyella sp.	1	1	1
Lyngbya aff. *limnetica*	1	1	1
Microcoleus chthonoplastes Thuret ex Gomont	1	1	1
Microcystis sp.	3	1	2
Myxosarcina sp.	1	1	1
Myxosarcina spectabilis Geitler	3	1	1
Myxosarcina concinna Printz	9	1	2
Phormidium foveolarum Gomont	6	1	2
Plectonema radiosum (Schiederm.) Gomont	2	1	2
Plectonema sp.	1	1	1
Pseudocapsa dubia Ercegovic	1	1	1
Pseudocapsa sp.	1	1	1
Schizothrix sp.	1	1	1
Stigonema muscicola Borzì ex Bornet et Flahault	1	1	1
Synechococcus sp.	4	1	2
Synechocystis sp.	2	2	2
Symploca dubia Gomont ex Gomont	1	1	1
Tolypothrix byssoidea (Berk.)	2	1	1
no spp	33	13	

Fungi	n° Tot presence	n° papers	n° sampling sites
Cladosporium sp.	1	1	1
Diplodia sp.	1	1	1
Lichenothelia intertexta Henssen	1	1	1
Lichenothelia globosa	1	1	1
Lichenotelia sp.	3	2	3
Ochroconis sp.	1	1	1
Phoma sp.	3	1	3
Trichoderma sp.	1	1	1
Ulocladium sp.	3	2	3
Coniothyrium sp.	1	1	1
Alternaria sp.	1	1	1
Hormonema sp.	1	1	1
no spp	13	5	
Lichens	n° Tot presence	n° papers	n° sampling sites
Buellia peregrina Bungartz & V.Wirth	1	1	1
Caloplaca alociza (A. Massal.) Mig.	5	2	2
Caloplaca sp.	1	1	1
Encephalographa elisae A. Massal	1	1	1
Petractis clausa (Hoffm.) Kremp.	1	1	1
Verrucaria baldensis A. Massal	1	1	1
no spp	13	3	

Algae	n° Tot presence	n° papers	n° sampling sites
Chlorococcum sp.1	3	1	2
Chlorococcum sp.2	4	1	2
Haematococcus pluvialis Flotow	1	1	1
Heterococcus caespitusus Vischer	1	1	1
Stichococcus bacillaris Nageli	1	1	1
Ulothrix sp.	1	1	
no spp	3	2	1

Tab 2 List of the species cited in the literature

Looking at the recurrence among the factors, we can observe once again the highest recurrence of the binomial (Mediterranean/cyanobacteria) and of carbonate and marble stones (Fig. 6). In particular we can also note that data on marble come only from the Mediterranean climate, and we have no other data on this material in other climatic context. In the case of carbonate rocks we have the highest wideness of recurrence in the different climatic contexts, but in all the conditions cyanobacteria are the most recurrent group. Lichens show in any case a not negligible recurrence.

In order to have stronger data we need to improve the information, considering case of studies coming from wider contexts.

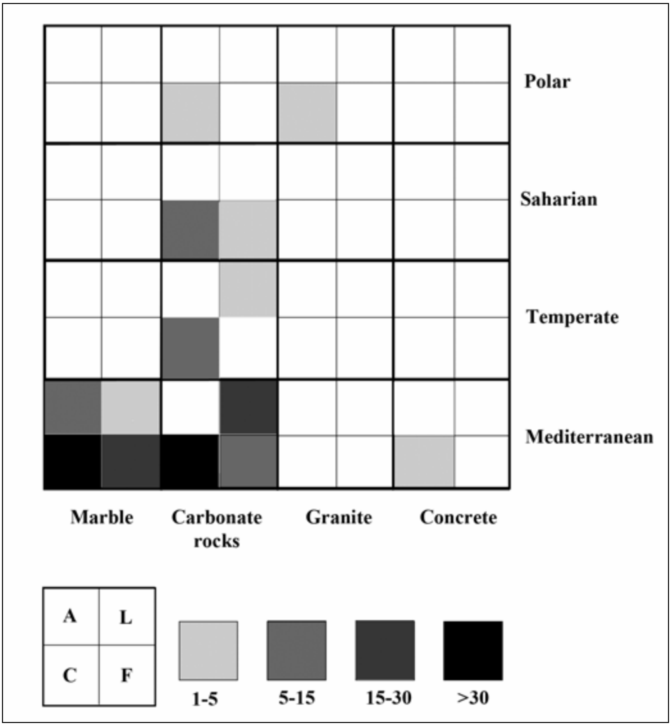

Fig 7 Distribution of the organisms detected, on different substrata in each bioclimate. The gray scale indicates the frequency of the organisms C=Cyanobacteria; A= Algae; L=Lichens; F=Fungi
White boxes indicate the absence of organisms

4. Discussion

4.1 Environmental conditions & stone biopitting

The predominance of the bioclimatic Mediterranean region in the selected samples probably does not reflect the real distribution of the phenomenon, because in the most general literature, that we excluded for the database due to the absence of the necessary requirements, many other localities are listed. As for example we can mention the diffusion of the phenomenon on the Dalmatian coasts (one of the first place of description due to Golubic *et al.,* 1975) or also in different Italian calcareous cliffs. Moreover the phenomenon is observed on exposed rocks both in cold and in hot deserts, as referred by many Authors. We also observed biopitting phenomena in temperate climate on limestone of the walls of a Georgian church near the Black sea (Caneva *et al.* in press) and N Europe, such as in Dresden (Germany). Even in tropical countries, such as Mayan calcareous monuments in Mesoamerica, stones colonized by endolithic cyanobacteria are sometimes detected. In those papers where the ecological conditions which favour this phenomenon are discussed, a certain climatic or edaphic xericity seems to be recurrent, as a common factor characterising the phenomenon.

The data of exposition and inclination of the surfaces suffering biopitting seems to stress a certain linkage of this phenomenon with conditions of dryness, because obviously as much the surface is vertical as less easily water is retained. Moreover southern exposition are more subjected to exsiccation, even if in case of Rome, vertical surfaces with this exposition are the only ones that receive water by the incident rainfall (Caneva *et al*., 1992), constituting in any case an extreme condition of xericity.

4.2 Stone & pitting

Marble is often described as the most affected material, but also calcareous rocks can be greatly weathered by this phenomenon. Looking at the literature in general, we need however to observe that a precise classification of stone type is one aspect in which a gap of knowledge exists in the studies on biodetetioration even if it is, on the contrary, an important passage to understand how the susceptible different types of stone react in different environmental conditions.

4.3 Pitting & organisms

Cyanobacteria are the dominant group associated with pitting, but for a more precise description of the phenomenon we have to consider that in general, the taxonomical information show a different level of definition. On the whole of the 249 samplings which have been uploaded in the database up to now, 99 go into species details while 45 give only indication on genus level, 62 stop at a general description as type of organism. For what concerns the remaining samplings, there is not a clear correspondence between each sampling and the organisms that are mentioned in the papers, so that it could prove to be arbitrary an attempt to assign a specific species to a single sampling.

Also in this case, we need to stress that another important point of heterogeneity which comes out from the bibliographic survey is the approach to the study of the diversity of the biological agents which may be found on stone. Here it seems that the differences reach the highest point. Qualitatively the organisms found are reported at all systematic levels from kingdom to single species so it results to be difficult to have a general view of the distribution of the different kinds. On the other hand quantitatively data (e.g. microorganisms /gr.stone) are generally not reported. Moreover it appears that, depending on the specific knowledge of the authors, are detected and thus reported only some kind of organisms, leading to a possible risk of artificial account of the biodiversity of the microbial communities growing on the site.

5. Conclusions

The existing bibliography on this subject is extremely heterogeneous, depending on the scientific approach used in studying this deterioration phenomenon.

Therefore, the data arising from the literature do not permit to use all the potentiality of the interactive database, due to the heterogeneity in the approach to the analysis of this phenomenon and to the missing of some information in many interesting papers.

The need of a normalization of the approach as suggested by many Authors trough national (NORMAL) and international (CEN/346, Conservation of Cultural Property) working groups on stone conservation comes with high relevance from this database.

Generic papers on stone deterioration focused on taxonomy or biochemical processes related to pitting could prove useful under a systematic or physiological point of view, but they usually do not report a series of information useful to give a defined ecological description of the phenomenon. Many papers report synthetic information of different cases without exact sampling references, which are useful to define the ecological niche of the associated microorganisms.

At the moment we can however evidence as the most recurrent etiological agents seems to be cyanobacteria, that the most recurrent environmental conditions are dryness, arising from the low porosity of the stone (the reason of such high recurrence of marble) and also from the exposure conditions (the reason of the preference for vertical or subvertical surfaces), and from the bioclimate (the reason of the high recurrence of Mediterranean and desertic climate).

For the future, a database which storage all these information will be made available on the network in order to serve as a useful tool in the field of conservation of cultural heritage.

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CHAPTER 3 – CHARACTERIZATION OF EUENDOLITHIC ORGANISMS AND WEATHERING PROCESSES

CHAPTER 3

Characterization of euendolithic organisms and weathering processes

3.1 Sample sites selection

The sampling sites represent macro-areas characterized by different bioclimatic conditions. These are: Georgia (Martvilli), Italy (North) (Hebrews' cemetery of Venice), Center and South Italy (archaeological sites of Rome such as Pantheon, Bernini's elephant statue and Mercati Traianei, the cliffs of the Amalfi Coast and the archaeological site of Filiano, Basilicata); Israel (Negev desert).

The case of studies of the deterioration of the Curch of the Virgin of Martvili and of the archaeological site of Filiano, will be described in details in Chapter 4, due to the fact that there already have been published some papers about the subjects.

3.1.a Church of the Virgin in Martvili, Georgia

The Church of the Virgin in Martvili (7th- 19th century) is one of the most significant monuments in Samegrelo (Western Georgia), and see of the Episcopal Chair of the Georgian Orthodox Church from its origin (10th Century).The climate of the Martvili area shows a Temperate-Boreal Macrobioclimate and an Oceanic - Semicontinental Bioclimate, with superior Thermo-moderated Termicity index and inferior Humid Ombrothermic index. The main lithotype of the exterior stone cladding of the church is represented by a fairly soft limestone.

3.1.b Hebrew's cemetery tombstones, Venice, Italy

We select Venice as a sampling location because, according to the Rivaz-Martinez Bioclimatic classification (Rivas-Martinez & Rivas-Saenz, 1996-2009), Venice belongs to the Temperate Macrobioclimate and the Oceanic Bioclimate. Samples have been collected from the tombstones of the Hebrews cemetery and sent to us by Dott. Salvadori of the Soprintendenza per i Beni Artistici e Storici of Venice. The stone is an Istrian stone and appears of light grey colour.

3.1.c Carrara’s Quarry, Massa-Carrara, Italy

Dott. Salvadori has also collected the samples coming from the Carrara’s marble quarry. This sampling location shows a Mediterranean Macrobioclimate and a Mediterranean Pluviseasonal Oceanic Bioclimate. Stone is marble and appears grey, dark grey or black in the different samples due to the presence of black material which surrounds occasionally calcite crystals which also show a high decohesion.

3.1.d Archaeological sites of Rome

Rome has a Mediterranean Macrobioclimate and a Mediterranean Pluviseasonal Oceanic Bioclimate.

We collected samples in different archaeological sites in the urban area of Rome, in order to understand if, within one macroarea, it is possible to detect some diversity in the colonization.

We than sampled a pitting phenomenon on a column plinth in the Traianus Market. The plinth is positioned in an open area and only at the NE exposition there is a *Nerium oleander* plant at 1,30m of distance. The side more affected appears to be the SW exposed with an encreasing of the pitting concentration in the upper and left part of it. The less affected is the side which is screened by the oleander, while the W, NW and SE esposition show scarce but clear and deep pits.

We sampled also some pitting phenomena, on the Western and Estern sides of the Pantheon and on the Bernini’s elephant statue in the nearby square, on which the phenomenon appears all around the plinth.

3.1.e Calcareous cliffs of the Amalfi Coast, Italy

Also the Amalfi Coast has a Mediterranean Macrobioclimate and a Mediterranean Pluviseasonal Oceanic Bioclimate. Here we sampled in three different coastal location, natural rocks with almost vertical inclination. The one coming from Furore has W-NW exposition and the stone appears close-grained. The samplings from Erchie have been collected from South exposition from rock which flakes easily as it happened with the stone in Capo d’Orso from where are the last samplings collected, in W-NW and S expositions.

3.1.f The prehistoric site of Filiano (Basilicata, Italy)

The Rinaldi's shelter (Filiano) is located in the State's anthropological reserve placed in the Apennine hilly belt, in the South of Italy (Potenza, Basilicata Region). The shelter, facing South, surrounded, still nowadays, by a thick covering of mixed oaks forest, has an optimal exposure concerning the thermal values of this quadrant, but also suitable to offer a certain protection from the prevailing winds, as those associated with rainin. The rock paintings of Palaeolithic age, portraying deers and perhaps arboreous symbols too, are situated on a subvertical wall made of calcareous sandstone rock.

3.1.g Negev desert, Israel

Samples from Israel have been collected and sent to us by Professor Avinoam Danin of the University of Jerusalem, and are all natural rocks. The first is from Argaman site, between Jericho and Tiberia, which records 300mm annual pluviometry. The sampling has been collected on a rock block, on the South facing side, slightly inclined.

The second is from the Negev Highland, at Rakhama Crest near Yerokham. The mean annual rainfalls here is 100mm, the stone is soft and highly crystalized. The sampling has been collected on rock outcrop South facing and gently inclined.

The third sampling is from the Dead Sea, at the Ruins of Khovart, Medin. The average rainfall here is 100mm, the stone is hard limestone and the sampling has been collected from a rock block South facing and gently inclined.

3.2 Samples collection

Samples are represented by scales or chips where it is possible or, otherwise, in case of monuments where the removing of fragments, whatever little, could damage the art piece, they are representend by powder collected from the colonized surface with an aseptic scalpel, even though this will exclude inevitably these samples from morphological analysis of the deterioration. This is the case of the archaelogical monuments of Rome.

While sampling some parameters have been always recorded. These are: the cover percentage of deterioration phenomena and characteristics;

stationary parameters (extension area of the sampling surface, exposure and surface inclination, heigh from soil, presence of vegetation in the surroundings); where possible, time since stone exposure, also.

3.3 Samples analysis

The collected samples have been analyzed under several point of views and using different methologies in order to try to asses the stone characteristics, the kind of organisms involved in the phenomena and the relationship between these organisms and the stone substrates.

3.3.1 Petrographic analysis

Some petrographic analyses have been carried out. Stone density have been measured using an helium pycnometer (AccuPyc 1340 micrometrics GAS Pycnometer). Helium pycnometer allows to measure the volume and true density of solid objects, without damaging samples, assuring maximum accuracy penetrating the finest pores. With helium are filled also the smallest pores (pore size 1E-10 m). So the density of powders and porous materials can be determined.

We than measured, with the use of a mercury porosimeter (Poremaster Quantachrome) the stone porosity (by means of total porosity, specific surface area/ porosity and pore-size distribution) (Tab.1). Both these substances do not interfere with stone nature.

	DENSITY	**POROSITY**
Martvili	2,23 g/cm^3	23,28%
Negev 3-4	2,41 g/cm^3	0,45%
Negev 2	2,33 g/cm^3	8,72%
Negev1	2,35 g/cm^3	4,00%
EN1	2,38 g/cm^3	3,40%
EN9	2,14 g/cm^3	1,75%
EN3	2,44 g/cm^3	2,91%
EN10	2,06 g/cm^3	1,36%
EN5	2,51 g/cm^3	2,21%
Furore1	2,32 g/cm^3	0,61%
C.d'Orso2	2,53 g/cm^3	3,51%
Erchie	2,52 g/cm^3	5,22%

Tab.1 Density and Porosity values of the samples collected

Density and porosity are considered two important parameters in order to classify stone types more clearly for deterioration purposes.

Tomaselli *et al*, 2000, have stressed the importance of uneven surfaces and porosity in favouring the susceptibility to colonisaiotn by photosynthetic microorganisms.

In particular, the specific area/porosity/pore-size distribution together with density measures are considered important properties known to influence the stone's susceptibility to agent of damages such as sulphate deposition, salt cycles and freeze-thaw cycles.

3.3.2 Samples polyphasic cyanobacterial characterization

In this research project we focused our attention on the systematic characterization of the cyanobacterial communities found in association with the deterioration phenomena.
Modern research in cyanobacterial taxonomy and phylogenetic classification it is based on what is called a “polyphasic” approach which combines modern ultrastructural, ecophysiological, and molecular characters with knowledge from the previous 150 years research of cyanobacterial morphological diversity (Komarek, 2006).
The combination of both molecular and morphological approaches for modern cyanobacterial taxonomy is considedered essential because while molecular studies are considered more predictive and a precise phenotype identification requires considerable time and experience, it is still necessary for the correct evaluation of the ecological function and the morphological variability *in situ,* the ongoing adaptational processes and the continual origin of new cyanobacterial eco- and morphotypes.
In order to acheive a genetic characterization by means of a phylogenetic analysis, starting from environmental samples we selected those which, after observation through optical microscope, appeared to contain more biomass. We than, proceeded through the molecular sequencing methodology using two different protocols: the UltraClean TM Microbial DNA Isolation Kit and than the improved methods based on xanthogenate-SDS nucleic acid isolation (XS) (Tillet & Neilan, 2000; Yilmaz & Phlips, 2009). Both of these didn’t succeed in isolating sufficient DNA to be sequenced.
After revitalizing the samples in water, these have been sowed on Petri dishes to be grown on Agar soil enriched with BBM medium. This in order to obtain species segregation to perform a proper isolation. Plates have been placed at room temperature under constant light provided by fluorescent lamp 40w. After two month, recognizable organisms have been sowed separately on Petri dishes with Agar soil enriched with BBM medium. We tried also to sow those samples from which apparently no organisms developed in Petri dishes containing Agar soil enriched with a different medium, that is BG11, to see if this would have made some differences.
From 43 starting samples we than obtained 86 plates. These have been transferred at the University of Rome, “RomaTre”, and placed in a phytotrone cabinet to grow at non-stop light provided by fluorescent lamps and controlled temperature. While checking them we happened to observe

mould growth in some of the plates; thus we resowed the samples of the moulded plates and decided to move all the dishes in a position in which the condition would have been more similar to those in which they have been left to grow the first time.

Notwhitstanding these efforts, we didn't succeed to obtain sufficient biomass to perform DNA isolation and amplification in order to run a phylogenetic analysis.

It has to be said that the traditional isolation techniques were developed in the area of aquatic microbiology and extended to terrestial habitats. The extreme conditions in which endolithic organisms develop are not easy to recreate under artificial situations, thus the changing of some parameters could determine the failure of growing. Moreover the artificial conditions could favour other more competitive and fast growing organisms thus leading to the possibility of obtaining the growth of different communities which do not represent the natural colonizators. It has been observed (Crispim *et al.*,2003; Sigler *et al.,* 2003) that such methods can result in the detection of artificially low numbers, due to the presence of inhibitory and predatory organisms, such as fungi, bacteria and protozoa. It is apparent that many cyanobacteria species which grow in dry environments are lost in culture because of the activity of fungi and it has been recognized that many kinds of microorganisms present in the environment are not detected by common culture techniques. Thus the diversity of strains detected in a population can be reduced by selective culture conditions. It has been reported also the artificially low phototrophic biodiversity after the use of these traditional culture and enrichment techniques. Moreover coccoid cyanobacteria are considered difficult to isolate in unicyanobacterial cultures which are necessary in order to identify strains basing on their nucleic acid sequences. Finally, some cyanobacteria produce exopolysaccharide sheaths or capsules to which other eubacteria attach, making the isolation of these species in axenic culture even more difficult.

We than decided to try to characterize the organisms using only the morphological based methodology.

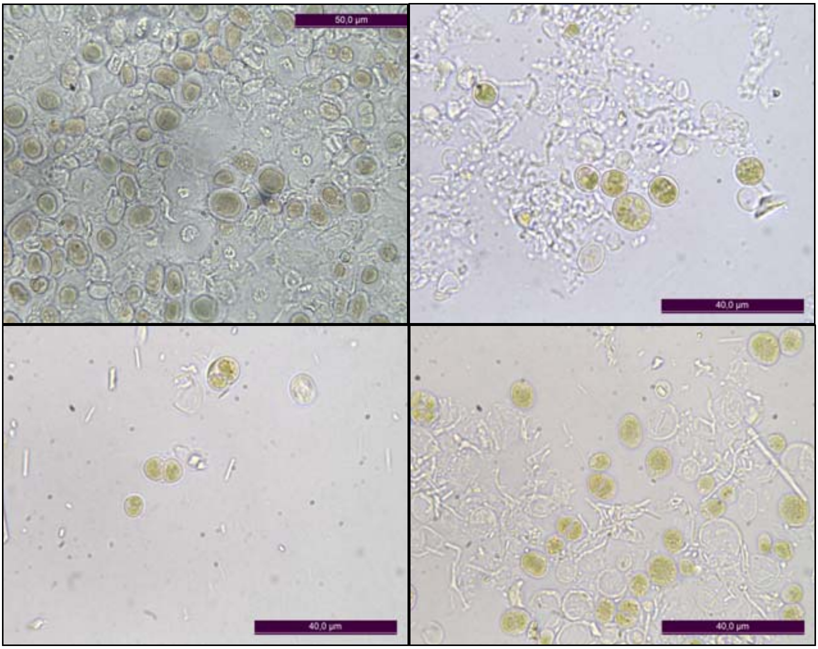
The morphological characterization of the cyanobacteria communities have been carried out following in our observation the determination keys based on cell morphology (Komareck & Anagnostidis, 2008) through observation under microscope with immersion objective at 100x magnification. We also photographed the organisms oberved at 20, 40 and 100x magnification producing a wide photographic documentation.

The possibilty to rely only on morphological approach, though, has limited the confidence of our identifications. Firstly because often the cell

structures on which species discrimination is based, are not easy to recognize. Secondly, because this methodology needs all the growth stages of the studied organisms to be observable in the culture plates.
Due to what just said, we obtain a satisfactory species identification of only 2 cyanobateria species among the observed.

Cyanothece aeruginosa (Nageli) Komarek 1976

Found in association with pitting phenomena in Martvili, Negev desert, and Amalfi coast.

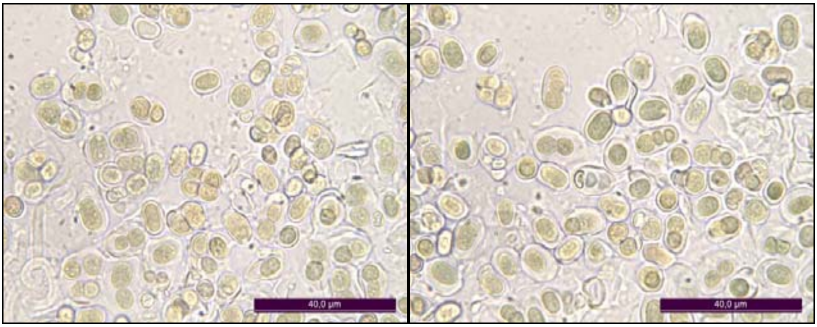


In Komarek & Anagnostidis, 2008 is described as follow.
Cells solitary or in twos during division, oval to shortly cylindrical with widely rounded ends, blue-green, rarely yellowish, with finely granulate content and usually visible reticulately keritomized chromatoplasma, (9) 10-45 (50) x (7) 10-30 (36) µm. Sometimes a narrow fine colourless gelatinous layer around the cells.

Occurence: Freshwater, in clear, cold moorland waters, raised bogs, on wet rocks and in swamps (pH below 7), rarely secondarily in cryoseston, from lowlands to mountains; probably cosmopolitan in ecologically corresponding biotopes, particularly in both temperate and circumpolar zones (whole Euroasia, N. America, Australia, Argentine, Kerguelen Islands, New Zeland, Antarctica, etc.); data from other biotopes (thermal waters, salines, crusts and dry areas, polluted waters) and from tropical regions are uncertain and usually related to other species.

Gloeothece palea (Kützing) Rabenhorst 1865

Found in association with pitting phenomena in the archaeological site of the Traianus Market.



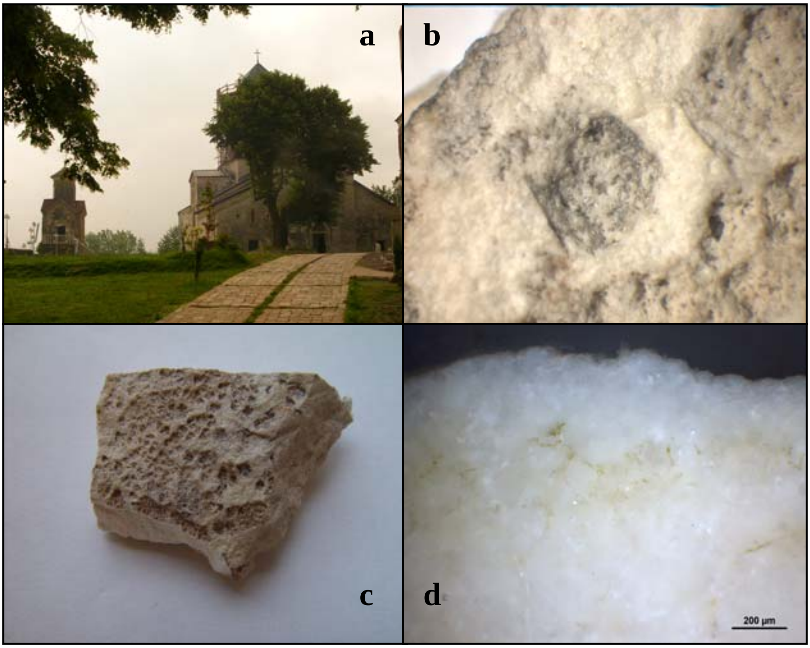
In Komarek & Anagnostidis, 2008 is described as follow.
Rarely sheatedsolitary cells, usually small, regular colonies of 2-4 (many) cells, united into gelatinous, dirty blue-green macroscopic agglomeration. Envelopes around cells distinclty delimited, more or less wide, not lamellate or only with 1-2 indistinct layers, colourless or slightly yellowish. Cells oval to cylindrical, blue-green but sometimes very pale, with homogeneous content, 3.8-13.5 x 2.5-4.5 µm.
Occurrence: Aerophytic and atmophytic, on wet rocks and walls, among mosses, in the vicinity of thermal springs, rarely on wet soils; known from the whole European temperate zone; aquatic or various tropical habitats recorded from Burma, Brazil, etc., probably concern other species.

3.3.3 Samples morphological analyses

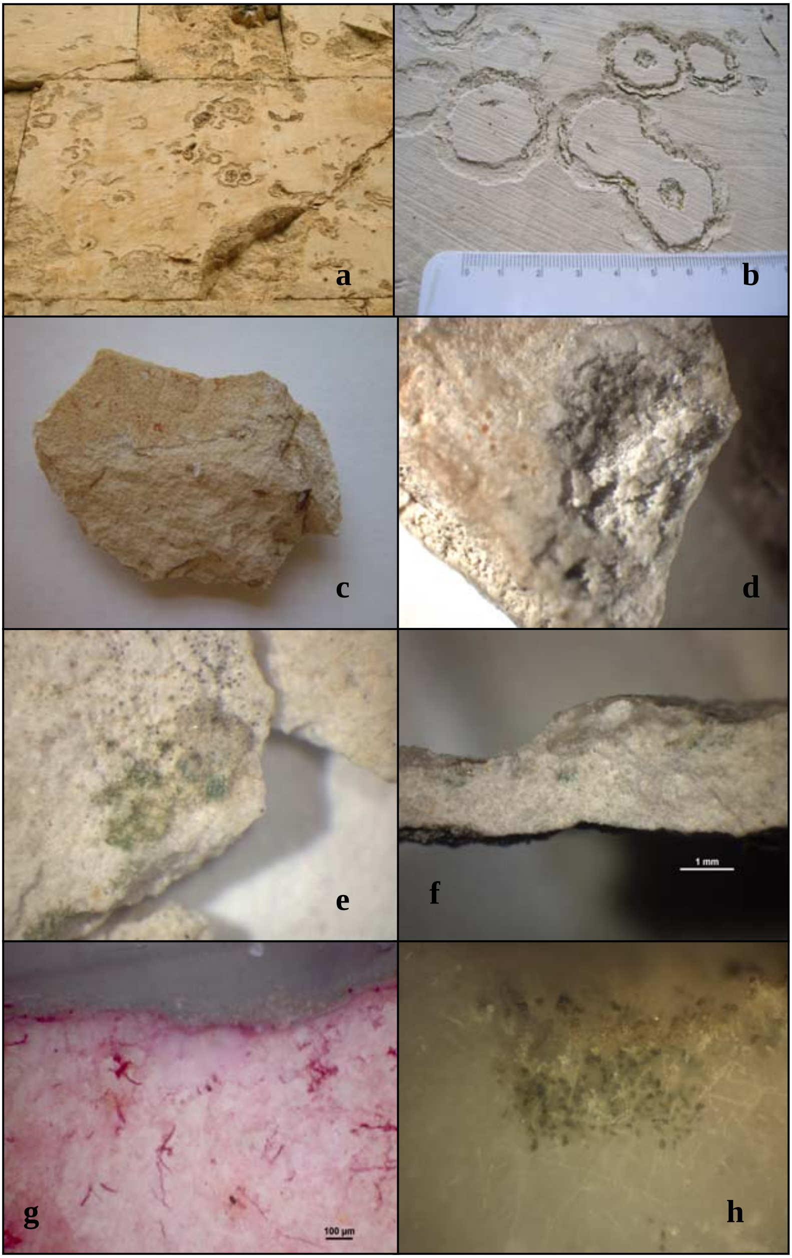
All the sample fragments and scales that have been possible to collect, have been observed and photographed with the use of a stereo microscope both on the superficial surfaces and on the sides. Parts of the samples fragments have been used in preparing polished cross sections. These in part, than have been observed as they are under a reflected light microscope and in part have been stained with the PAS staining method following UNI procedure (NORMAL).

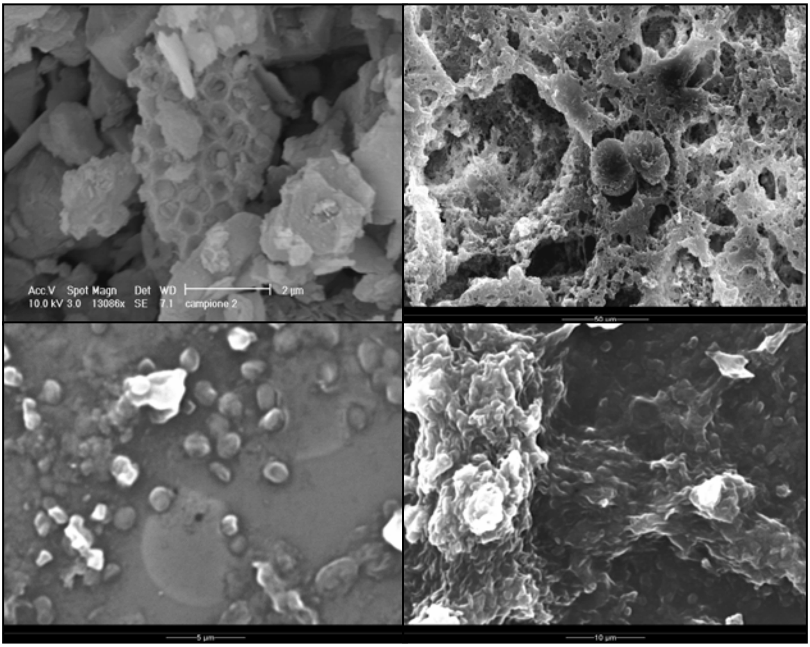
Other fragments have been solved using HCL solution which removes the calcareous matter. Finally a part of the remaining fragments have been observed and photographed with the use of electron scanning microscopy.

Church of the Virgin in Martvili, Georgia



Church of the Virgin of Martvili – *Sampling site (a); stereo photographs of pitting phenomena detected (b,c); polished cross section (d)*


a
b
c
d
e
f
1 mm
g
100 µm
h

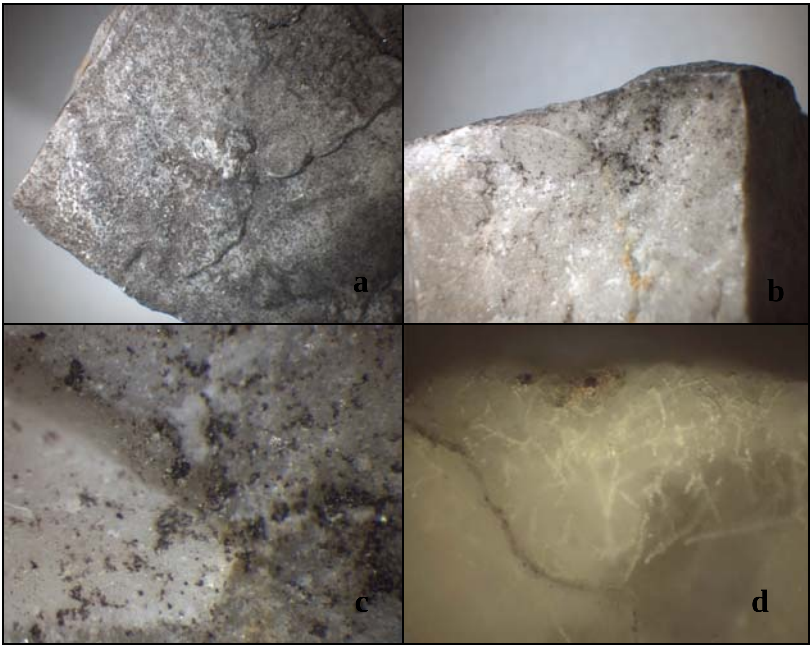


Church of the Virgin of Martvili - *SEM photographs of the pitting*

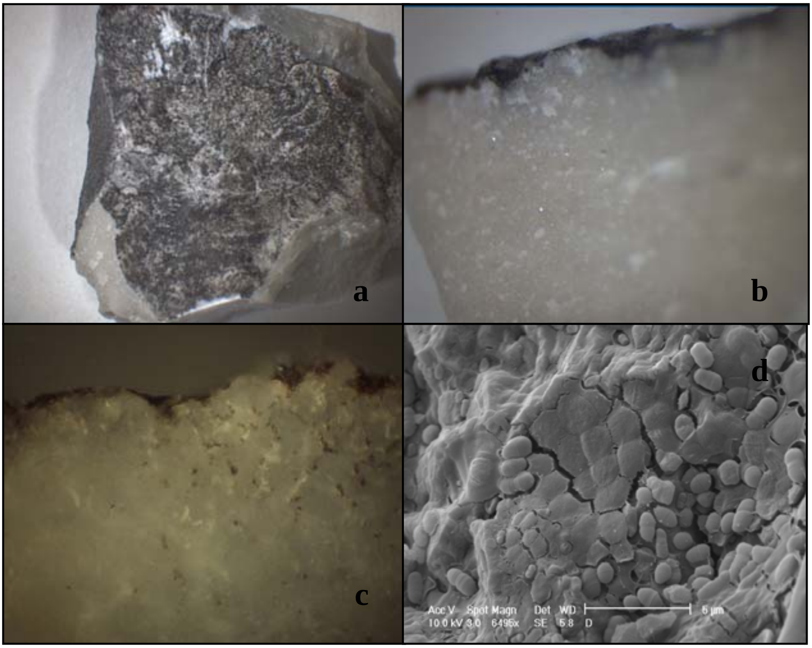
(in the previous page) ***Church of the Virgin of Martvili -*** *Anomalous deterioration phenomenon (a,b); stereomicroscope photographs (c,d,e,f); polished cross section (g); polished cross section stained with PAS (h)*

Hebrew's cemetery tombstones, Venice, Italy

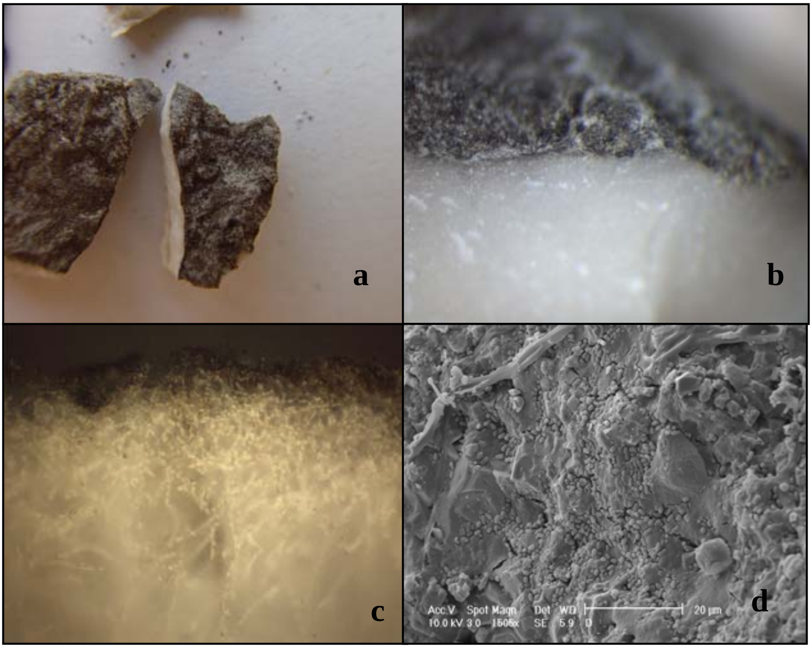
EN3 *- Stereomicroscope observations of the surface and of the side fragment (a,b); particulars of the pits on the surface (c,d); polished cross section (e); SEM image (f)*



EN5 *- Stereomicroscope observations of the surface and of the side fragment (a,b); particular of the surface (c); polished cross section (d)*

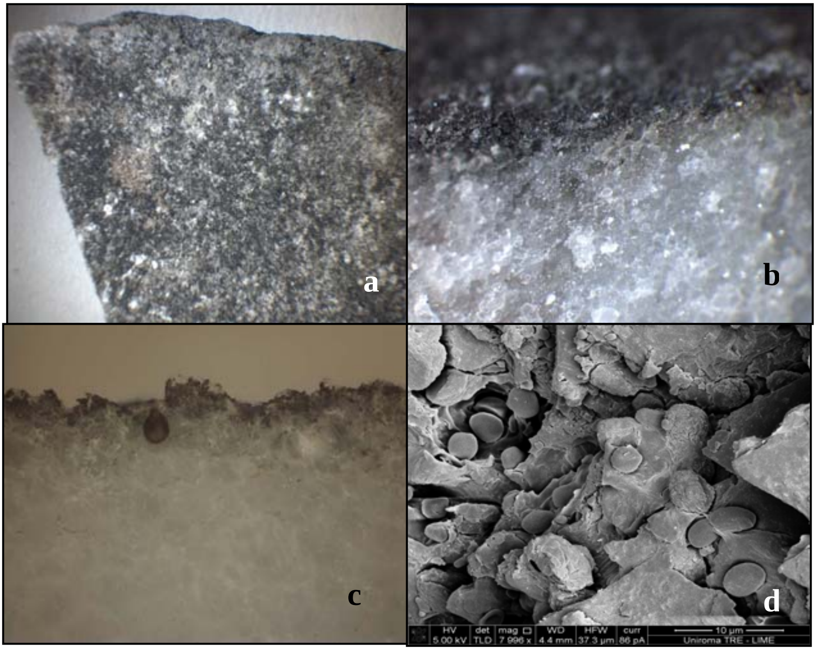


EN9 *- Stereomicroscope observations of the surface and of the side fragment (a,b); polished cross section (c); SEM image (d)*

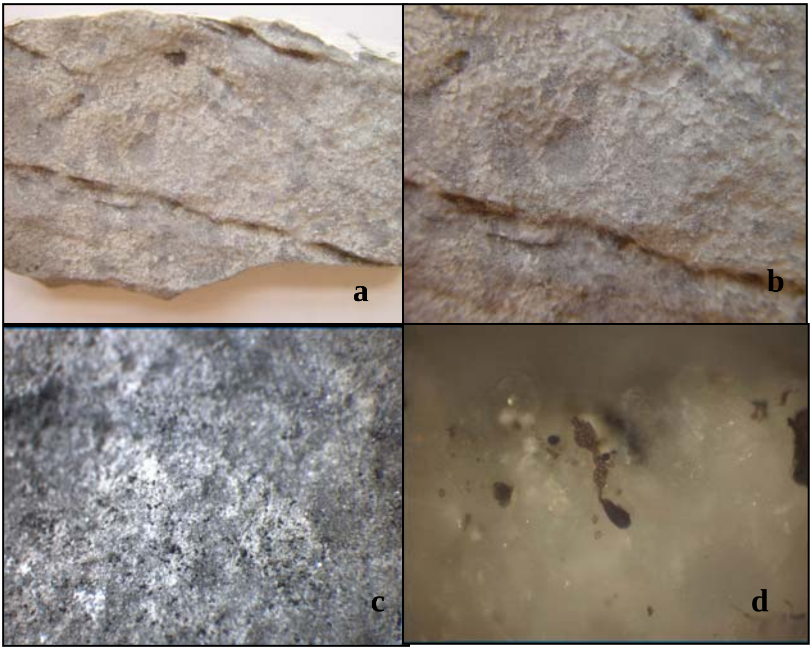


EN10 *- Stereomicroscope observations of the surface and of the side fragment (a,b); polished cross section (c); SEM image (d)*

Carrara's Quarry, Massa-Carrara, Italy



EN1 *- Stereomicroscope observations of the surface and of the side fragment (a,b); polished cross section (c); SEM image (d)*



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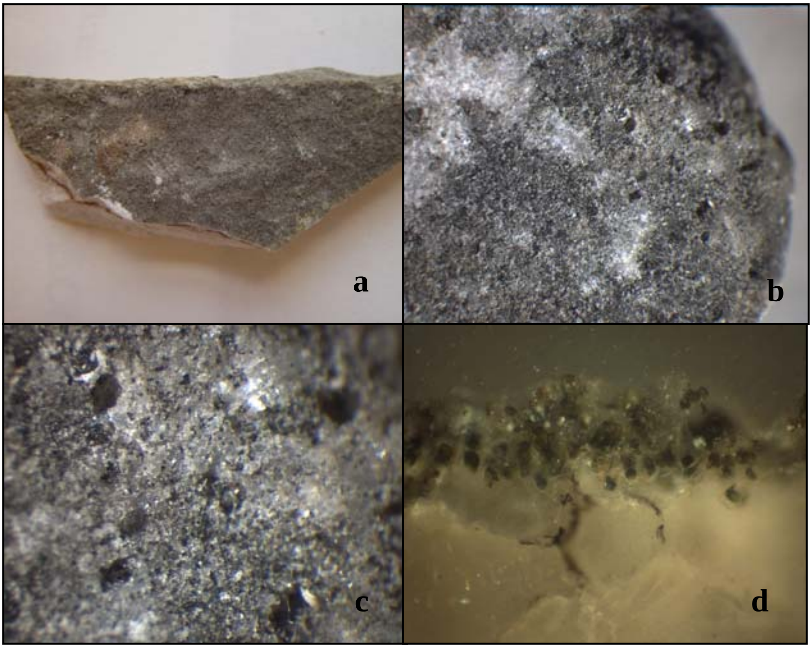
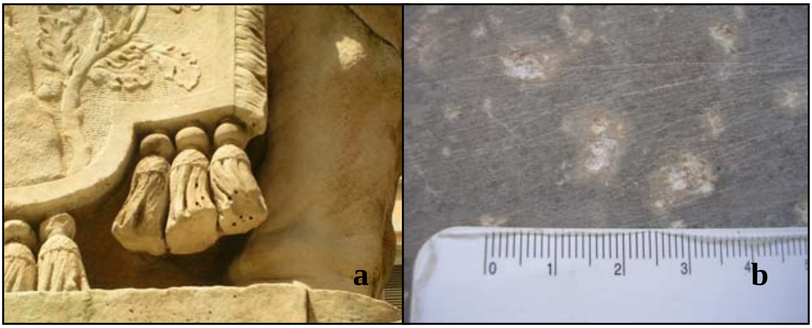


EN4 *- Stereomicroscope observations of the surface and of the side fragment (a,b); particular of the surface (c); polished cross section (d)*

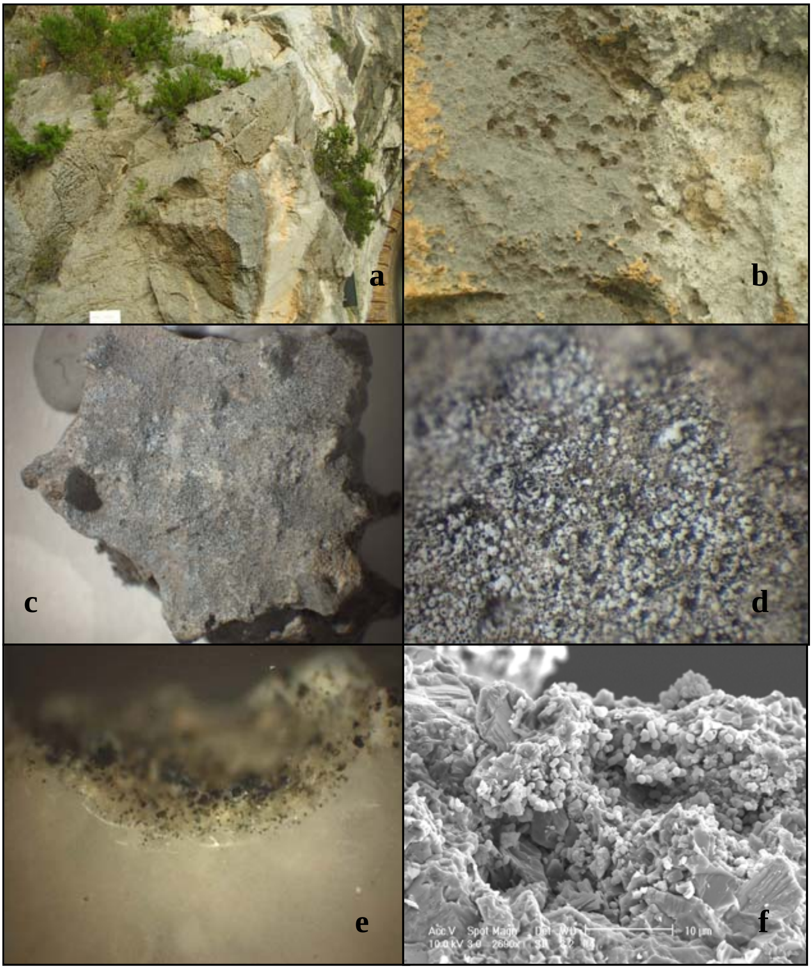
Archaeological sites of Rome

Column plinth, Trainus Market *– Sampling site (a); detail of the deteriorated part (b)*

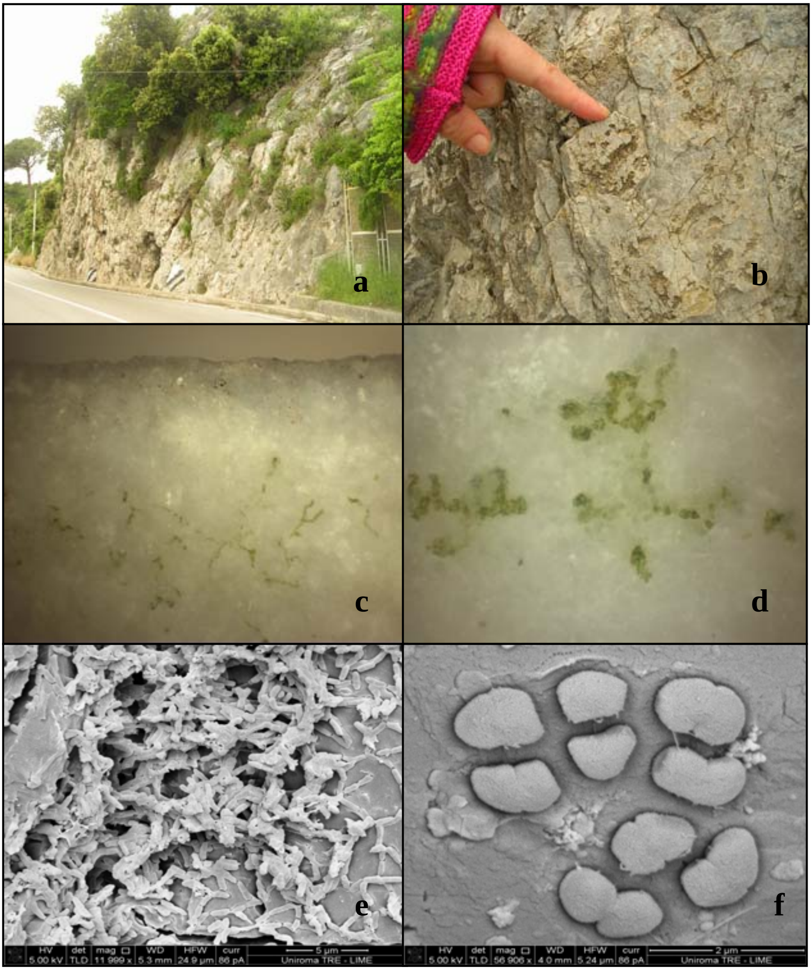


Deterioration phenomena on Bernini's Elephant statue *– Area with pits (a); details of a deteriorated part (b)*

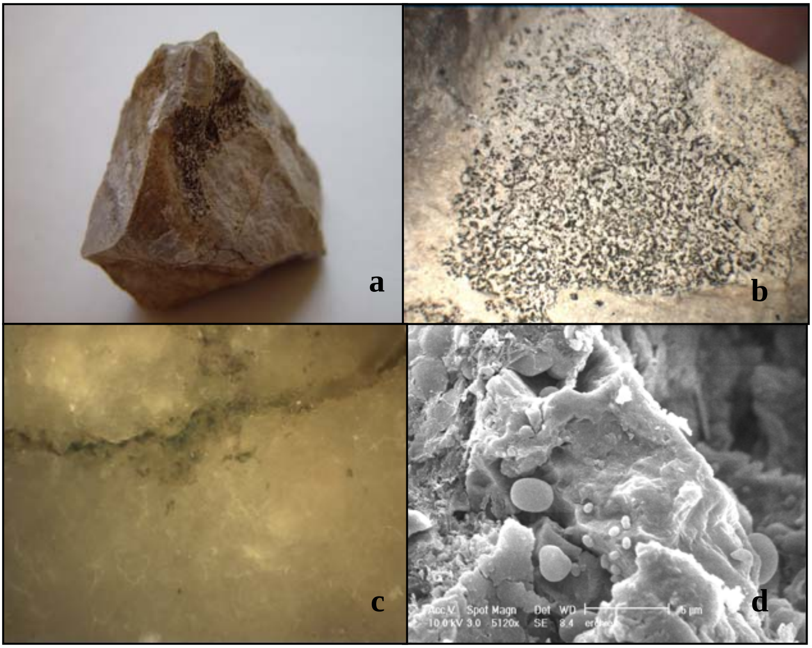
Calcareous cliffs of the Amalfi Coast, Italy



Furore *– Sampling site (a); detail of the sampling area (b); surface and detail of the sampling (c,d); polished cross section (e); SEM image (f)*

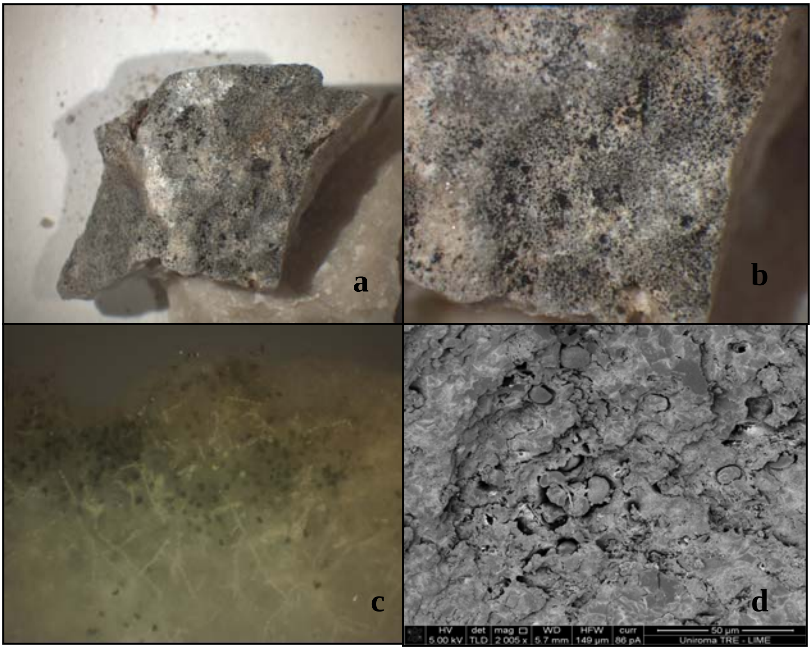


Capo d’Orso *– Sampling site (a); detail of the sampling area (b); polished cross section (c,d); SEM images (e, f)*



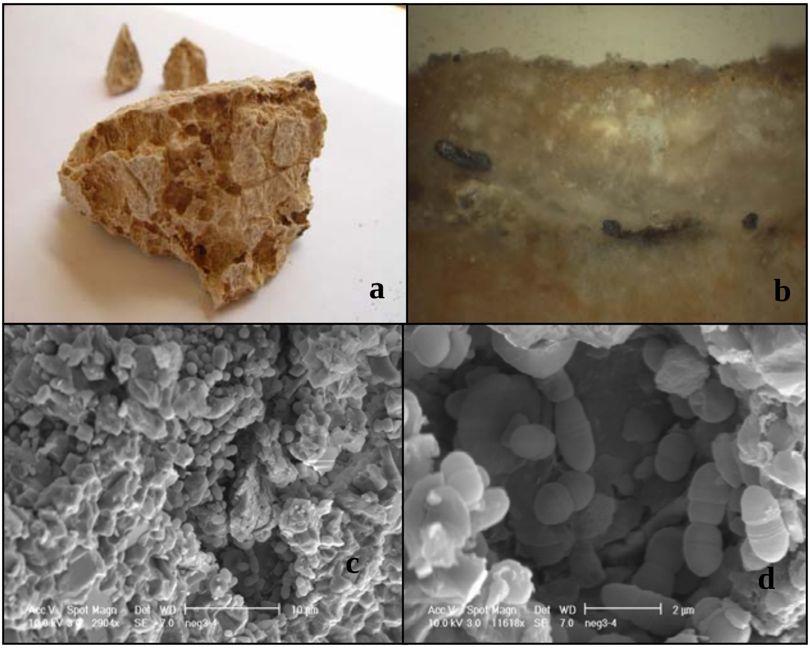
***Erchie** - Stereomicroscope observations of the surface and of the side fragment (a,b); polished cross section (c); SEM image(d)*

Negev desert, Israel



Neg1 *- Stereomicroscope observations of the surface and of the side fragment (a,b); polished cross section (c); SEM image(d)*

Neg2 *- Stereomicroscope observations of the surface and of the side fragment (a,b); polished cross section (c); SEM image(d)*



Neg3 *- Stereomicroscope observations of the fragment (a); polished cross section (b); SEM images (c,d)*

CHAPTER 4 – CASE OF STUDIES

CHAPTER 4

4.1 - The erosion of the carbonatic external walls of the “Church of the Virgin” in Martvili (Samegrelo Region, Republic of Georgia) by endolithic organisms: preliminary results

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Abstract

The biodeterioration phenomenon of the outside walls of the Church of the Virgin in Martvili has been studied with a floristic and ecological approach. This problem is particularly relevant in the cases of the northern, the southern and eastern expositions, sparing completely the western wall and preferring areas not shaded from sunlight and without incident rainfall. It also shows some peculiarities in the way the colonization takes place and different stages can be detected, suggesting an “in progress” phenomenon. Firstly, whitish to blackish spots on stone can be observed, indicating where the colonization has started; than perforations all along the perimeter of an almost circular area appear, which end, in the third stage, with the detaching of the scale in the middle of it. After this, the process starts over again burrowing deeper into the stone. The biological colonizations were studied through optical, light transmitted and SEM microscopy and species have been determined following morphological and molecular approaches. Observing fresh samples with optical microscopy has permitted to associate the diffuse growths of green and orange patinas due to algae (Trentepohliaceae) and cyanobacteria (Chroococcales) and meristematic fungi. The presence of a thin continuous green layer under the surface of the stone fragment indicated the presence of the euendolithic organisms. The use of thin polished section and PAS stain techniques has helped in understanding the biological origin of the deterioration phenomena, the penetration in three dimensions of the organisms and their potential destructive mechanical action. Ecological and environmental data have been also collected and analysed in order to highlight the macro- and microclimatic parameters which have favoured their establishment.

Keywords: stone conservation, endolithic microorganisms, biodeterioration, cyanobacteria, meristematic fungi

Introduction

Biodeterioration of stone is a not negligible phenomenon, even if its relevance is often underestimated, and sometimes confused with other weathering processes (Th. Warscheid & J. Braams, 2000).

The susceptibility of stones to deterioration is influenced by their chemical nature, their physical structure and geological origin, in addition to other environmental factors. In particular the porosity and roughness of the stone surface are also greatly relevant for the growth of the organisms (C. Urzì *et al*., 1994; G. Caneva *et al.*, 2009a).

The phenomenology and the growth forms of biodeteriogens vary a lot and in certain environmental conditions the presence of endolithic microorganisms (mainly cyanobacteria and fungi) is the most diffuse cause of alteration of stone. Usually their colonization gives rise to discoloration of stone to grey-black, more or less enhanced, and creates evident damage due to the formation of micro-holes resulting from the solubilization of the substrata (G. Caneva *et al.*, 2008).

In highly xeric conditions, such as those of walls of monuments not receiving directly an input of water, the endolithic environment has many advantages (water retention is greater, radiation from the sun lesser and not limiting for phototrophs and, to a certain degree, the action of wind is also limited), and therefore represents an environmental niche for many microorganisms (S. Golubic *et al,* 1981; A. Danin & J. Garty, 1983; W. Pohl, J. Schneider, 2002).

Within a project of monuments conservation, funded by the Getty Foundation and implemented by the Georgian Arts and Culture Center, we had the opportunity to investigate various biodeterioration processes present on Georgian monuments regarding mural paintings and natural stone materials. A very relevant case was the conservation of frescoes in the western porch of the Martvili Cathedral that was the object of a previous study (G. Caneva *et al*., 2009).

Moreover, in that occasion, we had the opportunity to observe that the external part of stone cladding suffers of different physico-chemical and biological problems.

In particular, some facades of the church, and especially of the tower, show biodeterioration problems due to the growth of lichens, algae and cyanobacteria. Their presence seems linked to the effects of incident rainfall and differential wetting in the various expositions.

In other areas the soft limestone appears to be very highly degraded, with severe cracking, splintering and detachments, in a similar shape of those of macropits arising from an endolithic growth.

The aim of this study is therefore to give a first contribution to the knowledge of this weathering process in order to contribute to the conservation of this monument and to add new data on the understanding of the phenomenon of endolithic colonization.

Study area

The Church of the Virgin in Martvili (7th- 19th century) is one of the most significant monuments in Samegrelo (Western Georgia), and see of the Episcopal Chair of the Georgian Orthodox Church from its origin (10th Century). It is located 288 m above see level on an hill south the town of Martvili. The grounds of the church are flat and composed by stone walls, but some phenomena of water infiltration from the ground happened in the past, which have been solved in the recent conservative intervention.

Different constructive phases of the churches and restoration activities are reported in the documents, but some difficulties emerge in stating the exact dates especially about the conservative intervention carried out in the past centuries.

The climate of Martvili area is only partially influenced from the one of the Black sea coast, where high humidity and heavy precipitation give rise to subtropical climate features; the macrobioclimate is Temperate-boreal and the bioclimate is oceanic - semicontinental, with superior Thermo-moderated Termicity index and inferior Humid Ombrothermic index.

The total average precipitation (from 2001 up to 2005 years) shows a total amount of 1024,8 mm and is characterised by a quite homogenous distribution, with an increase during summer (300,1mm), average values in autumn (278,9 mm) and spring (291,1mm) and a reduction in winter (154,7 mm) (G. Caneva *et al*., 2009b).

Fig. 1 - The entrance of the church in correspondence with the western porch

Methods

The pitting phenomena and the biological colonizations were studied observing the phenomenology and their distribution in the field, and later collecting small samples close to the pits areas. Polished cross sections were prepared and stained with Periodic Acid Sciff (PAS), according to UNI 10922 and UNI 10923 (2001). Sections were observed with a Leitz Orthoplan microscope and digital photographs were taken with Leica DC300 camera. Selected specimens were observed with a SEM (Philips SEM505). Petrographical analysis were also carried out with a gas pycnometer (ACCU Pyc 1340) and a Quantachrome mercury porosimeter, in order to define density and porosity of the stone.

The identification of species has been carried out following morphological investigation and, with regards to cyanobacterial systematic, also with molecular approaches.

For this aim two different isolating methods were tested at the department of Botany of the University of South Bohemia in České Budějovice, Czech Republic (RNDr.J. Kastòvsky). These were the UltraClean TM Microbial DNA Isolation Kit protocol and an improved method based on

xanthogenate-SDS nucleic acid isolation (XS) (D. Tillet & B.A. Neilan, 2000) (M. Yilmaz & E.J.Phlips, 2009).
After revitalization in water, samples have been sowed on enriched solid soil (Agar + BG-11medium). Plates were grown at room temperature and non-stop light was provided from fluorescent lamps and after two months, the plates have been checked and isolated organisms sowed on solid enriched soil (Agar + BBM medium) obtaining pure strains. These have been left once again to grow under the same conditions as before waiting the biomasses to be sufficient in order to permit satisfactory molecular DNA isolation.

Results and Discussion

The fairly soft limestone constituting the main lithotype of the exterior stone cladding of the church appeared in some areas very highly degraded, with diffuse pitting phenomena and detachments. The time of the exposition of the surfaces is probably variable, and in any case in the order or centuries. The limestone showed petrographical characteristics comparably with those reported in the literature with relatively high values of porosity.
We observed that the pitting phenomenon was particularly relevant, especially in the cases of northern, southern and eastern expositions, sparing completely the western wall. It also seemed to prefer areas not shaded from sunlight and not subjected to incident rainfall (differently from the other biological colonization, forming ephilithic patinas on the surfaces).
Two main kinds of pits were observed: one characterised by microscopic holes (micro-pits) which join together forming larger micro-pits (Fig. 2); the second one characterised by round shaped spots, with detachment of the central scales (Fig. 3).

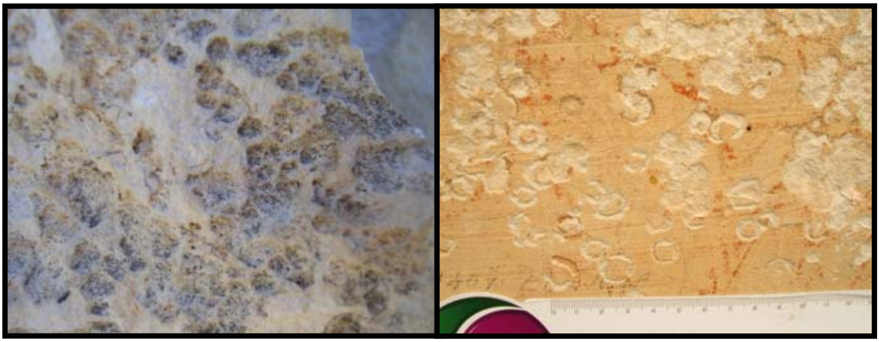

Fig. 2 and 3 - The two main phenomenologies of pitting on the stone cladding of the church of Martvili. .

The first one seems a typical "pitting", with depressions in the rock surface that are at the maximum 1cm deep and with a diameter of relative higher magnitude. The average diameter and depth of the micropits inside each pit is 150µm, creating a spongy-like floor. Looking at the stone surface under magnification, the microorganisms can be seen lodged in the micro-canals and cavities, which were formed by themselves dissolving the stone, forming thus the micropitting.

The second phenomenon appeared more frequent than the first one and it seemed to be more distributed on all the façades, except the one with western exposure. The pitting showed different stages of growth, suggesting an "in progress" phenomenon. Firstly, whitish to blackish spots on stone could be detected, indicating where the colonization had started; than we observed perforations all along the perimeter of an almost circular area ending, in the third stage, with the detaching of the scale in the middle of it. After this, the process seemed to start over again burrowing deeper into the stone.

The microscopic observation of fresh samples of the detached scales permitted to associate the diffuse growths of green and orange layers, on the scale's surfaces facing the inside of the walls, to algae (Trentepohliaceae) and cyanobacteria (Chroococcales). Often, the presence of meristematic fungi has also been detected (Fig. 4).

Fig. 4 - Green colonies of coccoids algae and cyanobacteria together with meristematic fungi.

Moreover, inside the lower layers of stone close to the pits, the observation of the polished cross sections permitted frequently to detect a thin continuous green layer at an average depth of about 2µm from the surface of the stone fragments, thus indicating the presence of the euendolithic organisms. This techniques and the use of PAS (Periodic Acid Schiff's reagent) stain techniques have helped in understanding the biological colonisation and the penetration in three dimensions and their potential destructive mechanical action (Fig. 5).

Fig. 5 - Penetration of endolithic microrganisms detected through PAS (Periodic Acid Schiff's reagent) stain techniques.

Dealing with biodeterioration processes, it can be observed that chasmoendolithic and crypto-endolithic organisms develop in the interior of stone, in cavities and fissures respectively, whether these are pre-existent or generated by the organisms themselves; in both instances, the increase in the biomass inside the stone results in pressure which in the end gives rise to a fracture corresponding to the colonized area, with consequent detachment of a scale of the material.

According to the literature, in these pitting phenomenologies, different microorganisms are associated, both photosynthetic (cyanobacteria, green algae, lichens) and chemoorganotrophs (fungi, especially meristematic ones) (L. Hoffman, 1989; A.A. Gorbushina *et al.,* 1995; O. Salvadori, 2000). In relation with the detected species, the depth of penetration varies between a few tens to a few hundreds of microns. Cyanobacteria and green algae (both free and as photobionts) will generally reach depths of 100-300 µm, even though some filamentous species can in fact penetrate up to a depth of a few millimetres. Fungi as mycobionts can penetrate further, pushing themselves a few millimeters into the stone (Caneva, G. *et al*., 2009). The calcifying cyanobacteria play probably an important role, as they reprecipitate calcium as calcium carbonate in the polysaccharidic sheaths and act as centers of nucleation for the neoformation of minerals (X. Ariño *et al.* 1997; P. Albertano, 2002).

Regarding the Martvili samples, at the moment we could not go further into details of the taxonomy of systematic groups identified trough the morphological analysis. This is due to the difficulties in extracting DNA from environmental samples and the slow growth of these organisms under controlled conditions.

Moreover, we cannot now exclude the presence of a lichenic process to in certain kind of weathering process, such in the phenomenologies of II kind, producing at the end macropits. In general, endolithic lichens, as well dissolving the stone through the penetration of the thallus, also cause grave damage to the substrate through the development of ascocarps (apothecia or perithecia). When they die, the holes remain on the surface of the stone (mesopitting), the diameter varying according to the species (C.K. Gehrmann, W.E.Krumbein, 1994; D. Wessels, L. Wessels, 1995). As it has already been observed on many monuments (for example the south front of Trajan's Column, or the lower section of the southern face of the Caestia Pyramid) the presence of macropitting is not negligible due to its recurrence and entity of damage (G. Caneva, *et al*., 1992; 1994).

The taxonomic recurrence of *Trentopohlia sp.* confirms also the frequency of species of this genus as colonizer on surfaces monuments. In fact these species were often detected in Mediterranean, temperate and tropical countries, and their action in the weathering processes were also proved. In the case of a study carried out on a Scottish castle built of sandstone, and colonized principally by *Trentopohlia aurea*, it was indeed demonstrated as it can contribute to the mechanical and to the chemical deterioration of stone through the production of lactic acid which acts as a chelating agent, and mobilising calcium ions (M. S Jones, R.D. Wakakefield, 2000).

Conclusions

The pitting of the external walls is of biogenic origin, and to be connected to various kind of microrganisms, such as cyanobacteria, algae and meristematic fungi, not excluding, in some cases, also lichens.

The biodeterioration processes on Martvili external facades appear therefore relevant and complex and the potential damages not negligible, considering also the soft quality of the stone.

In order to understand the ecological factors permitting the colonization of the various group of organisms, a systematic study have been undertaken, but some more specialistic analysis are still in progress.

Finally the exact comprehension of the various phenomena and their ecological linkage (e.g. with incident rainfall/ rising damp/isolation) will give preventive and/or conservative suggestions.

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4.2 - Biodeterioration problems of mural paintings in rock habitat: the prehistoric site of Filiano (Basilicata, Italy)

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ABSTRACT

Rock settlements often suffer from the damages characteristic and specific to hypogean environments due to infiltrations of rain waters, high level of humidity, condensation phenomena, abundance of salts and others resulting from the use of the caves as human and animal shelters. Biodeterioration is here favoured from the environmental conditions and from organic nutrients linked to the use of fertilisers in agriculture and the excreta of grazing animals.

The Rinaldi's shelter (Filiano), which presents important traces of prehistorical paintings, results to be an emblematic case of delicate balance, achieved throughout the centuries, between the environment and the artwork. The shelter, facing South, surrounded, still nowadays, by a thick covering of mixed oaks forest, has an optimal exposure concerning the thermal values of this quadrant, but also suitable to offer a certain protection from the prevailing winds, as those associated with rain. Over the plurimillenarian history of the site, important events have taken place, such as the collapse of a part of the vault that covered the shelter and fires that have left burning marks over quite a few concretionary layers. With the use of microscopic and cultural analyses, it has been possible to detect several biodeteriogens present on the rocky habitat, such as algae, cyanobacteria, mosses, lichens and vascular plants, apart from fungi, which are distributed following the edaphic and microclimatic gradients and, in particular, the presence of water and its fluctuations over time. Among all of these biodeteriogens, endolithic fungi with meristematic growth, which appear as little black spots, represent the most critical risk factor, with their less water demands and a not negligible progression. Considering the fragile balance of the site, it is suggested to avert seepage water from the sides of the painted area, but also to operate with extreme caution in modifying the general ecological conditions. The building of a covering, positive concerning some aspects, has to be studied carefully because it will modify the microclimatic and local ventilation conditions.

Keywords: mural paintings, biodeteriogens, meristematic fungi, rock settlements

1. INTRODUCTION

The conservation of mural paintings in rocky habitats shows high difficulties due to the more or less severe environmental conditions. A lot of weathering factors in fact characterise rock settlements, and they often suffer from the damages characteristic and specific to hypogean environments: infiltrations of rain water, condensation, abundance of salts, use of the caves as human and animal shelters, close contact with the underlying and overlying soil often enriched with organic nutrients, etc [1,2,3].

Biodeterioration phenomena are widespread in such conditions and the biological communities more frequently observed are composed by photosynthesizing microflora, with populations constituted by epilithic and endolithic species of cyanobacteria and green algae, and by lichen thalli. Microfungi, heterotrophic bacteria, and in particular actinomycetes, are also detectable when an enrichment of organic material occurs.

The Rinaldi’s shelter (Filiano), which presents important traces of prehistorical paintings, seems to be an emblematic case of delicate balance, achieved throughout the centuries, between the environment and the artwork, but several factors of risk can be detected and therefore controlled.

The aim of this work is to analyse biodeterioration with an ecological approach, in order to contribute to a correct planning of the conservation activities.

2. THE STUDY AREA

The shelter is located within a mixed deciduous forest with predominance of turkey oak, in the State’s anthropological reserve placed in the Apennine hilly belt, in the South of Italy (Potenza, Basilicata Region). The rock paintings of Palaeolithic age, portraying deers and perhaps arboreous symbols too, are situated on a subvertical wall made of calcareous sandstone rock, in an area probably presenting a dense forestal covering already at the time of the settlement.

The paintings, made using pigments identified as red ochre based on haematite, have been realized following a prehistoric parietal technique which consisted in laying the colours directly on the rock walls without earlier plastering.

Among the events occurring during the multimillennial history of the site, there have to be mentioned, firstly, those which led to the collapse of a part of the vault that protected the shelter. Concurrently, the blackening located in some areas of the shelter resulted to be carbonaceous matter mixed with sulphur, incorporated within the different concretionary layers, which is to be interpreted as fire traces due to the ancient anthropic attending of the shelter. The presence of widespread concretionary layers detected by chemical analyses has been interpreted as the result of dissolution and re-crystallization cycles of the sandstone carbonaceous cement triggered by water permeation into the sediment.

In several parts of the shelter, traces of organic substances can be detected as the widespread presence of phosphate and calcium oxalate testifies. This can be related with the store of bird’s guano and of pasture animals excrements.

Fig. 1.The current state of the shelter, which highlights the thick forestal covering.

3. METHODOLOGY

For a climatic characterization of the site, meteorological data collected by the weather stations nearby have been utilized, due to the lack of local ones. In particular, the thermal and pluviometrical data referring to daily precipitation, and daily temperatures of the period of time that goes from 1993 to 2004 have been processes. Winds have been analysed also, through a comparison of the winds and precipitation timing graphs recorded, in order both to determine the prevailing winds and calculate their frequency and distribution in the different quadrants and also to evaluate the risk of driving rain on the shelter.

In order to investigate biodeterioration phenomena, various samples of the main alterations observed in situ, have been collected.

In the case of the phototrophic microflora, sterile samplings collection have been performed using scalpel and plug and the taxonomic characterization has been carried out up to species and, in some cases, genera level, with the use of dichotomous keys commonly used in the systematic characterization [5,6,7,8].

The microbiological analyses, carried out in order to favour the growth of the cyanobacterial and algal species found in the samples, requested the use of liquid growth medium, BG 11, specific for photosynthetic microorganisms, according to Normal 9/88 (1990), still taking into account that artificial growth media penalizes some types of species strictly linked to natural environments.

For the identification, the heterotrophic microorganisms have been collected removing the superficial part of the alteration using a sterile scalpel and then fresh specimens to submit to optical microscopic observations (Leitz DMRB) have been prepared.

Concerning the identification of lichen thalli, in situ and laboratory surveys have been made through observations with stereomicroscopy and optical microscope following the usual analytical keys [10,11,12].

For what concerns the vascular plants, in situ visual sampling has been carried out together with a collection of samples for the morphological characterization following the analytic keys of the flora of Italy [13].

In the case of endolithic forms of alteration, microanalysis and scanning microscopy have been used also. To this aim, the samples have been submitted to dehydration process, through subsequent staying of the material in alcohol with increasing concentration, critical point drying and metallization by a thin gold layer.

In order to observe the possible deep penetration of the organisms, polished cross sections have been prepared using the samples collected as scales. This preparation requires the samples embedding in polyester resin followed by abrasion and polishing of the specimen, trying to obtain plain surfaces to be observed at reflected light microscopy.

The distribution of all the alteration forms has been mapped in order to highlight their links with the principal ecological factors and to identify the highly risks zones.

4. RESULTS

The processing of the climatic data shows that the shelter, facing South, has an optimal exposure due to the higher thermal values of this quadrant, but it also results suitable to offer a certain protection from the prevailing winds of the Western quadrants, as those associated with rain of the Eastern and Western quadrants.

The harshness of winter is not to be neglected in the context under exam, actually often during this season below-zero values are registered, which can lead, if water is present, to the formation of ice that favours the rise of fractures of the lithic substrate.

Concerning the biological colonisation, it can be hypothesized that already in the past some kind of growth could have taken place, in particular in correlation with water presence on the lithic surfaces. While in areas subjected to driving rain or percolation of water, lichens, mosses and ferns development tends to occupy all stone surfaces, the higher dryness in the shelter seems to not favour the biological development, neither of cyanobacteria or epilithic algae.

By the comprehensive analysis of the results, it can be pointed out, in fact, that the major part of the deterioration phenomena detected in the area close to the shelter are not linked to the development of photosynthetic microflora. The few species found belong to the taxonomic assemblages of cyanobacteria (*Chroococcus lithophilous*, *Gloeocapsa sanguinea*, *Calothrix parietina* e *Lyngbya* sp.) and of eukaryotic microalgae *(Pleurococcus vulgaris e Muriella terrestris*), ubiquitarian components of the microbial communities which develop over ground or rocks surfaces.

However, a more xerotolerant microflora, which is detectable as little superficial black spots, should have taken place before time and shows itself also in some areas nearby the paintings (Fig. 2). They are due to the development of meristematic fungi, frequently found on lithic materials exposed to open air in hostile environment conditions. These organisms show a characteristic kind of growth with cells enlargement and thickening of cell walls on which melanin is deposited.

The presence of endolithic fungi with meristematic growth is, in fact, the most critical conservation phenomenon for the paintings and appears to be not negligible. It can be explained with the nutritious enrichment typical of this kind of forest habitats and it is highly probable that the presence of fungi is the causal factor for the widespread of oxalate patinas detected in several areas of the shelter.

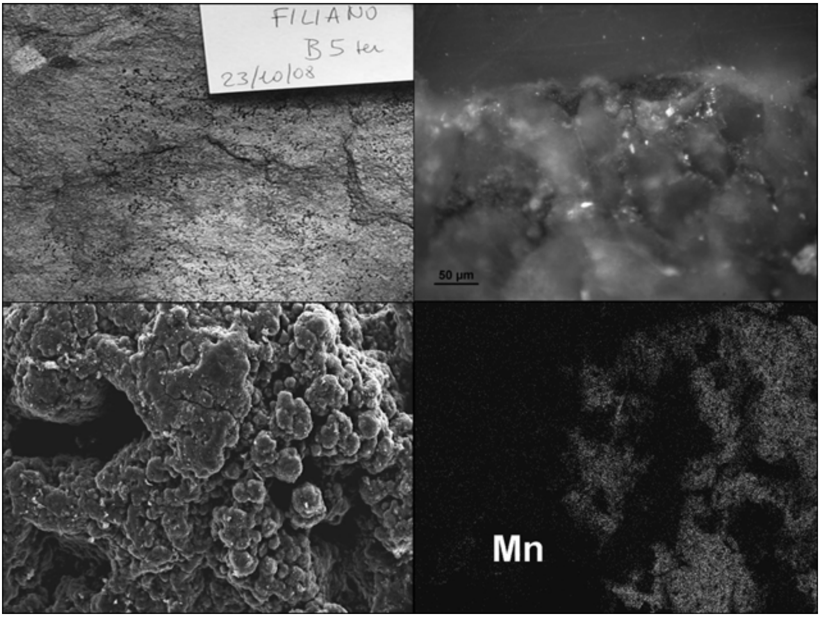

Fig. 2 Microorganisms associated to the black spots (a)and several neighbouring areas and polished section (b); fungal structures observed by SEM microscopy (c) and map of Mn accumulation.

SEM observations of fungal mats and the presence of Mn accumulation in these zones, as revealed by microanalysis, recall what observed with the dark patinas called "rock varnish", often described among the deterioration processes of rocks in desert areas. These patinas are frequently composed by clayey minerals cemented on the lithic substrate by manganese and iron hydroxides and to them could be associated both microcolonial fungi and several bacterial species.

Special attention should be paid to the presence of vascular plants which can be found close to the rocky paintings or standing on them. The damages on the stability of the site that can be produced by these organisms are linked both to their biological form (that is if they are woody, shrubby or herbaceous species) and to the characteristics of the root system.

The roots of the vascular plants, in fact, present a complex structure which exert highly pressures on the substrate. This is due to the fact that during the growth they tend to penetrate where there is less resistance, thus giving way to breaking mechanisms which lead to structural physical-mechanical damages that sometimes result to be heavy. Moreover, the effects can be seen also away from the implant point.

Some of the found species, in particular the woody ones as Turkey oak (*Quercus cerris*) and ash tree (*Fraxinus ornus*), show a high level of potential aggressiveness of the root system due to its widespread development and to its big dimensions, but by now risk factors do not appear to be present. On the contrary the neighbouring forest covering seems to exert a protective effect from prevailing winds erosion and the effects of sun radiations.

5. CONCLUSIONS

Microorganisms distribution appears to be linked mainly to edaphic and microclimatic gradients and, in particular, results to be depending on water presence and its fluctuation on time as well as on the presence of organic enrichments, secondly.

Light seems not to be the main influencing factor in this context, because the recorder values seem not to be thresholds and because the phototrophic microflora plays a secondary role in comparison with the heterotrophic one.

It is clear that ferns and mosses are, in general, the more hygrophilous organisms and also that the lichen covering seems to be favoured by a certain input of water. Among the phototrophic microflora, cyanobacteria appear to be dominant in less hygrophilous conditions, while green algae seem to be correlated to higher water values.

The not negligible presence of endolithic fungi is favoured by conditions of still greater relative dryness in the sheltered areas and it could be explained with the enrichment of nutrients typical of this forest habitat.

Due to the fragile balance of the shelter, it is suggested to intervene with extreme caution in changing the favourable ecological conditions, acting especially in improving seepage water courses nearby the painted area, averting them from the mass of stone. This intervention will have to be carried out from the above of the shelter, closing and diverting the ways of flowing down of water.

The building of a cover which could further remove the risk of water seepage from the lithic substrate during rain phenomena has to be studied carefully, considering that if it is not properly reamed, it could somehow modify the microclimatic conditions and lead to the formation of micro air currents which besides exerting an erosion action, speeding up deposition and thus decay processes, could give rise to evaporation phenomena with consequent dissolution risk of the cementing matrix and loss of adhesion of the pigment from the support.

On the other hand, a covering which protects from rain could be an effective barrier from chemical and biological weathering processes and it could interfere with the endolithic growth already insisting on the site as well as the recolonization of most of the lichen species which need a direct water supply.

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CHAPTER 5 – GENERAL CONCLUSIONS

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General Conclusions

In relation to the appointed goals of the study, it can be summarised that:

The building up of the database has permitted to understand that the existing bibliography on pitting phenomena is extremely heterogeneous, depending on the scientific approach in studying them.

Due to this heterogeneity and to the missing of some information in many interesting papers, the data collected from the literature do not permit to use all the potentiality of the interactive database. Infact, generic papers on stone deterioration focused on taxonomy or biochemical processes related to pitting, could prove useful under a systematic or physiological point of view, but they usually do not report a series of information useful to give a defined ecological description of the phenomenon. On the other hand, many papers report synthetic information of different cases but without exact sampling references, which are useful to define the ecological niche of the associated microorganisms.

This suggests the need of a normalization of the approach as already proposed by many Authors through national (NORMAL) and international (CEN/346 Conservation of Cultural Property) working groups on stone conservation.

We can however evidence, according to the knowledge acheived up to now by the literature, that the most recurrent etiological agents seems to be cyanobacteria and that the most recurrent environmental conditions are dryness due to the low porosity of the stone and also from the exposure conditions (the reason of the preference for vertical or subvertical surfaces), and the bioclimate (the reason of the high recurrence of Mediterranean and desertic climate).

The application of the polimorphic approach to the cyanobacteria taxonomy has highlighted the existing difficulties in cultivating and isolating of these organisms, more stressed if these come from endolithic environment. This suggest the need of testing more efficient ways of DNA isolation and specific in particular for these extreme peculiar environments, which could eventually avoid or at least reduce the “error” introduced by cultivating under laboratory conditions these organisms.

The case of the deterioration of the church of the Virgin of Martvili in Georgia and of the archeological site of Filiano in the South of Italy have highlighted that the pitting phenomenon is more widespread than what resulting from the database, suggesting a linkage not much with macroclimatic conditions, geographic location or rock type substrate than with surface microclimate which, preventing microbial growth, due in particular to an extreme dryness condition, has permitted the development of these highly adaptive and tolerant to severe stresses organisms, which in other moderate conditions fail to compete with more fast growing organisms.

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