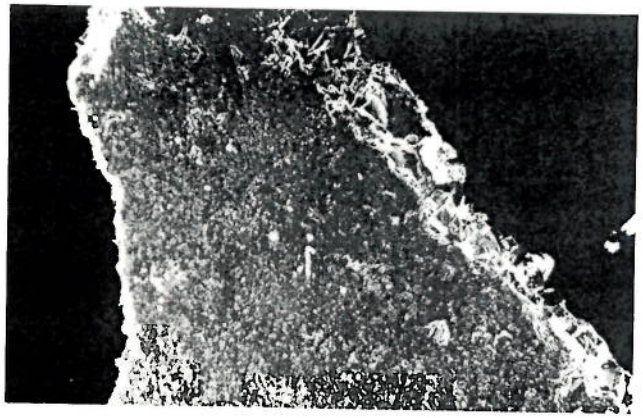

Figure 5 : Verrucaria on brickwork (S.W.M. 50x)

Figure 6 : Xanthoria Parietina on marble (S.E.M. 50x)

MICROBIOLOGICALLY INDUCED DETERIORATION OF DOLOMITIC AND CALCITIC STONE AS VIEWED BY SCANNING ELECTRON MICROSCOPY

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Abstract

Biodeterioration of stone has been the subject of numerous studies in recent years. This report presents conclusive evidence obtained with scanning electron microscopy (SEM) of fungal attack on dolomitic and calcitic stone. The attack by Trichothecium sp is rapid, deep into the stone, and has a characteristic appearance.

This study originated from a program to elucidate the contributions of micro-organisms to the deterioration of specific regions of the Spanish Apse of Fuentiduena. The Apse was constructed from a soft yellow dolomitic limestone. Two areas, where water run-off periodically inundates the stone, has severe decay. Large populations of micro-organisms are abundant in these areas.

Samples of micro-organisms from the Apse were obtained and cultured in the laboratory. The micro-organisms: a Trichothecium sp. fungus, a Chlorococcales alga, and a cyanobacterium, were cultured on: 1) single crystals of dolomite; 2) single crystals of calcite; and 3) thin sections prepared from the limestone of the Apse.

The extent and type of deterioration obtained was studied after 5 weeks in culture. Optical, epi-fluorescent, and scanning electron microscopy were employed. Implications of the contribution of biodeterioration to the overall decay of the limestone of the Apse are discussed.

1. INTRODUCTION

Biodeterioration of stone has been the subject of numerous studies over recent years. It has been shown that bacteria and fungi in culture can break down minerals mechanically [1], or chemically [2-7]. The earliest observations of fungal attack on calcite were done in the last century [8]. It was found that the hyphae penetrated the calcite crystals without any regard for crystal planes. Deterioration was attributed to chemical secretions by the hyphae. Numerous other studies have implicated micro-organisms in stone deterioration [9-17].

This study was carried out to elucidate the nature of micro-organismic attack on stone: the rapidity, extent, and characteristics of the attack. The concern for it arose due to the presence of an apparent biodeterioration problem on a monument at the Metropolitan Museum of Art (MMA). This monument is the Apse of the church of San Martin de Fuentiduena, originally from a small town of the same name in the province of Segovia, Spain. The Apse, believed to

have been constructed sometime before the thirteenth century, was moved to and rebuilt at The Cloisters, MMA in 1958-60. It is composed of Spanish dolomitic limestone blocks.

Currently the Apse is surrounded by a temporary fiberglass screen which is due to be removed. Despite the presence of the fiberglass wall there are two areas of severe localized decay associated with extensive growths of fungi, algae, and bacteria. Both of these areas are inundated by water run-off every time it rains. Should these micro-organisms contribute significantly to stone decay, then when the covering is removed, and the whole exterior of the Apse is subjected to periodic wettings, a large scale attack by micro-organisms could cause major decay of the stone.

To assess the importance of the micro-organisms the following sets of experiments were designed: (1) culturing and identifying of isolates from the wetted surfaces of the Apse; (2) growth of the isolates on: (a) polished sections of the Apse; and (b) individual crystals of dolomite and calcite, the principal components of the Apse limestone.

This report presents results of the culturing experiments on single crystals of dolomite and calcite, and preliminary results on thin sections of the Apse.

2. MATERIALS AND METHODS

Samples from the Apse with apparent biological growth were selected aseptically and cultured in Sabaroud's medium (for fungi), liquid and agar, and in soil extract medium (for algae and bacteria), liquid.

Cultures of a fungus, an alga, and a bacterium were isolated and tentatively identified.

Samples of crystals of dolomite (from Eugui, Pamplona, Spain) and calcite (from Cherokee County, Kansas) were placed in culture vessels with triple-centrifuged and washed isolates of the fungus, alga, or bacterium. Culture conditions were: 22°C, 100% RH, day/night 14/10 h, for five weeks.

Epi-fluorescence was utilized with a Zeiss Photomicroscope II fitted with FT 580/LP 590 exciter filter/barrier filter combination for algal chlorophyll. Bacterial presence was confirmed by using DAPI, 0.01 µg/ml, for a five minute stain [18].

Samples of crystals for energy dispersive x-ray spectrometry (EDS) were air-dryed, mounted on spectroscopically pure carbon stubs with spectroscopically pure carbon paint, coated in a vacuum evaporator with approximately 10nm of spectroscopically pure carbon, and examined at 20 kV. Scanning electron microscope (SEM) specimens were treated as above but then coated with approximately 10nm of pure gold. SEM observations were conducted at 20 or 30 kV.

3. RESULTS

Cultures from the Apse revealed the presence of an ascomycete, perhaps a Trichothecium with a monoverticillate spore head fungus, a chlorophyte, belonging to the chlorococcales, and a cyanobacterium,

Lyngbya sp.

Epi-fluorescent illumination revealed extensive growth of the alga and bacterium on the surface of the crystals and petrographic sections after only five weeks in culture. No qualitative or quantitative differences were observed between growths on the calcite and the dolomite crystals.

Extensive fungal growth was also evident on the fungi-inoculated samples and, since the cultures were not axenic, algal and bacterial growth was also present. In all cases the alga and bacterium had no visible effect on the crystals in five weeks.

Figure 1 illustrates the extensive growth of fungi found on the crystals and the deeply etched surface.

Figure 2 illustrates a partially etched surface next to an area which was only water-weathered.

Figure 3 is a higher magnification of a severely etched surface with fungal hyphae and growing tips.

Figure 4 is a freshly cleaved inner surface. Extensive fungal growth is evident on the surface perpendicular to the exposed surface with numerous hyphae extending into the former interior. Also present are hundreds of fungal spores. The fungal attack has extended approximately 1 mm into the crystal in this region.

Figure 5 is a detail at higher magnification of a freshly cleaved surface. In this micrograph, the top surface was the exposed one. Fungal hyphae have found their way into the crystal.

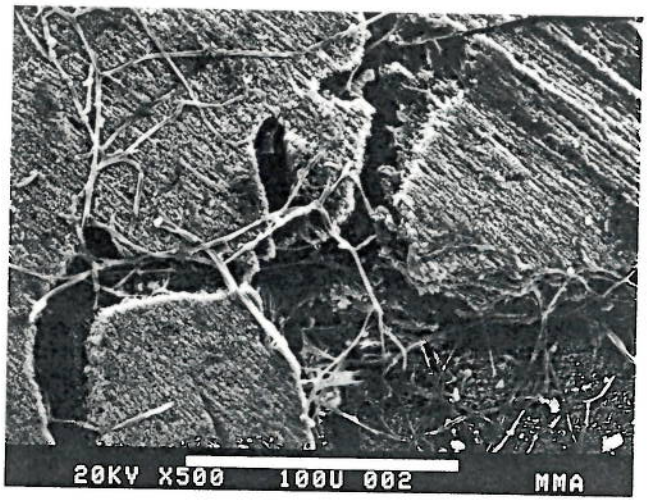

Figure 1 : SEM photomicrograph of fungal-etched dolomite crystal.

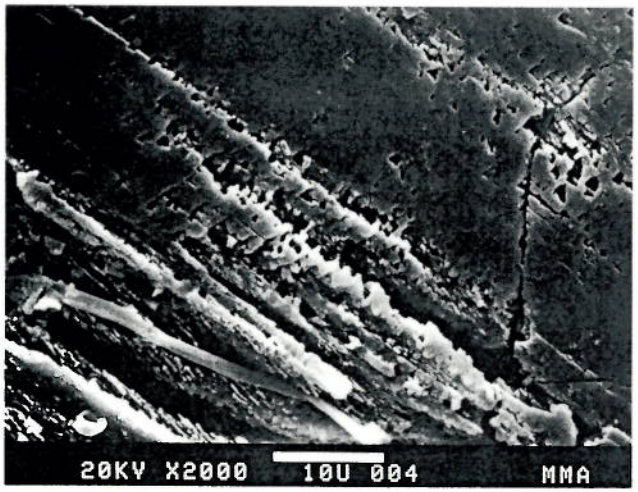

Figure 2 : SEM photomicrograph of water-weathered surface (upper right) and fungal-etched surface (lower left). Note the fungal hyphae running parallel to the deterioration.

Figure 3 : SEM photomicrograph showing detail of etching. Note the fungal hyphae running parallel to a crack in the crystal and the deep etching.

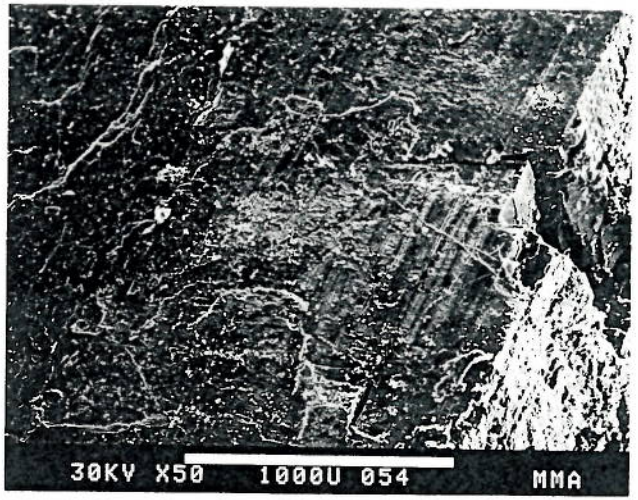

Figure 4 : SEM photomicrograph of freshly cleaved inner surface. Note the extensive fungal growth on the surface perpendicular to the exposed crystal (on the right side of the micrograph). Hyphae and numerous spores are found at least 1 mm inside the crystal.

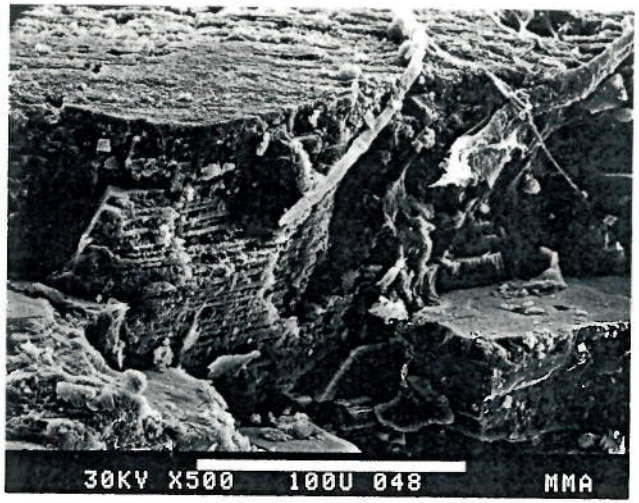

Figure 5 : SEM photomicrograph showing detail of a freshly cleaved dolomite surface. The top surface was the exposed one. Fungal hyphae have found their way into the crystal. Note the difference between the etched (left) and the unetched (right) surfaces.

Figure 6 is a photomicrograph of a fractured surface of the limestone used in the construction of the Rudolf Hospital in Vienna, in 1884. This specimen was taken from a fairly undeteriorated area 1-2 cm towards the interior of the stone. Note the presence of fungal hyphae in the lower left corner and the similarity of etching in this micrograph as compared to the previous ones.

Figure 7 is a enlargement of the gypsum crystals produced by the bacteria on petrographic sections of the Apse stone.

Energy dispersive x-ray analysis (EDS) showed accumulations of Ca, Mg, Cl, Fe, Na, Si, S, P, K in the fungus, and Si, Ca, Mg, Fe, Al, S, K, C, and P in the alga, in decreasing order of concentration. This reconfirms the ability of fungi and bacteria to concentrate environmentally scarce elements within themselves.

4. DISCUSSION

SEM examination clearly demonstrated the extensive corrosive ability of fungi on limestone. After only five weeks in culture the fungal hyphae have penetrated at least 1 mm into the crystals. It is apparent that the fungus has corroded channels along and in front of its path. Trichothecium sp are known to secrete oxalic acid, citric acid, and gluconic acid among other products [19,20]. These secretions could chemically attack the dolomite or calcite and account for the observed etching. For a general literature review of secretory products of micro-organisms see [21]. Water attack, at least in the form of high humidity, does not corrode the crystals as quickly or as severely as the fungal attack (see fig. 2).

The channels created by the fungus provide new avenues of attack deep inside the crystal, and a vastly increased surface area susceptible to attack by water and/or dissolved acids in the water, such as nitric and sulfuric acids from air pollutants. The increased surface area will noticeably decrease the time needed for dissolution to take place [22]. In addition, the roughening of the surface provides new micro-habitats for bacteria and algae, and eventually footholds for higher plants.

Extensive growths of the fungus are apparent on the petrographic sections as are large populations of the alga and the bacterium. The micro-organisms coat the stone with cellular secretions, nutrients accumulated from the environment, and miscellaneous material fortuitously trapped by the organisms. Actual attack of the stone by the fungus is evident by the presence of etched surfaces similar to that on the crystals. These findings make it possible to identify fungal caused deterioration in actual stone, as seen in figure 6. This micrograph shows that fungal attack can occur in relatively unweathered stone, contrary to previous observations [3].

The bacterium is also found extensively on the petrographic sections as are newly formed gypsum crystals (fig. 7). The gypsum is presumed to be a product of the bacterium. The production of soluble salts by the bacterium will also be a contributing factor in deterioration of the stone. Gypsum will be dissolved by water and carried further into the stone, through the channels made by the fungus, where it can recrystallize as the water evaporates creating mechanical pressures in the stone [23]. Gypsum crystals have been found on all types of porous building materials [9,24], and frescoes [14].

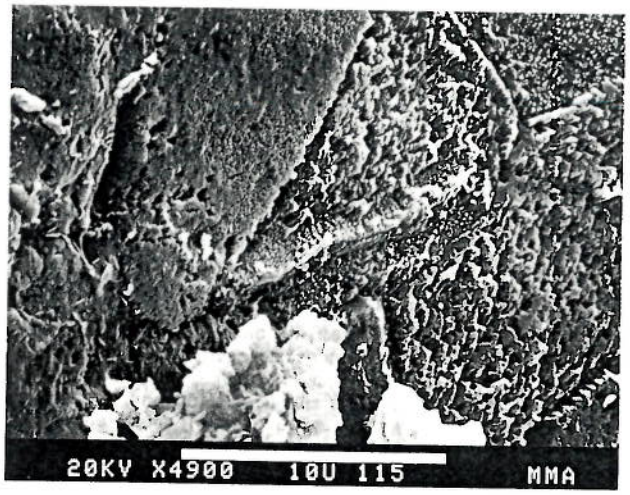

Figure 6 : SEM photomicrograph of limestone from Rudolf Hospital, Vienna. This relatively unweathered area is 1-2 cm inside the stone. Note the presence of fungal hyphae in the lower left corner and the etched appearance of the stone.

Figure 7 : SEM photomicrograph of gypsum crystals produced by the cyanobacterium on petrographic section of the Apse.

It is probable that some of the gypsum is derived from bacteria. This has been proven to be true on external surfaces of marble tombstones by determination of the S^{34}/S^{32} ratio. Biologically produced gypsum gives a higher stable isotope ratio than does atmospherically produced gypsum [25].

The contribution by the alga to deterioration in our study is unclear at present. The alga creates a mass of slime which could hold moisture against the stone surface aiding in dissolution by water. In addition, the alga may provide maltose, glucose, and other secretions that could provide nourishment for the fungus. The sugars could also act as chelating agents for calcium.

If an appropriate alga, usually, Trebouxia or Pseudotrebouxia, is present then a lichenous association could result [26,27], with consequent attack of the stone by the lichen [3].

The regions of the Apse from which the micro-organisms were isolated show extensive efflorescences containing calcium, potassium, sodium, magnesium, sulfates and nitrates. These salts may have resulted in part from the action of the bacterium and/or fungus and in part from acid air pollution attack. The overall decay could have been produced by the combined effect of different deteriorating agents: water run-off, chemical dissolution by rain water containing acid air pollutants, and microbiological attack. The microbiological attack causes a significant increase in surface area due to the deep etching it produces. The increased surface area permits increased dissolution action by water run-off, a more significant factor in dissolution of calcareous stones than "acid rain", for pH above 3 [28]. That such a synergistic effect has taken place could explain the extremely fast deterioration of these areas in which vertical gullies have been carved into the wall of the Apse within 15 years.

5. CONCLUSIONS

It is clear from our preliminary investigation:

(1) that a Trichothecium sp fungus causes rapid, deep, and extensive damage to dolomite and calcite stones;

(2) the bacterium can produce gypsum which will then be available for dissolution and recrystallization.

Continuing studies will attempt to: assess the rate of deterioration of the crystals by the fungus (correlated with water and acid deterioration); the contribution of the alga to deterioration; the stimulatory or inhibitory effect stone consolidants might have on micro-organismic growth, and the effect of the micro-organisms on the consolidants.

6. ACKNOWLEDGEMENTS

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THE CERTOSA OF PAVIA: A CASE OF BIODETERIORATION

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Abstract

Heterotrophic bacteria which produce a red pigment were isolated from deteriorated moldings and statues which decorate the facade of the Certosa of Pavia, North Italy.

1. INTRODUCTION

Many moldings and statues which decorate the facade of the Certosa of Pavia, North Italy, are covered by pink-to-orange red stains(Fig.1). These stains are present only on the decorations carved in Carrara marble, never on those carved in other stones.

Since by chemical and petrographic analyses no difference was found in the composition of the stained marble pieces, when compared with sound marble, the suspicion arose, that the colouring might be due to microorganisms. In fact, it is known that microorganisms can often be one of the major causes of stone deterioration (2,3,4,5,6,7,8,11,12).

For this reason, we undertook a microscopical and microbiological study of the stained marble. The preliminary results of this study are given here below.

2. MATERIALS AND METHODS

Fragments of sound and stained marble were taken with a scalpel, under sterile conditions,as recommended in "Raccomandazioni Normal 9/82" (10).

Part of the samples were observed by scanning electron microscopy, part were crushed and either observed by photomicroscopy or inoculated into agar plates or liquid media, to see if they gave rise to microbial growth.

Other samples were taken by gently brushing the marble surface with a velvet dab, and processed in the same way.

The colonies obtained in this way were repeatedly transplanted into different culture media, until axenic cultures were obtained. The isolated microorganisms were observed by phase-contrast or transmission electron microscopy.

The culture media used were those recommended in (10) except the last one:

- Plate Count Agar (7 days at 28 °C), for aerobic heterotrophic bacteria
- Plate Count Agar with addition of $CaCO_3$ for heterotrophic bacteria capable of solubilizing inorganic salts
- Malt Agar with addition of 100 µg/ml oxytetracycline (7 days at 28 °C) for fungi

Laboratoire de conservation de la pierre
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