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Section IX: Biodeterioration

Study of the biodeterioration zone between the lichen thallus and the substrate

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SUMMARY

Studies relating to biogeophysical and biogeochemical weathering are summarized in relation to the historical application of specialized techniques to the biodeterioration zone. Recent results obtained from SEM equipped with a back-scattered electron detector, in which thalli of *Aspicilia cinerea* growing on granitic rock were examined are described. Aspects of biodeterioration of monuments are considered here, since study of this type of weathering presents unique problems, such as difficulties in sampling. However, investigation of monument biodeterioration can be carried out with techniques similar to those applied to lichens on natural rock substrates.

Introduction

The existence of mineral particles attached to, or embedded among hyphae when both dark and light hypothalli are removed from their rock substrates was first described by Fry (1922). More than fifty years later the role of lichens in weathering remains only partially understood. Recently Viles (1987) has suggested that lichens may be best viewed as one component in a complex weathering system which in some circumstances play a dominant role.

Observations of the specific occurrence of biological and biochemical weathering processes are not always easy since hydrolysis and dissolution by water is prevalent all over the earth's surface. As a result, it is necessary to study weathering microsites at the interface between living organisms (in this case lichens) and minerals (Robert & Berthelin, 1986). It is evident that besides lichens, many bacteria, cyanobacteria, algae or fungi can be found on rock surfaces, which could also be involved in the degradation and formation of minerals and rocks (Eckhardt, 1985).

Some authors (Klappa, 1979; Danin *et al.*, 1982) have considered that the value of lichens and algae in weathering should be recognized as an important indicator of palaeoenvironmental conditions.

In the contact zone between the lichen thallus and the substrate two types of weathering processes can take place: biogeophysical and biogeochemical. Biogeophysical weathering of rocks was studied more intensively in earlier lichenological investigations, while study of the biogeochemical weathering developed later.

The present work reviews the research on both types of weathering and considers the methodology used. Although reference is made to the work of other authors, a thorough review of the literature is not presented here; a more in-depth treatment may be found elsewhere in this volume Wilson (1993).

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Biogeophysical Weathering

In physical weathering, two processes, the penetration of rhizinae and thallus expansion and contraction are the most important mechanical processes. Lichens with a foliose thallus segregate minerals from the rock as a result of the adherence of rhizinae and of part of the thalli. These minerals remain fixed (joined) to the rhizinae or simply to the external surfaces of the thalli (Figure 1).

Polarized light microscopy reveals very clearly the position of the minerals attached to the thallus surface and this technique also allows observation of the minerals microdivided in the inside of the lichen thallus (Figure 2).

In the case of crustose thalli, investigation of the possible mechanical action by the thallus seems to be of more interest. Light microscope observation is interesting (Figure 3) since it allows identification of the rock minerals which are in contact with the thallus. The relatively recent technique of scanning electron microscopy with a back-scattered electron detector (BSE) allows a better understanding of the contact between the hyphae and the substrate (Figure 4). [The artificial colour was introduced to clarify the observation.] The emission of back-scattered electrons is strongly dependent on the atomic number of the target. This permits a good distinction of components in the thallus and in the lichen-rock contact zone. Moreover, the back-scattered electron scanning images, have higher resolution compared with petrographic micrographs (Bisdom & Thiel, 1981), which allows the study of the lichen-rock zone at the submicron range.

In Figure 5 it is possible to observe how thallus areolae of *Aspicilia cinerea* are situated on granitic material. The hyphae are encrusted with all the superficial minerals which finally are microdivided and embedded (Figure 6). Where the thallus covers a zone rich in micaceous material, the mica seems to be broken up following exfoliation planes in which hyphae are found (Figure 7). In Figure 8 a less magnified image of the same areole and its neighbouring areoles is shown. Here it is necessary to emphasize Two points need emphasizing here: firstly, the hyphae surround the minerals as a flowing stream; secondly, the hyphae penetrate deeply inside the rock. On the surface of the rock the penetration of a big bundle of hyphae results in the breakage of contact between minerals. When some minerals are surrounded by a stream of hyphae it is possible to distinguish lines of alteration in the embedded mineral (Figure 9). The bundles of hyphae become narrower in the interior of the rock and finally only one hypha is present in the deeper fissure (Figure 10). According to the above results it is possible to conclude that mechanical action seems to exist, because the visible part of the thallus is encrusted with fragments of minerals among its hyphae and a bundle of hyphae penetrates amongst the minerals of the rock [isolating minerals between them.] It is not absolutely clear whether the hyphae produce fissures in the rock or whether the hyphae exploit existing fissures. This point has to be more exhaustively investigated. However, it seems to be clear from the images shown that a close relationship between hyphae and minerals exists and this close relationship can favour biogeochemical weathering.

Biogeochemical Weathering

These processes are related to the dissolution, the precipitation and the formation of new minerals.

Dissolution phenomena present in a great variety of aspects. In Figure 9 the patterns of dissolution on quartz substrates can be clearly distinguished. The processes of neogenesis and reprecipitation seem to be more uniform and have received more attention. This could be due to the fact that several years ago it was very difficult to present convincing observational evidence showing the weathering effects of lichens on underlying rocks using the light microscope and ordinary chemical techniques. However, at this moment the study of the lichen-rock biodeterioration zone has benefitted from the combination of direct observation techniques, as Light Microscopy (L.M), Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), and analytical techniques such as X-ray Diffraction (XRD), Energy Dispersive X-ray Analysis (EDX), Infrared Spectroscopy (IR), Atomic Absorption Spectrophotometry and Flame Photometry.

Experimental Studies

The experimental studies concerning the role of biological factors have made very important contributions to knowledge of the biodeterioration zone. Weed *et al.* (1969) showed experimentally the alteration of mica to vermiculite by fungi. In the light of work by Robert and Berthelin (1986), it seems logical to ascribe to the lichens a chemical ability similar to microorganisms and fungi in the processes of weathering. Moreover, there have been a number of important experimental studies carried out with lichens. Schatz (1963) established that *Parmelia conspersa* thalli on an aqueous suspension reacts with mica and granite to produce a red coloured supernatant in 3–4 hours. *P. stenophylla* and *Umbilicaria arctica* also reacted with this silicate material, while *Xanthoria elegans* did not. The author attributed the pedogenic role of lichens to the action of lichen acids.

Following this line of investigation, the ability of four lichen compounds from *P. conspersa* thalli to react with granitic rocks and their minerals has been investigated (Ascaso & Galván, 1976). The ability of lichen substances to chelate Al, Fe, Ca and Mg cannot be entirely correlated with their water solubility, since stictic acid was more active than norstictic acid, although both compounds have similar water solubilities. There also exists a certain degree of extraction of the elements Al, Fe, Ca and Mg when rocks and minerals are incubated with pieces of the thallus (Ascaso *et al.*, 1976). When rocks and minerals were incubated with thalli, these fragments of thalli were more effective inducing colour-complex changes than both combinations and single solutions of selected lichen substances. Other substances besides lichen acids can produce biochemical weathering, including carbon dioxide and oxalic acid (Syers & Iskandar, 1973). The effect of oxalic acid has also been investigated under experimental conditions. Labradorite treated with oxalic acid showed extensive surface etching (Jones *et al.*, 1980). Etching was manifested by deep overlapping rectangular pits and by pronounced ridges and furrow patterns. The incubation of chrysotile fibres with oxalic acid leads to magnesium oxalate dihydrate formation (Wilson *et al.*, 1981), whilst incubation of crystalline dolomite with oxalic acid induces formation of calcium oxalate monohydrate (whewellite) and magnesium oxalate dihydrate (glushinskite) (Ascaso *et al.*, 1982). Some crystals which might correspond to calcium oxalate dihydrate (weddellite) were also observed. According to Jones and Wilson (1985), all such investigations can be considered as indirect evidence of weathering.

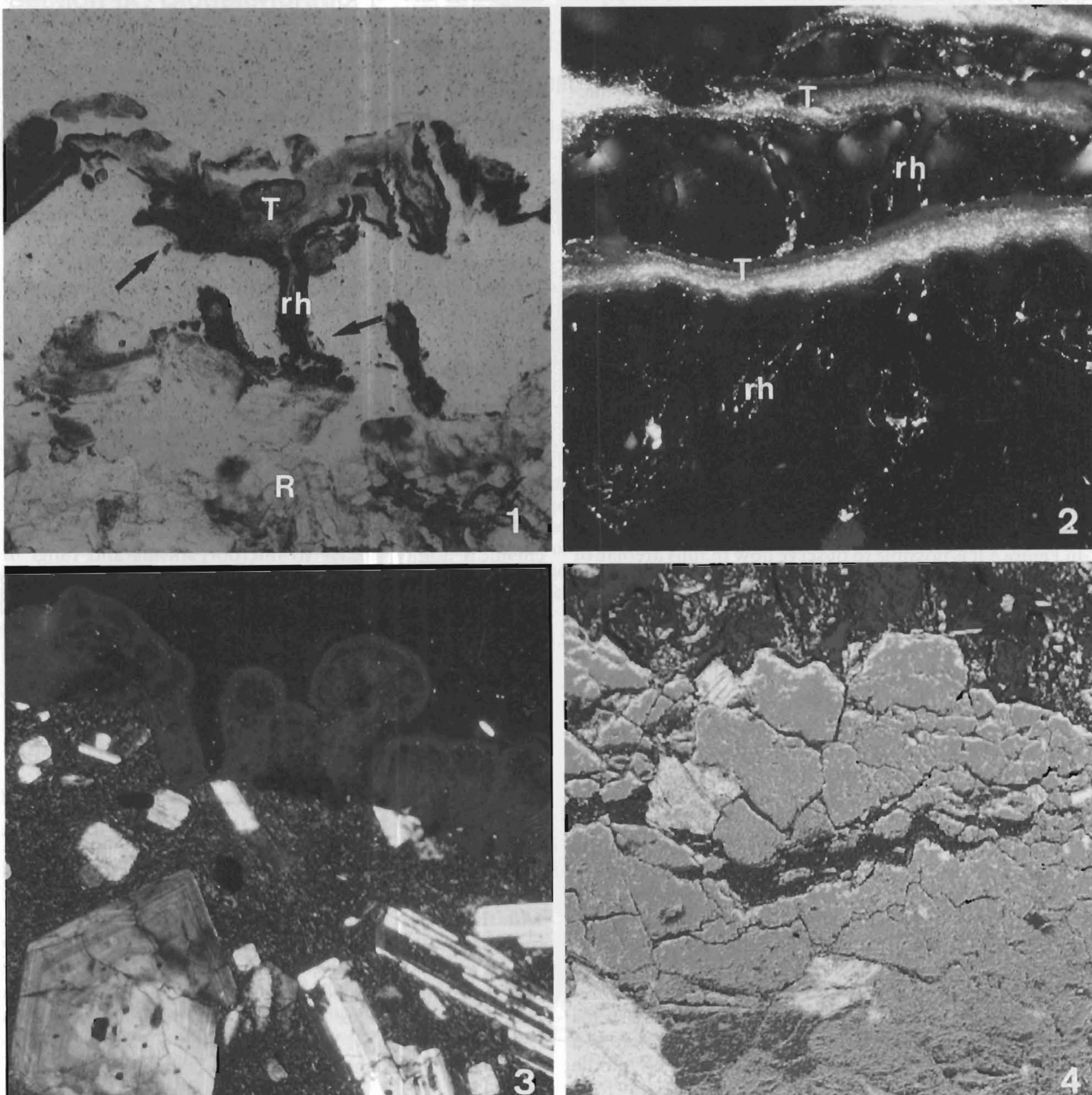

Fig. 1, 2, 3 and 4. Figure 1. Transverse section (light microscope) through *Parmelia conspersa* thallus on granitic rock. There are minerals (arrows) adhering to the lower part of the thallus and to the rhizines. R, rock; rh, rhizine; T, lichen thallus. ×50. Figure 2. Different fragment from the same thallus as that in Figure 1 in transmitted light between crossed polars. T, lichen thallus; rh, rhizine. ×50. Figure 3. Transverse section throught *Xanthoria elegans* on volcanic rock. In transmitted light between crossed polars. ×50. Figure 4. Back-scattered electron scanning image (BESI) of transverse section through *Aspicilia cinerea* on granitic rock. Artificial coloured micrograph: red – hyphae of the thallus, green – feldspar, blue – mica and rose – quartz. ×90.

Field Studies

Thallus

Some of the products formed experimentally in the laboratory have also been observed under natural conditions. These products are deposited extracellularly internally or on the surface of the thallus, or in the lichen-substrate contact zone. In nature, some metal-lichen acid complexes do occur at the rock-lichen interface which might involve direct leaching of metal cations by the lichen thallus, but some of the complexes do not occur in the zone adjacent to the rock surface. The accumulation of products in the interior of the thallus induces the accumu-

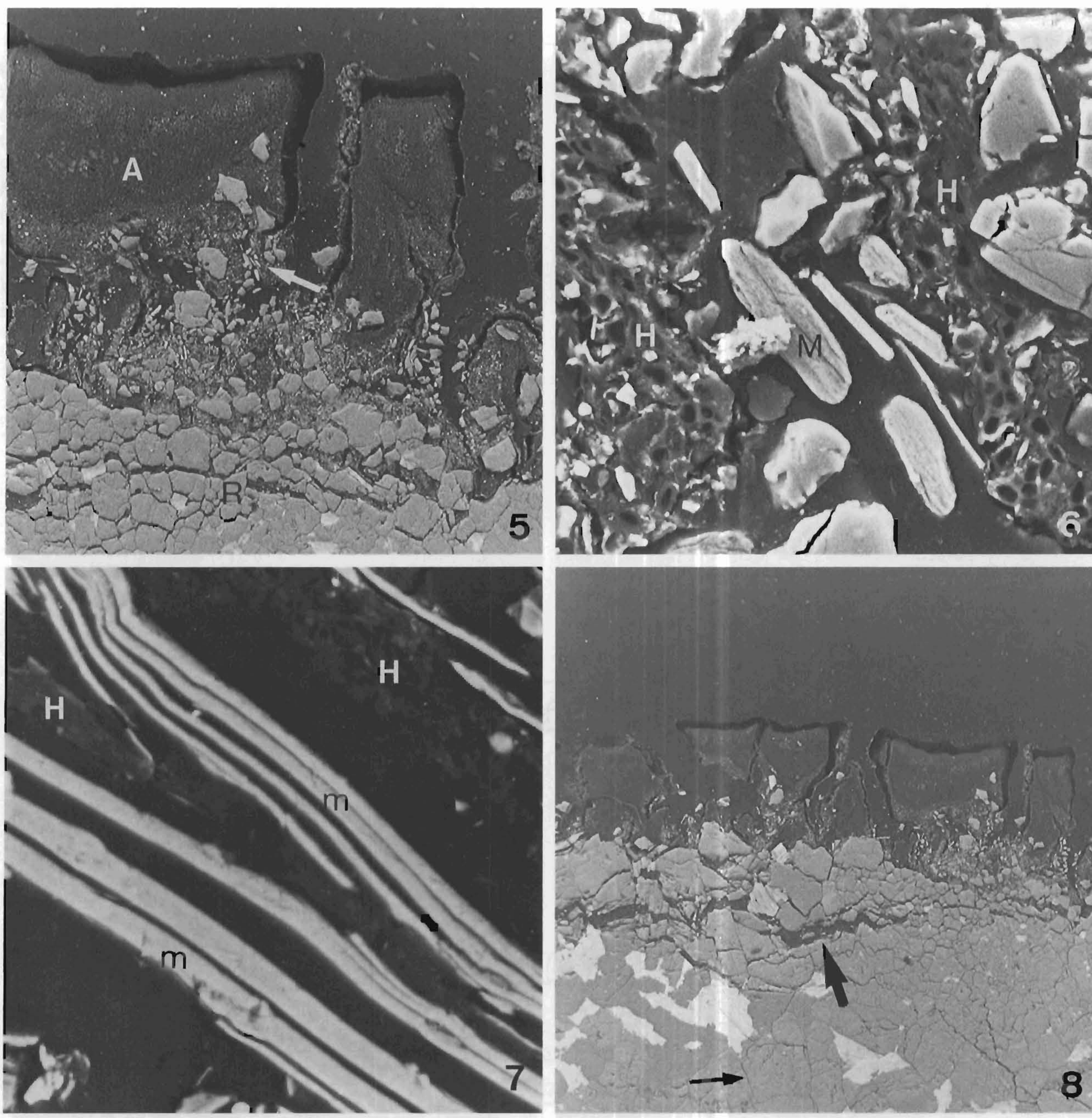

Fig. 5, 6, 7 and 8. BESI of transverse section through *A. cinerea* on granitic rock. Figure 5. Thallus areola embedding rock superficial minerals (arrow). A, areola; R, rock. ×100. Figure 6. Microdivided minerals within hyphae. Detail of arrowed part in Figure 5. H, hyphae; M, mineral. ×1000. Figure 7. Plates of mica with hyphae. H, hyphae; m, mica. ×770. Figure 8. General view showing interaction of several lichen areolas with rock surface. Heavy arrow = minerals surrounded by a stream of hyphae. Light arrow = deep penetration of hyphae inside the rock. ×40.

lation of a range of metal cations (James, 1973; Richardson & Nieboer, 1980). Ferric oxide has been identified in the ochraceous crust of *Acarospora sinopica* (Weber, 1962), and aluminium has been detected in the ferruginous crust of *Tremolecia atrata* (Jones et al., 1981). Purvis (1984) observed copper oxalate in *Acarospora rugulosa* and *Lecidea theiodes* (= *L. lacker*), and complexing of Cu by norstictic in the cortex of *L. lactea* (Purvis *et al.*, 1987) and Cu-psoromic acid in the epithecium and hypothecium of the apothecia of *Lecidella bullata* (Purvis *et al.*, 1990). This extraction of metal cations can induce a certain degree of deterioration of substrate; however, it is also important to stress that the formation of a metal-lichen acid complex within a lichen does not necessarily mean it is produced through the direct action of the lichen acid on the underlying substrate.

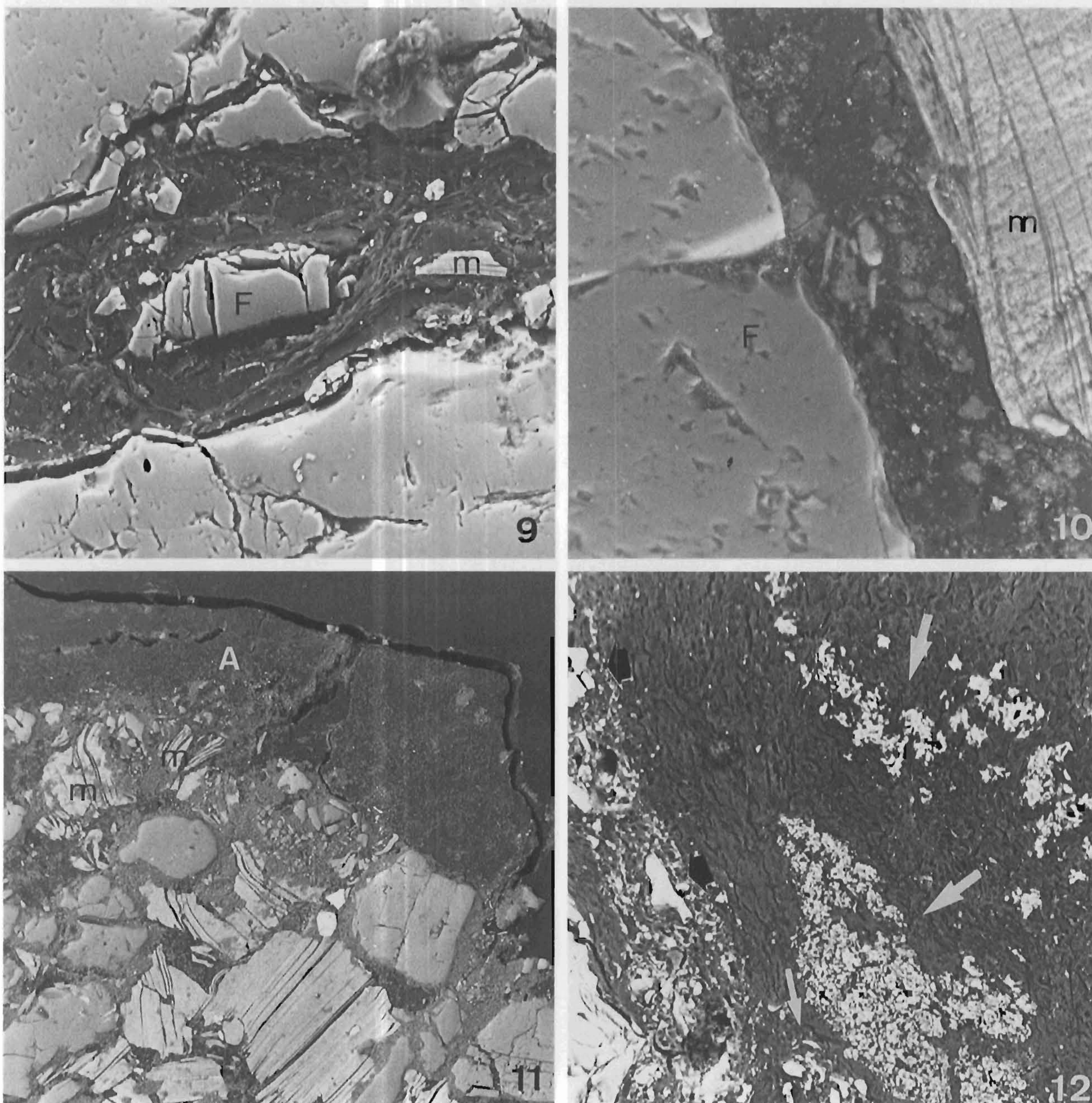

Fig. 9, 10, 11 and 12. Figure 9. Detail of arrowed part of Figure 8. F, feldspar; m, mica. ×50. Figure 10. Detail of Figure 8. Fissure between feldspar and mica minerals occupied by hyphae. F, feldspar; m, mica. ×600. Figure 11. BESI of transverse section of *Rhizocarpon geographicum* on granitic rock. Hyphae of areola are encrusted with areas of mica. A, areola; m, mica. ×100. Figure 12. BESI of *A. cinerea* on granitic rock showing different types of inclusions (arrows). ×300.

Interface

Several techniques have been used in the study of this zone. The chronological use of these techniques is shown in Figure 13. Analytical techniques have been applied to the study of the interface for 20 years. Doorman (1967) used infrared spectroscopy in an attempt to distinguish between the mineralogical material that exists on the surface of the unweathered rock and the material located beneath the lichen thalli covering the same rock.

At that time, these techniques were of great interest in understanding the mineralogy of the biodeterioration zone. Other techniques of direct observation were developed subsequently. Using high resolution microscopic techniques Hallbauer and Jahns (1977) were able to observe the biodeterioration zone beneath the thallus of *Dimelaena oreina*, and reported that the hyphae penetrated mica and quartz and produced "chemical boring". The thickness of the lichen-rock interface was estimated at 4–10 µm. The application of EDX helped to differentiate between mineral grains and the living organisms. Around the same time, the mineralogy of the interface between thalli of *Parmelia conspersa, Rhizocarpon geographicum* and *Lasallia pustulata* and granitic rock was investigated by Ascaso *et al.* (1976), using analytical techniques such as X-ray diffraction combined with TEM. The material used was obtained by scraping methods until a sufficient quantity was collected, not only the crustose thallus from the rock but also the minerals and organic matter present there. The mixture of thallus and the above-mentioned residues are treated with hydrogen peroxide to obtain a sample of inorganic material. Kaolinite and amorphous silica were found in the interface of *Parmelia conspersa* and granite. Halloysite and goethite crystals were also found under *Rhizocarpon geographicum.* The same techniques were used to study the interface of metamorphic rocks and *P. conspersa, P. tiliacea, Lasallia pustulata* and *Ramalina protecta* (Galván *et al.*, 1981). The mica is preferentially retained in the lichen thalli whereas quartz is depleted. The abundance of micas in the scraped off material of the interface observed by XRD has also been seen by the application of BSE on a transverse section of *R. geographicum* over granite. Figure 11 shows the aspect of mica embedded in the thallus areola. Feldspar and chlorite

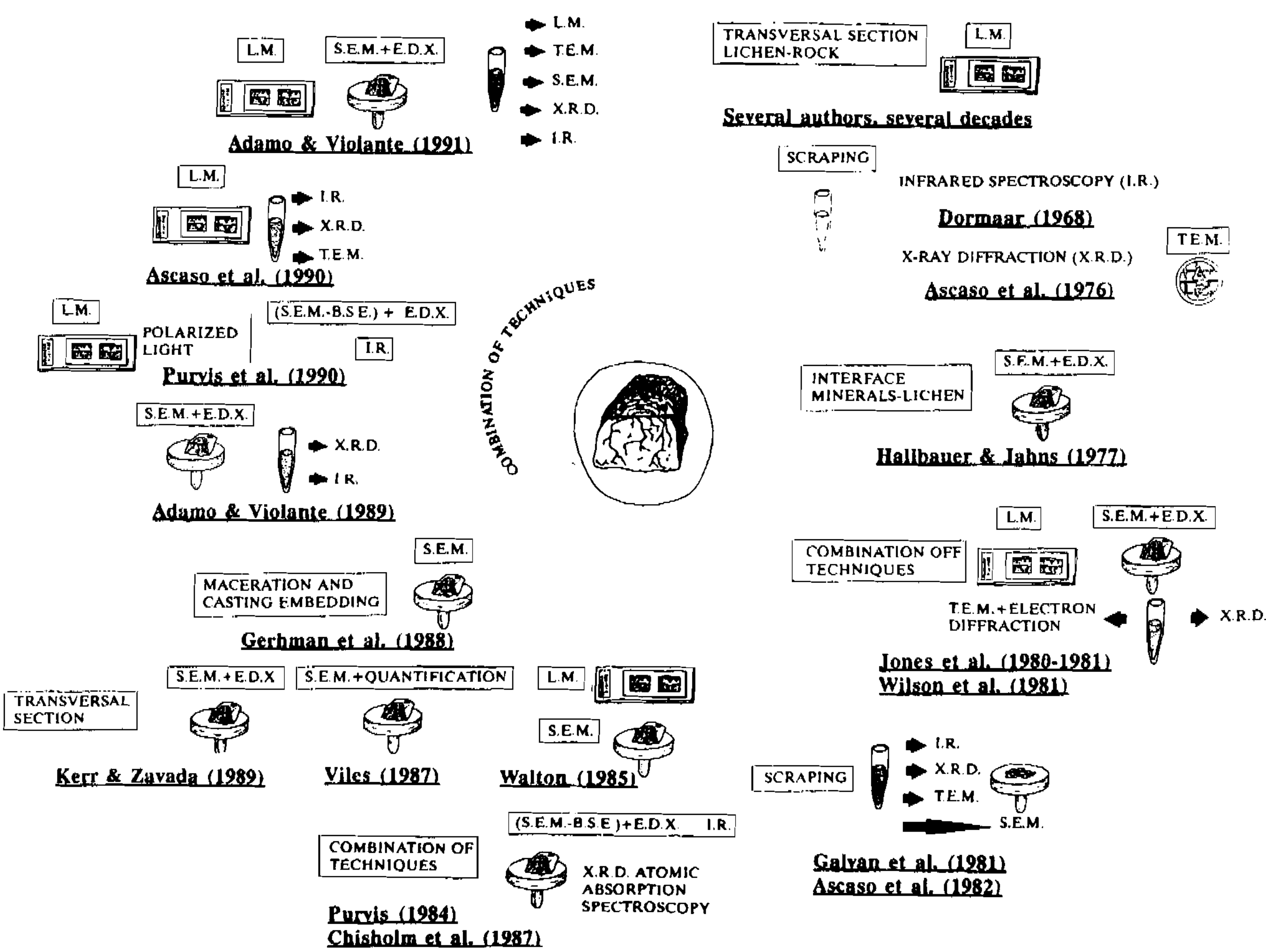

Fig. 13. Techniques used in the study of the lichen-rock interface.

from the rock are altered and the characteristic products of weathering are goethite and amorphous gels. The alterations of feldspars and chlorites seem to suggest dissolution processes and accumulation of certain elements. In this aspect, Kerr and Zavada (1989) investigated the differences between unweathered rock and rock with lichen thalli. The relative abundance of rock-forming elements on both surfaces has been studied. There is a depletion of Si on the studied substrates, while the amount of Al, Ca, Cl, Fe, K, Mg, P and S on the areas occupied by the lichen thallus increases.

Recently a study with Antarctic lichens has been carried out using LM techniques on a transverse section of lichen-rock, and also by IR, XRD and TEM analysis on scraped off material (Ascaso *et al.*, 1990). The species investigated, *Xanthoria elegans, Lecidea lapicida, Rhizocarpon geographicum* and *Bacidia stipata*, have a volcanic andesite and a volcanigenic sediment as substrate. Light microscopy with parallel and cross nicols facilitated the idenrification of the rock minerals over which the thalli wer growing and also permitted observation of mineral accumulation in several thalli. *R. geographicum* and *X. elegans* accumulate minerals in the interior of their areoles, but this effect was not observed in *L. lapicida*. This technique was also used by Walton (1985) on Antarctic lichens. The application of the IR technique shows that beneath the thallus of *Xanthoria elegans*, the bands of the IR spectra ascribed to the parent rock minerals, plagioclase and pyroxenes, decrease greatly or even disappear. However, bands appear with a maximum of 1050 cm^{-1}, indicating possible phyllosilicate formation. In all the IR spectra obtained, it can be observed that bands that were present in the rock disappear in the interface, whereas other new bands appear. When XRD is applied, the peaks observed for the scraped off material are fewer and less intense than for the parent rock. However, *R. geographicum* presents a stronger intensity in the feldspar peak in the scraped material. Other minerals, such pyroxenes, hydromagnesite and phyllosilicates, found in the X-ray diagram of the rock are not present in the X-ray diagram of scraped off material. Beneath the *Lecidea lapicida* thallus, quartz, chlorite and decayed micas of the illite type occur. It is not known if the mica of the illite type could be a newly-formed clay mineral. Calcium oxalate ($CaC_2O_42H_2O$) (at 2.5 H_2O) (weddellite) was found under the thallus of *L. lapicida* and *B. stipata.*

The presence of calcium oxalate in different forms is well documented in lichen thalli. The abundance of calcium oxalate monohydrate in the scraped off material of *Caloplaca calopisma* on crystalline limestone and dolomite was reported by Ascaso *et al.* (1982). On dolomite, large amounts of ferric oxalate ($Fe_2C_2O_2$) were also observed. In this study dealing with several lichen species, the greatest effect on the two carbonate rocks was produced by *Diploschistes ocellatus*. Calcium oxalate dihydrate was not evident in the X-ray diffraction diagrams, but a few crystals were observed by SEM. It is interesting to note the presence of other oxalates in the interface zone. As well as ferric oxalate, copper oxalate has been found in the medulla extending to the rock surface in the case of *Acarospora rugulosa* growing directly on the Cu-containing minerals atacamite and brochantite (Purvis 1984; Chisholm *et al.*, 1987).

TEM has permitted observation of imogolite, allophanes and amorphous material beneath the *X. elegans* thallus and bacteria beneath the *B. stipata* thallus. The presence of cryptoendolithic bacteria on continental Antarctic rock is well known (Friedman 1982; Friedman & Weed, 1987). These bacteria could exert effects in combination with those of the lichen thallus.

All these microscopic and microchemical techniques have been applied to scraped off material or to transverse sections from the lichen-rock contact zone, according to the literature available. Scraped material has been investigated by IR, XRD, TEM, SEM and EDX. Transverse sections of lichen-rock have been investigated by LM, SEM, BSE and EDX. In the authors opinion, one of the most promising and extremely valuable techniques is the observation and microanalysis in the back-scattered electron mode in the SEM equiped with EDX spectrograph. This technique permits not only the localization of the inorganic compounds in the interior of the mass of hyphae, or at the contact zone of the lower thallus with the rock minereals, but also the identification of their chemical composition. More recent investigation by BSE techniques of *Aspicilia cinerea* has permitted the observation of different types of inclusions within the hyphae (Figure 12). BSE scanning images provide compositional information of inclusions according to brightness (Figure 14) which indicates differences in atomic number of components and their morphology (Figures 15 & 16). The application of SEM with BSE detector and EDX spectrograph has shown that the composition of different structures found next to the rock surface or in the interior of the areola have elements such as Ca and Fe. Structures rich in calcium and iron could correspond to oxalate, as demonstrated in other thalli by other techniques, but many inorganic structures remain to be identified. Some are simply fragments of rock mineral microdivided and embedded within the hyphae, but others could correspond to iron oxides, alumino-silica gels and even clay minerals. Therefore the effect of biochemical weathering on pedogenesis might be considerable and consequently it must be taken into account in future works on this subject.

Biodeterioration in monumental rocks

The research reported so far was developed during the 1970s and 1980s. It was not until 1981 that results of research on the biodeterioration of monuments by lichens were published.

Among the techniques used in these investigations, the best known are: SEM, and Electron probe X-ray microanalysis (EDX). Nevertheless, other techniques such as TEM (with embedded and ultrathin sectioned rather than scraped off material), BSE, and Raman microscopical analysis, have also been employed (Figure 19). All these methods are particularly interesting since they are considered nondestructive. Although only the methods shown have been used in the study of the biodeterioration zone, there are other microanalytical techniques that have been useful in elucidating the causes and mechanisms of monument damage (Van Gieken *et al.*, 1991) and could be employed in lichen research in the near future. These authors proposed techniques like secondary ion mass spectrometry, particle-induced X-ray emission and laser microprobe mass analysis. Related to the investigations of lichens on monuments, there is one study involving fungi which must also be taken into account. Eckardt (1985) investigated biodeterioration of sandstone monuments and frescoes. The solubilization of cations from anphibolite, biotite and orthoclase by yeast and filamentous fungi is shown as well as the solubilization of cations from biotite by *Aspergillus*

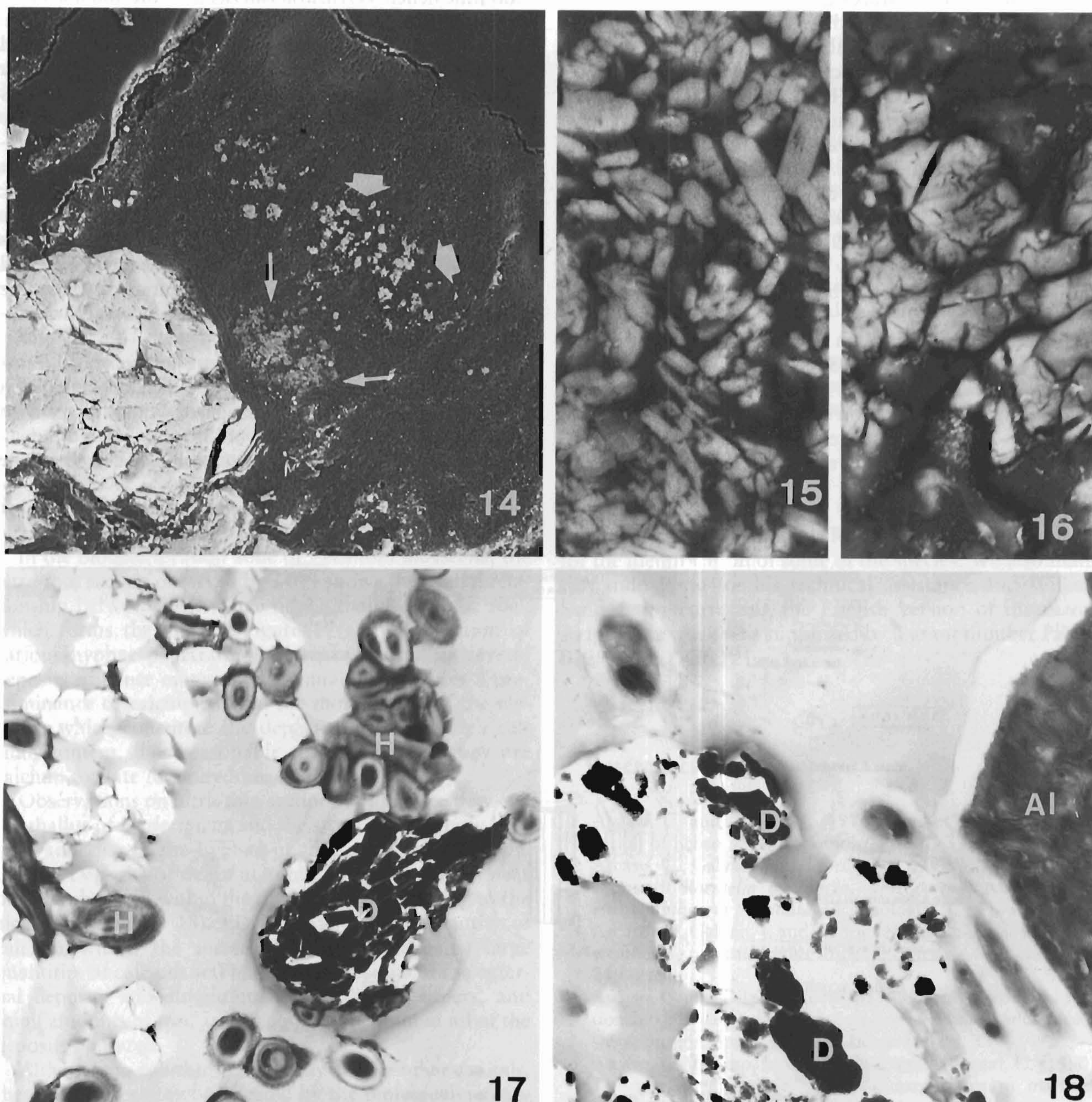

Fig. 14, 15, 16, 17 and 18. *A. cinerea* as in Figure 12. Figure 14 showing differences in brightness (atomic number contrast) of inclusions (see arrows). ×150. Figure 15 and 16 showing differences in morphology. ×3000. Figure 17. Ultra-thin transverse section of *Lecanora albescens* on rock. TEM study. D, dense deposits; H, hyphae. ×5000. Figure 18. Ultra-thin transverse section of *Caloplaca albescens* on rock. TEM study. Al, algae; D, dense deposits. ×6300.

niger. This author suggested that "The ability of these organisms to degrade minerals can be demonstrated experimentally. An increased use of bacteria and fungi in biotechnology and bioengineering also demonstrates that microoganisms are capable of conducting processes on a large scale".

Investigations of the biodeterioration zone of lichens on monuments show on the one hand aspects known from the study of lichens on naturally occurring rocks and on the other hand new features generated by urban environmental conditions. One of the aspects already known is the presence of calcium oxalate monohydrate and dihydrate. Living and/or dead encrusting lichens are found associated with "scialbatura" (Del Monte & Sabioni 1987). This patina has the two calcium oxalates as main components. These compounds are also found beneath some thalli on marble columns of the Basilica of Santa Maria Assumta (Venice) (Salvadori & Zitelli, 1981) and also beneath the thallus of *Dirina massiliensis* forma *sorediata* in the Palazzo Farnese (Seaward & Giacobini 1989; Edwards *et al.*, 1991). Among the new aspects of this investigation, the urban environment has been extensively investigated by Seaward *et al.* (1989).

The influence of the urban environment was taken into account by Saiz-Jimenez (1981) when the detrimental effect of *Lecania erysibe* on the Giralda of Sevilla was studied. The biodeterioration zone is defined by a thin film of about 1–2 mm of substrate detached when the lichen is removed. According to this study, the pollution affected the lichens, but the high pH of the calcareous rock can have a buffer effect. Recently, the calcareous walls of Jerusalem and marble of Roman monuments have been investigated by Danin & Caneva (1990). The fact is that in the biodeterioration zones in an urban environment, the chemical compounds of pollutants act on the one hand while on the other hand a special microclimate is generated, which can change the ability of water retention. Ciarallo *et al.* (1985) carried out an investigation on the endolithic lichen *Verrucaria calciseda* on tile and marble in relation to its ability to retain water. The hyphae penetrate to different depths in both materials (1 mm in tiles and 0.2 mm in marble). The mechanical damage is caused by the great inhibition ability of the lichens (up to 35 times their own weight) and the resulting variation of turgidity of their endolithic parts, which behave like wedges. Therefore the interaction between the urban environment with the thallus action is of great complexity. Seaward *et al.* (1989) summarized this problem: "Since damage can be brought about by a multitude of both physical and chemical processes, exacerbated in urban environments, as well as through biodeterioration, it is essential to determine that damage to any particular monuments is indeed attributable to lichen action".

Fortunately many monuments are located outside urban environments and the action of the lichen thalli on these monuments can be investigated without an urban influence. Modenesi & Lajolo (1988) investigated the action of *Aspicilia contorta* on a marble collected in the

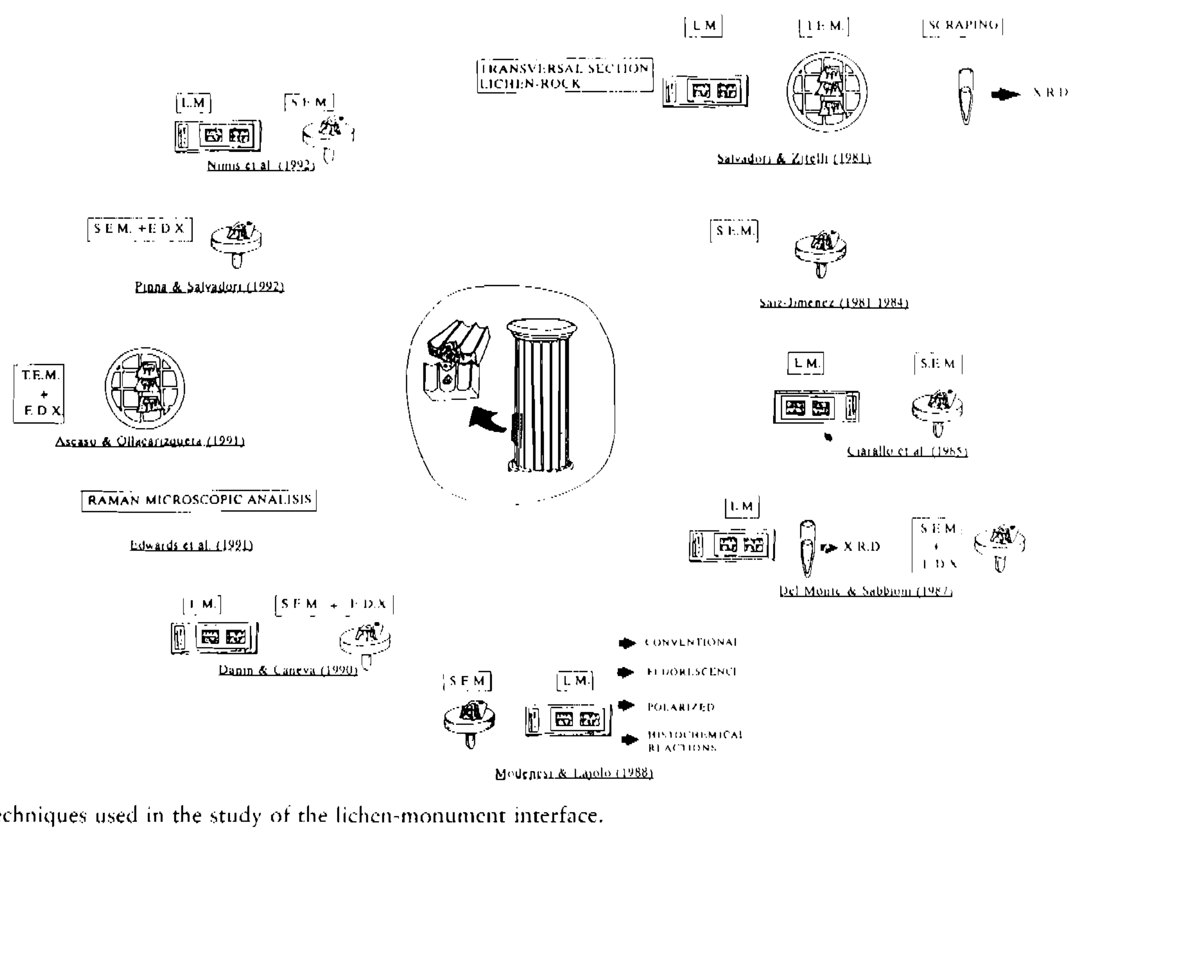

Fig. 19. Techniques used in the study of the lichen-monument interface.

Carrara quarry. The study of the biodeterioration zone was carried out by means of histochemical techniques. The structural and morphofunctional study was carried out by parallel observations with polarized light and epifluorescence. The author tried to identify lichen metabolic products affecting mechanical or chemical stability of the substrate. These techniques reveal mucopolysaccharide substances that can contribute to thallus hydration. Observations by SEM showed that hyphal branches do not penetrate the hard crystals of calcite as they are eroded superficially. The hyphae penetrate deeply between the grains, separating them further. The penetration of bundles of hyphae, probably due to the pre-existent disaggregation of the grains, is observed. Groups of algae carried by hyphae are observed at a depth of 400–500 μm.

A monument situated outside the urban environment was investigated at Silos Monastery in Burgos, Spain (Ascaso & Ollacarizqueta, 1991). *Lecanora albescens* was collected on the abacus of capital number 63. In the zone of the capitals themselves, no lichen thalli were found, with the exception of capital number 5, from which *Caloplaca decipiens* was collected. The biodeterioration zone was investigated in this work by TEM observation of ultra-thin sections. In nearly all previous works of this nature, TEM was used to study the lichen-rock interface by obtaining scrapings. Scraping is a suitable technique with respect to mineralogical aspects, but it is a destructive technique. The application of TEM to the study of monuments is advantageous due to the small amount of material required. EDX technique coupled with TEM allows the determination of the elemental composition of the structures observed.

In the biodeterioration zone of *Lecanora albescens*, the ultra-thin section observed by TEM shows the spatial relationship between the hyphae of the thallus and the rock which forms the abacus. Figure 17 shows a section of various hyphae penetrating the rock, as well as several deposits of dense material. EDX analysis indicates a predominance of calcium. Given the morphology of the elements which constitute the deposits, and their high calcium content, it is reasonable to suppose that they are calcium oxalate monohydrate crystals.

Observations on ultra-thin sections at interface between the thallus of *C. decipiens* and the stone capital show bacteria adhering to the hyphae of the lower surface of the thallus. Deposits of dense material are seen in zones near these hyphae and within the thallus itself, extending to the algal layer (Figure 18). EDX reveals a large quantity of calcium within the intrathalline dense deposits, large quantities of calcium and magnesium in some of the external deposits and aluminium and silicon in others, and small amounts of iron and traces of potassium in all of the deposits analyzed.

Although no epilithic lichens grow on the other capitals, these substrates may be affected by the biological activity of bacteria, fungi or certain endolithic lichens whose presence may be difficult to detect because of severe limitations on sampling.

Capital 53, which showed no apparent epilithic lichens, was chosen for petrographic study, since it was among the most deteriorated within the cloister complex. SEM revealed unidentified filaments with an externally segmented appearance. To determine whether these filaments were of a biological nature, they were prepared for TEM observation. This revealed structures with walls similar to those of fungi in appearance and thickness. Within the part which could represent the cellular cytoplasm are clear zones which resemble the concentric bodies characteristic of lichenized fungi.

EDX study of dense deposits occurring in the vicinity has shown an elemental composition similar to that described for dense deposits external to the thallus of *C. decipiens.*

The presence of calcium-rich deposits in the interior of *L. albescens* and *C. decipiens* thalli, and their laminar appearance which suggests calcium oxalate monohydrate, is in agreement with numerous studies of natural rock and monuments which have identified that compound within the thallus interior. The accumulation of other types of deposits externally in close proximity to thallus hyphae should be more thoroughly investigated. These results show that TEM combined with X-ray microanalysis is of great assistance in observing dense deposits within interior spaces of the different layers of the lichen thallus, and in determining their elemental composition. The technique is suitable for samples taken from monuments, where only very small quantities of material may be removed.

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