The Role of Photosynthetic Microorganisms on Ancient Monuments

A Survey of Methodological Approaches

Abstract

Among the factors that determine the deterioration of ancient monuments, biological forms have been extensively investigated in the last two decades, but in many cases their role has not yet been completely ascertained. In this field, it is mainly autotrophic and heterotrophic bacteria, lichens and fungi which have attracted the attention of biologists, and several investigations have been concerned with understanding their action in the transformation of the substrate, and in assessing their deteriorative function.

Prokaryotic and eukaryotic microalgae have been described from numerous different sites, nevertheless few investigations have been made on the origins, the ecophysiological characters and the deteriorating activity of the various species found, as well as on the interactions between these microorganisms and their geophysiological and biological environment.

The exposure of any type of material to the atmosphere leads, after some time, to biological growth, and the presence of light, together with humidity, allows the development of phototrophic microorganisms that can cover the surfaces producing epilithic or endolithic algal films or mats. Airborne cells and spores of the species present in the surroundings of the monument can be the first colonizers ; the production of slime by pioneer species can facilitate the adhesion of other organisms and dust particles, and the contraction and expansion of the extracellular polysaccharides can result in mechanical damage. Furthermore, the release of inorganic and organic metabolites by microalgae determines interactions either with the substrate, which can be corroded, or with bacterial and fungal populations that can utilize these compounds and increase deterioration. Finally, the protective function of algal films on monuments against the corrosion resulting from heavy air pollution must be evaluated.

An overview of methodological approaches to algal studies in relation to their role on monuments is presented together with some technical aspects.

Introduction

Since the first and famous studies on the « maladie verte », that affected the prehistoric mural paintings in the caves of Lascaux in France (Lefèvre, 1974 ; Lefèvre and Laporte, 1969) the presence of photosynthetic microorganisms has been reported by a number of different authors. Microalgae have been found on buildings and stones exposed to the open air (Anagnostidis *et al.*, 1983 ; Favali *et al.*, 1978 ; Giaccone *et al.*, 1976 ; Giacobini *et al.*, 1979 ; Pietrini *et al.*, 1985 ; Tiano, 1979 ; Wee, 1988 ; Wee and Lee, 1980), as well as on walls and paintings in caves, churches, hypogea, and more generally in confined environments characterized by high humidity (Albertano and Grilli Caiola, 1989 ; Andreoli and Rascio, 1982 ; Dupuy *et al.*, 1976 ; Giacobini *et al.*, 1979 ; Grilli *et al.*, 1987 ; Leclerc *et al.*, 1983 ; Tomaselli *et al.*, 1979 ; Van der Molen *et al.*, 1980). The colonization is most frequently due to cyanophytes and chlorophytes, which can also occur as free lichen phycobionts, but sometimes to diatoms, and less commonly to xanthophytes and rhodophytes. Under peculiar environmental conditions monoalgal populations can occur, but more often different algal species appear associated with bacteria, fungi, mosses and also higher plants. Generally the species recorded are typically subaerial and ubiquitous. A recent review of algae from terrestrial habitats with a useful distinction among the different lithophythic species has been made by Hoffmann (1989), who listed the numerous taxa found on stones, in soils and in caves. Grant (1982) reviewed the fouling of terrestrial substrates relating a variety of materials affected by algal growth and the possible methods of control. For coastal and saline environments the review by Fletcher (1988) on marine algae is also relevant.

Detection

The detection of the presence of photosynthetic microorganisms and the assessment of the lithophytic group they belong to (Hoffmann, 1989), represent the first steps in the investigation. The presence of microalgae is frequently revealed by variously coloured patinas on the substrate but microbiological investigations may also be required to reveal them.

The determination of the algal species usually comes from the microscopic examination of fresh samples scraped from the substrate in the least destructive way. Culture methods and isolation of monoalgal or axenic strains are often essential in order to define the taxonomic position of the species present (Albertano and Grilli, 1988b ; Giacobini *et al.*, 1979 ; Pietrini *et al.*, 1985). More commonly light microscopy, but also epifluorescence, scanning (SEM) and transmission (TEM) electron microscopy help in the identification of the taxa, as well as the definition of the relationships among microorganisms and substrate (Albertano and Grilli Caiola, 1990 ;

Alessandrini, 1982 ; Giacobini *et al.*, 1985 ; Grilli Caiola *et al.*, 1987 ; Koestler *et al.*, 1985 ; Rascio and Andreoli, 1982).

The need for quantification of the microorganisms present in a known area leads to the employment of different counting procedures (Raccomandazione Normal, 1982). To this purpose, a comparison of various enumeration methods and removal techniques applied to bacterial populations has been made by Lewis and May (1985). Moreover comparable numerical data on microbial growth on monuments would improve the data bank systems (Caneva *et al.*, 1985, 1989).

Origins

So far the origin of the species found on the different sites has been scarcely investigated. Some authors have used agar plate controls for the microflora present in the air or near to the substrate studied (Lefévre *et al.*, 1964 ; Jaton *et al.*, 1985 ; Schlichting, 1975 ; Wee, 1982). Airborne prokaryotic and eukaryotic microalge have been listed by Brown *et al.* (1964) and Lee and Eggleston (1989). This problem has also been considered by Brunet and Vidal (1980). Information on general theory and practice in studies on airborne microorganisms can be found in Cox (1987).

Interactions

The vegetative cells or spores transported by air, rain or animals are subject to environmental selection which is a product of the surrounding biological environment (species competition), climatological characters and the geophysical nature of the substrate. A survey has been made by Krumbein (1987) on the action of microoganisms on colonized substrates, with special attention to the definition of the terminology used in biological weathering. The possible mechanisms involved have been more recently reported by the same author (Krumbein, 1988) and by Eckhardt (1985).

Papers on weathering patterns have been published by Danin (1986), on the interactions between the biological, climatological and geophysical environments by Brunet and Vidal (1980), Del Monte (1990) and Leclerc *et al.* (1983), and on the action of lichens by Jones and Wilson (1985) and Gehrmann (1988). In this respect the effects of air pollution on the selection and succession of microorganisms must also be considered.

The destroying mechanisms of photosynthetic microorganisms on the various substrates (Grant and Bravery, 1985 ; Koestler *et al.*, 1985) have not yet been extensively tested. It is ascertained that the damaging effects on stones are produced by epi-, endo- and chasmolithic species (Krumbein, 1987 ; Schneider, 1976). A demonstration of calcium uptake by algal cells has been given by Bech-Anderson (1986). Studies on the presence of extracellular mucilages increasing disruption damage by physical contraction and expansion of the polysaccharides have been undertaken (Palmer, 1990), as well as investigations of their adhesive role in marine fouling (Chamberlain, 1976). Attention has also been focused on the metabolic relationships among the various microbial species present in algal associations (Albertano *et al.*, 1990 ; Krumbein, 1987).

Some quantification of the damaging action of microalge is needed in order to assess their negative role, and laboratory experiments should determine the fouling time, the decay action and the time of substrate decay. A methodological proposal has recently been put forward by May and Lewis (1988) for bacterial populations.

In the case of limited negative action on the substrate, the protective function of algal films against the effects of heavy corrosion due to air pollution can be considered (Krumbein, 1988).

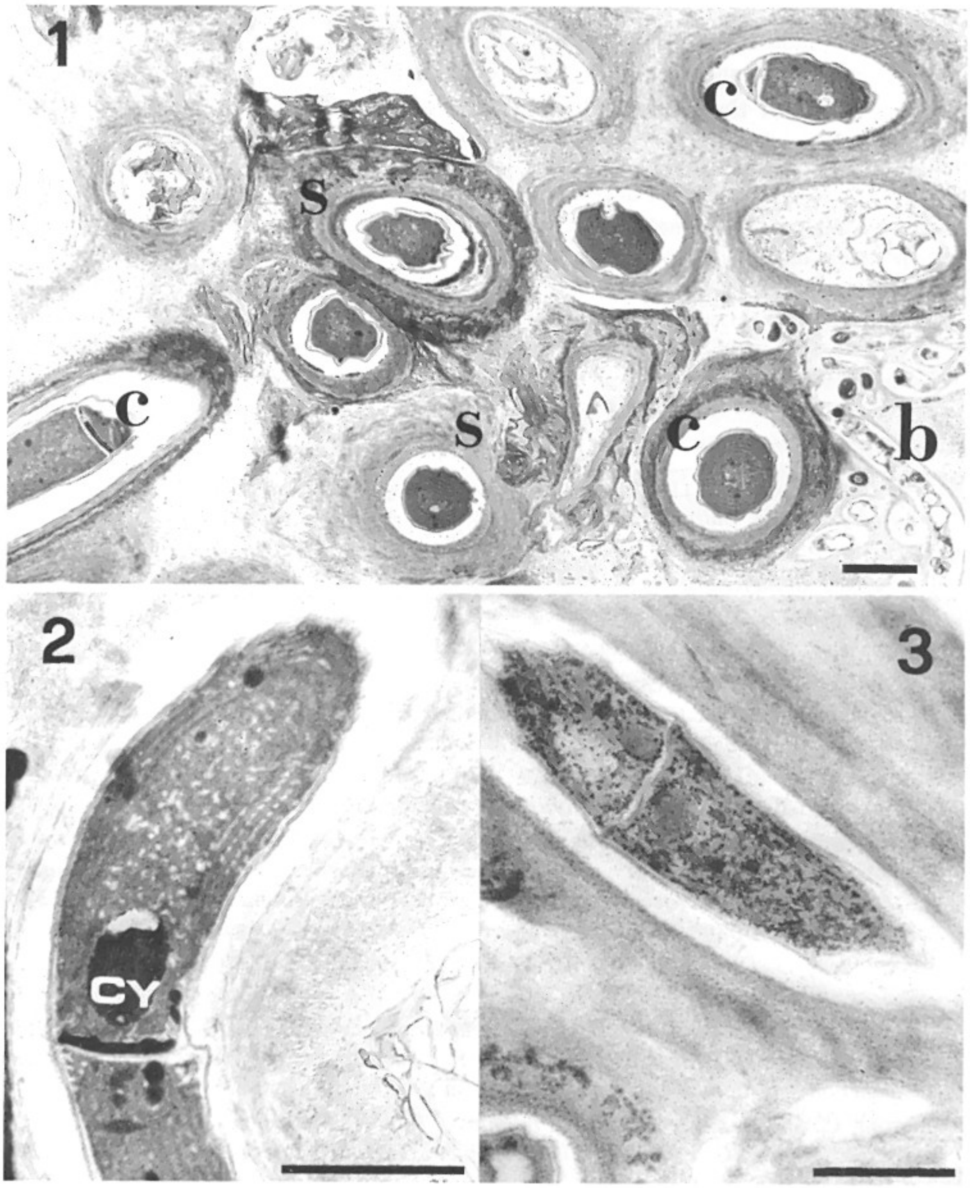
In addition to the above mentioned techniques, the methods employed by research workers in microalgal investigations also include casting-embedding-etching techniques (Krumbein, 1987), SEM-EDAX analysis of element biotransfer (Bech-Anderson, 1986) and cytochemistry for the characterization of cell structure and metabolic condition of the photosynthetic microorganisms (Albertano *et al.*, 1990) (Figs. 1-3). Progress in the identification of organic compounds released by microorganisms should be attained by the new techniques (Oelting *et al.*, 1988).

Protection

Several research workers have employed control methods to remove photosynthetic microorganisms from buildings, walls, paintings and other materials (Grant, 1982 ; Richardson 1987). The effects of algicides (Dupuy *et al.*, 1976 ; Pietrini *et al.*, 1985 ; Tiano, 1979), antibiotics (Lefévre *et al.*, 1964), gases and sprays (Lefévre and Laporte, 1969) and UV irradiation (Van der Molen *et al.*, 1980) have been tested in « in situ » and laboratory experiments, as well as the behaviour of protective plastic materials (Favali *et al.*, 1978).

Whatever the method chosen, before the employement its interference with the substrate, the environmental risk, the time of its effectiveness and the cost of treatment must be carefully evaluated. Moreover, after the experimental use of control methods, the recolonization processes and/or the changes in the biological composition of the associations must be followed.

In addition, investigations on the ecophysiological characters of the taxa involved can improve knowledge of specific ecological ranges, and thus allow a limitation of such environmental parameters as humidity or light (Albertano and Grilli Caiola, 1988a ; Albertano and Grilli Caiola, 1989).



TEM micrographs : bars = 2μm.

Fig. 1. Thin sections of samples scraped from roman frescoes in the Domus Aurea in Rome, where an algal association was present at extremely low light intensity. The presence of bacterial populations (b) among the filamentous cyanophytes (C) has been favoured by the mucilagenous sheaths (s) surrounding the trichomes.

Fig. 2. In the longitudinal section a large structured granule of cyanophycin (cy) inside the cyanophyte cell functions as a nitrogen supply.

Fig. 3. The PATAg reaction for the detection of polysaccharides provided evidence of the polysaccharidic composition of cyanophyte sheaths and of carbohydrate reserve among the photosynthetic membranes : glycogen appears as dark granules.

In conclusion, all results obtainable by the different methods employed need a prior knowledge of climatological data, the physical and chemical characteristics of the substrate, the biological environment and environmental pollution in order to be usefully interpreted.

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