Biodegradation of Cultural Heritage: Decay Mechanisms and Control Methods

Piero Tiano

CNR - Centro di studio sulle "Cause Deperimento e Metodi Conservazione Opere d'Arte", Via G. Capponi 9, 50121 Firenze, Italy.

1. INTRODUCTION

Our cultural heritage is made with almost all type of materials produced by the nature and used by men to realize several types of artefacts from very simple mono-components to complex structures integrating inorganic and organic matters. These cultural heritage objects, even if made with the more "resistant" stones and metals, are influenced by the environmental parameters, which can modify their structure and composition. Furthermore, being them inserted into the "biosphere" can be decayed by biological mechanisms. The biotransformation process has a worldwide diffusion and attains highest levels in warm-humid climates where the environmental conditions are extremely favourable to the growth of most organisms.

The role of conservators is to try to avoid weathering processes and of restorers to repair the damages undergone.

Cultural heritage assets are exposed to weather and submitted to influence of environmental parameters. Physicals, chemicals and biological factors interact with constitutive materials inducing changes both in its compositional and structural characteristics. A certain aspect of matter transformation is due to the metabolic activity connected with the growth of living organisms. This activity is needed to maintain in equilibrium the "matter transformation cycles" and contribute to very important aspect of "life" such as to transform hard rocks in soft soils (pedogenesis) or to reduce the complex biological structures into the originate simpler components. In fact, several kinds of micro and macro organisms can find a suitable habitat for their growth either on monumental buildings, archaeological remains and other works of arts made with wood, textiles, paper etc. The living species dwelling on these materials are ranging from microscopical bacterial cells to higher plants and animals. The growth process and vegetative development of organisms have a direct consequence on the conservation of cultural assets. The main types of damages derived from metabolic activity of organisms are related with *physical, chemical* and *aesthetical* mechanisms, while the intensity of these damages are strictly correlated with: type and dimension of the organism involved; kind of material and state of its conservation; environmental conditions, micro-climatic exposure; level and types of air pollutants.

Various methods have been used to classify and quantify the micro and macro organisms involved in the biodeterioration processes. The ecological and physiological parameters concerned with the development of specific biodeteriogens have been investigated together with the effect of their growth on material weathering. Conservative interventions using direct and indirect methodologies have been applied in order to stop or slow-down the biodeterioration process. The methodologies and products must be chosen taking in mind substrate conditions and species to be treated, standing to not cause negative interference with materials and with low environmental impact.

2. Inorganic substrata

The biodeterioration of inorganic materials used in the works of arts is essentially a process that involves monumental rocks and archaeological remains, being other materials such as glasses and metals practically unaffected by this mechanism. Only a case is reported in which the ancient glass windows of a medieval church have been etched by algae, while some corrosion pattern of buried metals can be attribute to an indirect action due to the surrounding microbial activity.

2.1 Stones

The decay of rocks and freshly exposed monumental stones is a complex process in which physical and chemical mechanisms are usually considered the main factors, it generally starts with alteration processes due to the synergetic action of rain, wind, sunlight and freezing/thawing cycles. Thus the initial smooth and clean stone surface becomes progressively rough and more porous, with the formation of micro cracks and fractures within crystal grains. Moreover, the pollutants, whose concentration level is continuously increasing in our atmosphere, form deposits of particles, black encrustation and leave secondary reaction products on stone surfaces. The consequence of these combined actions is a loss of cohesion with dwindling and scaling of stone material and with general weakening of the superficial structural strength. |1-6|.

We consider in particular the biodeterioration mechanism and the role played by several biological agents (Biodeteriogens) both in the weathering of rocks |7-10| as well as in the decay of stone monuments |11-20|. In fact, different kinds of organisms can grow using the mineral components of a stone and its superficial deposits. The main consequence of their metabolic activity, such as the excretion of enzymes, inorganic and organic acids and of complex forming substances, is the dissolution of minerals of the substratum. Moreover, the growth and the swelling of some vegetative structures (e.g.: roots and lichenic thalli) induce physical stresses and mechanical breaks.

2.1.1 Bioreceptivity Of Stone Materials

The concept of *bioreceptivity* was focused by Guillitte |21| as the aptitude of a material to be colonised by one or several groups of living organisms. Otherwise, the mere occurrence of organisms on stone surfaces does not automatically imply destructive action but in some cases could be considered: as only aesthetic detrimental appearance |22|; perceived aesthetically pleasing or credit these with a protective role against weather induced aggression |23|.

The *bioreceptivity* must be related with the totality of material properties that contribute to the establishment, anchorage and development of flora and/or fauna. In stony material it relates mainly with surface roughness, moisture content, chemical composition of the outer layers and with the structure-texture of the rock.

The "Primary or Intrinsic Bioreceptivity" is connected with the initial potential of colonization of a sound stone. Then, following the evolution over time of surface characteristics, under the action of colonising organisms and environmental factors, it becomes the more important "Secondary Bioreceptivity". The conservative treatments applied on stone induce a "Tertiary Bioreceptivity". Moreover, we can have "Extrinsic Bioreceptivity" when the stone colonization is essentially due to the presence of settled matter not related with the beneath stone |21|.

One can assess the bioreceptivity of a material to an organism by artificially inoculating the material with the diaspores of the organism itself and placing them under optimal environmental conditions (growth chamber). Thus a specific "Bioreceptivity Index" could be determined for a stony material related to its *susceptibility to biodeterioration.* To achieve this result a multidisciplinary team must operates to conduct integrated studies, as standard as possible, choosing the most suitable parameters for measuring the bioreceptivity either for quantify the microbial mass or to characterise the stony material |24|. However these studies are always indicatives, as many types of colonisation are part of a synecological mechanism and not all the organisms present on exposed stone surfaces can develop on artificial conditions, even if very close to those present in nature. Hence the test made with a single organism or few isolated strains can become either impossible or completely atypical. In fact, the results reported in few lab experiments can be considered pertinent only for the stones used, the organisms tested and the incubation conditions applied |24-26|.

2.1.2 Ecology of stone substrata

We must take into consideration that the chemical composition and physical characteristics of stone surfaces change over the time of exposure to weather. The initial exclusively mineral composition progressively is implemented with organic matter, contemporary the physical structure changes from almost smooth and whenever polished to a rough finish with increase of the stone porosity, and in the worse cases "sugar" decay with sandy transformation of the rock can takes place. In general, the kind of biological agent that develops on a stone is affected by the rock type (e.g.: alkaline limestone or acidic sandstone) and its surface conditions, together with the environmental location and the climate to which the monument and the stone are exposed. The various nutritional and finishing conditions of an exposed stone substratum influence the temporal and spatial distribution of the biocoenosis, which can develop on it.

2.1.3 Ecological succession

The pioneering micro organisms that develop on a surface, which is strictly mineral, without any presence of organic matters, are those lithophilous, photoautrophic, chemiolithotropic and the lichens. A fertilized surface with very small amounts of organic matter and growth factors (*Oligotrophic* or *Copiotrophic*,), indifferent of their origin either from airborne particles or derived from previously developing organisms, can support the growth of oligotrophic, heterotrophes bacteria, actinomycetes and microfungi. The development and the death of successive generations of micro organisms, together with the accumulation of ammonia and phosphate compounds coming from the atmosphere, enriches the stone with organic matter and essential growth factors (*Eutrophic*) allowing the development of heterotrophic micro organisms. Then with the progressive worsening of the physical surface conditions, a “soiling” effect is induced which allows the implantation and germination of the most common vegetal reproductive bodies deposed from the air on surfaces. These range from cryptogames spores up to higher plants seeds. Finally a more complete ecological niche is established with the presence of some very adaptable specialized microfauna. Some micro organisms can growths only on stone surfaces (*Ephilitic*) others prefer more protect habitats like small crevices and fissures (*Chasmolitic*) or inside the stone structure (*Endolithic*).

Favourable environment and the presence of energy sources with organic and inorganic nutrients allow the biological settlement of an exposed stone surface. When we analyse this colonization we find that a photosynthesis-based consortium of organisms generally composes it. It shows seasonal changes, following the different levels of humidity and the life cycles of the single species, taking into account that many survival strategies have been developed to survive unfavourable living conditions |18|. The pioneering colonization steps, of a single or very few strains, can be followed in time observing fresh exposed stones or cleaned surfaces after restoration interventions.

The metabolic activities of these organism such as the production of extra cellular polymers; the liberation of chelating compounds and of organic/inorganic acids; together with the presence of coloured pigments and to the mechanical pressure exerted by growing structures or shrinking/swelling phenomena induces different types of damages:

physical (abrasion, mechanical stress);

chemical (solubilisation, new-reaction products);

aesthetical (coloured patches or patinas and crusts).

The consequence of the biological activity on stone surfaces is the formation of biofilms, coloured patinas, encrustations and the presence of vegetative and reproductive bodies. Moreover, the stony structure can be interested to dwindling, erosion, pitting, ion transfer, and leaching processes.

2.1.4 Organisms

The principal groups of organisms investigated in relation with the biodeterioration of monumental stones, with the specific methodologies of study and their relative importance in decay processes are:

Bacteria

The researches have been mainly directed to make a statistical survey of the possible functional groups present on various monuments and to study their ecological and physiological significance |27-33|. In fact several species of bacteria have been detected and isolated from a stone monument either from its surface or at a depth down to several millimetres, using microbiological techniques. The micro organisms investigated can be divided in two main nutritional groups:

a) Chemiolithoautotrophes

b) Chemioorganotrophes

a) The first very specialized group contains almost exclusively micro organisms belonging to the sulphur and nitrogen cycle. Various species in these groups give out, as their end-metabolic product, a strong inorganic acid (sulphuric and nitric respectively). Initially the attention was focused upon the sulphur-oxidizing bacteria (*Thiobacillus spp*.) |34-38| on the basis of the high level of slightly soluble calcium sulphate normally detected on decayed stones. Afterwards their importance was reshuffled |39|, except for very particular substrata |40|. In fact new results seem to confirm the purely chemical origin of the gypsum |41,42| and no experimental evidence has succeeded in demonstrating the direct action, on a specific monument stone decayed example, of this group of bacteria. Positive results have been obtained in lab experiments |43-45| in which the substratum was concrete and the reduced sulphur compound utilized by the bacteria was essentially H_2S. Even if this is a typical sewage environment, very different from that one of an exposed monument.

The nitrifying bacteria |46-48| on the other hand were investigated for their ability to oxidize reduced nitrogen compounds especially ammonia substrata (by *Nitrosomonas spp*.) originating from the atmosphere. While the very soluble nitrates are hardly detected on stone surfaces, it has been demonstrated in lab experiments the high level of nitric acid produced by *p*. from nitrites and its direct action on the binding material of a concrete block. Besides the nitrite has a catalytic effect on the SO_2 oxidation to sulphite |49|.

However the high level of NO_x existing in the polluted atmosphere could have a prevalent role in stone decay processes |50|

b) The occurrence of Chemioorganotrophes bacteria, has been investigated for their capacity to produce several organic acids that can solubilize the minerals components of stones |8,14,17,18,20,51-59|. Among the wide range of heterotrophes isolated, most could be assigned to the genera *Flavobacterius* and *Pseudomonas* |60|. The genesis of some red staining of stone could instead be attributed to species of genus *Microbacter* |61,62|.

All these microscopical organisms must be identified and quantified using laboratory methods, as they are macroscopically undetectable on stone surfaces. The most used procedure is the simple microbiological colture technique. But the data output must be correctly interpreted since the synthetic media normally used are quite different from the real nutritional conditions; hence there is always the doubt that the isolated strains are qualitatively and quantitatively very different from those effectively developing on stone monuments.

To directly detect the activity of bacteria on stone samples collected from monuments several instrumental methods have been applied such as: the *Fluorescent Antibody Technique* (*FAT*) |63| and the *Enzime-linked Immunosorbent Assay* (*ELISA*) |64| using antisera, raised on rabbit, of bacterial cell surface antigens present on previously isolated species from the same decayed stone, together with: *staining* |65| or *enzymatic* methods measuring the *DHA* |66,67| alone or coupled with the determination of protein content in filtrates solutions of powdered rock samples |68|, or through the determination of the metabolic activity with *FDA* and *INT* |69| *colorimetric* assays or with *bioluminescent* techniques quantifying the *ATP content* alone |70| or coupled with *Differential Flux Calorimeter* determinations (*DFC*) |71|. Also the application of *fatty acid methyl ester* analysis (*FAME*) |72| was used to identify the isolated strains. The direct visual observation of stone surfaces, either with optical *epifluorescence* |73| or scanning electronic microscope (*SEM*) |74-76| is surely an useful tool to ascertain the biological presence on stone samples, even if there is always the doubt that the organisms observed are viable.

The results obtained with all these investigations are surely very precise but, as they arisen from pure isolated strains incubated in laboratory conditions on highly energetic culture media, these cannot give a complete idea of the complex processes involved in stone deterioration. In fact, an exposed stone surface has generally a mixed interacting population, a copiotrophic nutritive level and it is subjected, in European climates, to long periods of unsuitable weather conditions. Therefore these results are very useful to understand the biodeterioration process in laboratory, but hardly can be fully extrapolated to an "in situ" situation.

Lithophilous Algae and Cyanobacteria

These atmophytis photoautotropic organisms can develop well on exposed stone whenever a suitable combination of dampness, warmth and light occurs. Growth is most prominent in the spring and autumn. During dry and cold weather these organisms will die leaving a dirt deposit of dead cells and reproductive bodies, which permit rapid new growth as soon as suitable weather returns. These micro organisms are very sensitive to moisture content as they develop and reproduce in a water film on the stone's surface. Bright green coloration develops on all areas that are sufficiently moist and not subjected to direct sunlight. Their presence always indicates a high moisture content of the substrate |9|. Depending on the kind of organism and on the cycle phase, dark green, brown, grey and pink coloured patinas may also occur |77|. In urban atmospheres pollution may prevent the development of algae and other organisms |78|.

The algae and cyanobacteria can be considered the pioneering inhabitants of a stone surface. The latter can develop with just CO_2, N_2 and salt minerals traces. In fact they are, together with some very specialized bacteria, the only organisms capable to directly do nitrogen fixation |79|. Survival strategies of terrestrial algae include the formation of a polysaccharide sheath that allows both fast absorption and slow releasing of the humidity. Moreover this sheath facilitates cell/surface adhesion and the overcoming of long dry periods |80|.

These organisms can adapt themselves to very particular substrata, changing in colour and morphology. The use of synthetic media could favour the development of one species at the expense of another, leading to wrong conclusions on their respective ecological importance. In this case, direct observation under optical or scanning electronic microscope can easily overcome these drawbacks |81|.

These micro organisms usually participate in numerous lichenic associations |82| and the most common genus for *Cyanobacteriaceae* are *Nostoc, Calothrix* and the boring species *Hyella.* Epiphytic green algae, *Pleurococcus spp* and *Chlorococcum spp*, that utilize humidity of the air for their hydric needs, can also develop on stone surfaces |83|.

The occurrence of micro algae and cyanobacteria on monuments |84-87| or plaster |88| has been investigated both for their ability to etch mineral components |89| and to form anaesthetically coloured patinas and in some case a reddish staining of marble |90|. But while their aggressive action in relation to the substrate is still a matter of controversy |84| the presence of algae patinas can assume great relevance, especially for monuments exposed in wooden environments |91|, even if, the metabolic activity and the remains of the dead cells promote the development of heterotrophic organisms and lower plants. |92|.

More recently new approaches for direct identification of phototropic organisms have been experimented using biomolecular methods such as the *Polymerase Chain Reaction* (*PCR*) |93| and the application of *Fluorescence Lidar* technique for the remote monitoring of the fluorescence spectra of photosynthetic pigments |94|.

Lichens.

The lichens are of great ecological importance because of their pedogenetic action on the lithic surfaces |94-106|. Through respiration they produce CO_2, which it is transformed, within the thallus into carbonic acid. With their metabolism release organic acids with chelating properties, which complex mineral cations present in the rock substratum.

These metabolic substances cause compositional change in the stone surface layer immediately beneath the lichen structure, with depletion of its main chemical elements (Al, Mg, Mn, Zn, Si, Ca, K, Fe) and the accumulation of some of these, especially Ca, inside the thallus |107|. Moreover they can cause physical damage |108| as a result of the hyphal deep penetration (up to 15 mm |89|) and periodical thallus contraction and swelling (up to 35 time its dry weight |110|) as a consequence of climatic changes. With their slow constant growth (about 1 mm per year |111|), they can cover large stone areas with undesired chromatic patches.

With respect to their morphology, crustose lichens are closely attached to the substratum and are more significant in stone weathering than foliose and fruticose lichens. Among crustose lichens the ephi and endolithic strains are very important for stone biodeterioration |112|.

A close relationship has been found between the growth and the distribution of the identified lichenic species and some environmental characteristics of an examined area |113,114|. In fact, these organisms have been proposed and used as biomonitors |115|. Different lichenic species develop selectively upon specific lithotypes |116| and their colonization can be supported by the contribution of the organic nitrogen present in bird excrements such as crows and pigeons |117|.

The genera of ephi and endolithic crustose lichens, frequently isolated from stone monuments, are *Protoblastenia, Verrucaria, Caloplaca, Aspicilia, Lecanora* and *Xanthoria* |118,119|.

Like algae these organisms are also sensitive to air pollution which exerts a selective action on the species present |120|. When lichens die they leave behind a pitting corrosion with etched negative image pattern due to the metabolic activity and to the incorporation of mineral fragments into the thallus |109|. In some cases the lichen crust can act as a protective cover against other decaying agents, like atmospheriles, substance dwindling and temperature and humidity fluctuations |109,120|. Moreover the lichens can have a plugging effect on very porous and soft stones |110|. For these reasons it is sometimes better to avoid eradicating lichenic thalli from stone surfaces |120|.

One of the most studied acids produced by lichens has been oxalic acid. Saxicolous lichens of crustose species can produce up to 50% of their total weight of this acid |121|, which being toxic for the organisms is neutralized forming a very insoluble compound, the calcium oxalate |122-124|. This and its different hydrate forms (Whewellite and Wedellite) have been greatly investigated for their relevance upon the formation of patina on stone monuments |109,110,113,116,125-129|. This patina in some cases was extremely continuous and homogeneous like a paint (scialbatura) |125,127|. A very interesting debate has been made, in two international specific congresses, upon its origin (biological or chemical) and significance and whether it has a protective function or not |128,129|.

Artificial laboratory growth is very difficult for these organisms and the use of optical microscopy must be used for their direct study on stone specimens |130,131|.

Actinomycetes and Fungi

The actinomycetes preferentially develop where a growth of algae and chemosynthetic nitrogen fixing micro organisms exists |132|. With their metabolic activity contribute to weathering and to enrich in organic matter the stone surfaces. Species have been isolated belonging to the genus *Nocardia* and *Streptomyces.* They prefer to grow in subterranean environments (crypts, grottoes, tombs) with high and constant level of relative humidity |133,134|.

The second group of strictly heterotrophes micro organisms, the fungi, also need organic matter to grow. The acidolytic and complexing activity shown by the lichens was due to the fungal component of this symbiotic association. This, as well as the reduction and oxidation of mineral cations are characteristics activities of fungi |135-138|. Some fungal species (*Penicillium* and *Cephalosporium*) have a greater biochemical decay potential than lichens |99|. The genera which have been demonstrated being more abundant on the monuments investigated are: *Cladosporium*, *Penicillium, Trichoderma, Fusarium* and *Phoma* |139,141|. They chiefly utilize the polysaccharides coming from: microbial cell walls, metabolic intermediates and storage materials that are available on stone surface.

Metal (Mn and Fe) bio transfer, operated by some fungal species, is involved in crust formation |142| as well as the formation of rock varnish |143|. The former can induce crustose exfoliation processes, while the latter hardens the surface layer, acting as a protective coating |144|.

Several fungal metabolic products are strongly coloured and the phenomenon of staining of damaged areas on monuments can be the consequence of fungal development. Dark spots are attributed to the presence of fungi of the family of *Dematiaceae* which contains, water and organic solvents insoluble, melanin pigments inside the mycelium |141,145|. Even if the formation of brown or black colour patinas could derived from the oxidation of cell component debris due to the cyanobacterial and algal colonization |146|.

Lower Plants and Weeds

The primary vegetal colonization of mineral substrata usually originates from more primitive lower plants which have a much greater capacity to extract mineral ions from "unavailable" sources such as rock surfaces |147|. This is due to the presence in their rootlets of higher concentration of H^+ ions |148|.

Liverworts and Mosses develop less rapidly and rarely on stone that is free from humus deposit. The principal source of such a deposit is the accumulation of atmospheric particles and from dead micro organisms. The mechanical and chemical action shown by these organisms for stone monuments is negligible, as they do not have true roots systems but only rhyzines which are not directly in contact with stone surface, even if the capacity of mosses to accumulate Ca^{++} ions could be related with a biodeterioration capacity |149|. These lower plants are indicators of wet conditions as they need very damp environment to complete their reproductive phase |150|. Heavy accumulation (cushion) of growth can obstruct the passage of rainwater from gutters causing stagnation, and consequently increase damage through freezing |111|.

Higher plants with a real root system and aerial vegetative apparatus may be of greater importance in the conservation of monuments, especially for the archaeological remains where they can obstruct both sight and accessibility |151| or for subterranean structures |152|. The action of these organisms may be physical, through the pressure exerted by roots growth (up to about 15 atm. |153|) and chemical through the production of acidity and exudates from their rootlets |154|.

The transfer of nutritive metal cations through a network of colloidal particles, by a contact-exchange mechanism with the H^+ ions of rootlets, starts with the attack of silicates and carbonates structures |155|. Among the organic exudates, secreted by roots, are found the chelating acids 2-ketogluconic, oxalic and citric |156|.

The pH measurements made directly on the rootlets of some species present on monuments, (*Parietaria diffusa, Capparis spinosa, Cymbalaria muralis* and S*onchus tenerrimus*) have shown values from 5.2 to 6.3 |158|.

In temperate climates the most frequently recovery of ruderal plants are herbaceous or shrub species, with calciophilous, xerophilous and nitrophilous habitus (*Cynodon dactylon, Melica minuta, Parietaria officinalis, Capparis spinosa*, *Ceterach officinarum, Hedera helix* |151|) and very rarely arboreal species (A*llianthus altissima*, *Ficus carica*, *Robinia pseudoacacia* |158| or *Ulmus minor* |159|.

The development of micro and macro flora is heavier and more noxious in tropical climates where high rainfall and temperature greatly increase the growth of vegetal, especially higher plants, on monuments |160,161|.

In some cases the roots can reach several metres (10 - 20) deep, as in the case of the very common rupicolous species (*Capparis spinosa*) which is able to penetrate into very compact substrata |162|. The deep penetration of the root system of tree species such as: *Pinus pinea* and *Quercus ilex* can be dangerous for hypogeous structures, provoking the detachment of plaster and the collapse of walls |158|.

Other factors must be taken in consideration as visual obstructions and the risk of fires |163,164|.

The biodeterioration of calcareous stones induced by plants, which accumulates high amounts of calcium inside, was checked using Energy-Dispersive X ray analysis (EDX) |165|, while the Scanning Electron Microscopy (SEM) was employed to study stone surfaces colonized by plants |166|.

Animals

Arthropodes communities as: the red mite *Balaustium murorum* with others acari (*Phauloppia lucorum*, *Trichoribates trimaculatus*) and the psocid *Cerobasis lucorum* were observed on stone building supporting algal and lichenic growth. This microfauna feeds on detached lichen particles and contribute to the diffusion and propagation of new colonies being the spore, soredia and algal cells entrapped among their legs and body hairs |167|.

The avifauna, especially pigeons and crows, can directly or indirectly damage stone substrata. The direct destructive action of birds is both physical, caused by trampling and grazing, and chemical, caused by the dropping of acid excrement (guano) containing high amount of nitrate and phosphate compounds. Indirect damage is made by organic substances accumulated on stone surfaces, which can serve as nutritive substrata for heterotrophic micro flora (bacteria and fungi) |154|. For grotto and caves the presence of bats colonies can induces discolouration of painted surfaces.

The passage of sheep and goats in archaeological sites with their trampling and feeding action can compromise floor and mosaic wholeness on structures with poor cohesion |154|.

The action of an insect known as the mason bee (*Hymenoptera*) which bores, for the purpose to building his nest, into stones and removes clay ground material from them has also been reported |168|.

Black spots due to the dense mosquitoes deposits (Diptera) excretes have been found on white marble surface |169|

The decay action played by sea boring animals |170| such as bivalves (*Molluscs*) or marine worms (*Polychetes*) is confined to the case of submerged monuments |153|.

3. Plaster, Mortar and Frescoes

The same organisms as on natural stones can develop and bio deteriorate on these substrata, at higher on lower extension depending if the object made with these materials is placed in outdoors or indoors exposure. A particular case is represented by the frescoes, which composition should be fully inorganic, but either for their collocation or for past restoration interventions some organic materials can be present in their structure, consequently them can be attacked also by the organisms developing on organic substrata |171|.

4. Organic substrata

The easel and panel paintings, wooden sculptures, library materials, prints and textiles are artistic objects whose composition is based on natural organic materials. All of them, due to their biodegradable nature, are usually maintained in confined environments. The biological attack of these materials occurs only under poor conservation conditions: high humidity level, soil contact, poor ventilation, and rare maintenance operations.

4.1 Wood

The susceptibility to the microbial attack of this material is depending on its moisture content. Microbial deterioration, operated mainly by microfungi, can starts when the water content is above the 20%. These can develop on surface or within internal structures inducing, through the production of exoenzymes, change in cell integrity. The structural biological polymers are destroyed chiefly by the activity of fungal strains |172-175|:

White rot (*Pholiota* sp., *Fomes*,sp., and *Pleurotus* sp);

Brown rot (*Merulis lacrymans*, *Poria* spp. and *Coniophora puteana*);

Soft rot (Chaetomium, Xylaria, Alternaria, Humicola Stenphylium

While the role of bacteria (*Pseudomonas* and *Achromobacter*) and actinomycetes (*Micromonospora*) is less important because they require an higher water content and have been found mainly in outdoor and marine environments.

Insects, however, are the most serious source of damage for wooden objects kept in museums or indoor environments; they use wood as a nutrient source, for shelter and egg deposit. In feeding, some insects utilize only the compounds obtained from the cell contents (sugars and starch) while others utilize even the cellulose. Among insects able to use cellulose, the way in which adult insects and/or their larvae digest wood varies. Some of them are able to digest partially decomposed cellulose, while the generality have a complex symbiotic relationship with some micro organisms that secrete cellulase enzyme complex in their gut (yeasts in *Anobidi* and protozoan in *Termites*). The main insects involved in wood decay are reported in Table 1 |174|.

Among *Coleoptera* or beetles, many species have larvae that destroy wood by living inside it and feeding on it, The life cycle of the beetles comprises four phases: egg, larva, pupa and adult. The beetles lay their eggs in cracks or sheltered places. After few days (10) the larvae emerge, bore into the wood and live into it (for 1 to 5 years) until they mature. After a short pupal stage (six weeks), they become adult, bore to the surface and fly away. The sexually mature beetle reproduces and the life cycle begins again. For most of their life cycle, these insects are not visible and infestations are usually detected only by the small flight holes or by the formation of little bore dust deposits.

Belonging to the *Coleoptera* order, the *Anobiidae* are the insects most frequently found in deterioration of furniture, sculpture and other wooden objects. High RH and moderate temperature favour their development. Unlike the Anobiidae, the powderpost beetles (*Lyctidae*) even thrive in dry conditions and attack mainly sapwood. Longhorn beetles (*Cerambycidae*) can cause serious damage, mainly to structural timber such as roofs or floors.

Table 1 - Insects frequently found on wooden materials

Orders	Families	Common names	Type of damage
Coleoptera	*Anobiidae*	furniture beetle	Winding and circular tunnels; circular emergence holes
	Lyctidae	powderpost beetle	Tunnel with oval section
	Botrychidae	wood borer	Circular holes and tunnels
	Cerambycidae	longhorn beetle	Large, oval tunnels and holes
Isoptera	*Kalotermitidae*	termites or white ants	Deep and crater-shaped holes; sometimes entire interior of object is destroyed but outer surface is left intact
	Rhinotermitidae	"	"
Hymenoptera	*Siricidae*	wood wasp	Circular tunnels and holes of wide dimension

Termites are most common in the tropics and subtropics because they require high temperature and humidity. They are social insects, forming communities that include reproductive individuals (queen and males), nymphs and wingless specialized sterile forms (workers and soldiers). Workers are confined to the ground or wood where they devote their energy to feeding and foraging for the community. They often hollow out the wood completely, leaving a thin outer shell of wood undamaged. Termite attack is often not detected until the whole structure collapses. This happens because many termite species are *photophygous* (heliophobic).

There is also a range of omnivorous insects that can cause incidental damage while scavenging for other food; cockroaches (Dictyoptera) belong to this group. These insects only cause surface erosion of variable depth.

A different situation occurs with Dermestidae, a family of Coleoptera, which are destructive for dry materials of animal origin and occasionally for wood and materials of vegetable origin; the larvae frequently bore "refuges" into timber adjacent to a food source.

In contrast, the damage caused by insects living inside the wood includes emergence holes of different shapes and sizes as well as superficial or deep tunnels. The presence of holes does not necessarily indicate an active infestation, but the bore dust produced from holes may give an indication. The main insect damage is due to the breakdown of the wood fibres, which affects structural strength.

Waterlogged wood is the term used to describe wood kept under soil or water, for example archaeological wood such as shipwrecks or pile-dwellings. Under these particular conditions (high water content and lowered oxygen pressure), wood can easily be attacked by microaerophilic and anaerobic heterotrophic microorganisms and, in sea sites, by some marine organisms.

The micro flora able to attack waterlogged wood varies in relation to the maintenance site (sea-water, fresh-water, water-saturated soil, etc.) and specific environmental conditions (oxygen concentration, temperature, concentration of mineral salts, etc.). In sea water, bacterial degradation of wooden materials can occur in water or in sediment up to a maximum of 60 cm deep; fungi occur under aerobic conditions in the sediment-water interface |176|.

The micro organisms that can degrade waterlogged wood are either some wood destroying species such as soft rot fungi, sea-water fungi or bacterial strains.

Among bacteria, the most common are cellulolytic aerobic *(Cytophaga, Cellvibrio, Cellfalcicula)* or anaerobic species *(Plectridium, Clostridium)* and common microorganisms such as strains of *Bacillus, Pseudomonas, Arthrobacter, Flavobacterium, and Spirillum*. Bacteria are also categorized according to the type of alteration they cause. Thus, there are erosion bacteria, cavitations bacteria and tunnelling bacteria |177|.

In sea-water and fresh-water environments, some micro-algae strains can also cause damage to immersed woods. They form slimy green patinas and cause superficial modification of wood characteristics. The alterations caused by these micro organisms are not too serious, but they contribute to chemico-physical decay.

In sea water, marine borer animals are the most destructive organisms for waterlogged wood. They are widely distributed in salt water, prevalent in warm climates, and include different species of *Mollusca* and *Crustacea*.

The main genera of Mollusc are *Teredo, Bankia* and *Martesia,* which produce tunnels that penetrate deeply within the wood. The tunnels may have a chalky-white shelly (calcareous) lining. The first two genera comprise a number of species known as "shipworms". After a free-swimming larval stage, the young organisms become able to attack timber and make very small entrance holes in the surface of wood, but once within it they increase rapidly in size. They use wood for shelter and feed on the minute organic particles and plankton found in the sea water. Their whitish, worm-like bodies are equipped with a pair of shelly valves at the anterior end that excavate wood. The molluscs of the genus *Martesia* are small clams with their bodies entirely encased within the bivalve shell. The damage caused is relatively small compared to the other two genera.

Within Crustacea the main wood-destroying genera are the isopods *Limnoria, Sphaeroma* (common name "wood lice") and *Cherula* (common name "sand fleas"). The wood-boring crustaceans differ from the molluscan borers either in their methods of attacking wood or in their general structure. They do not become imprisoned in the wood but, instead, are able to move about. The young and the adult are equipped with a pair of strong-toothed mandibles designed for chewing wood, which provides nourishment; they burrow in the timber, making galleries of various depths with narrower bores than those of Mollusca. They attack the timber in such great numbers that the infested wood becomes honeycombed and can be broken by the mechanical action of water at the point of attack. The damaged area is usually concentrated at the water line. The damage caused by these marine borers is more evident to inspection and proceeds more slowly than that of shipworm, so it is less spectacular and serious |178,179|.

4.2 Paper

Paper is primarily composed of cellulose and other substances related to the origin of the raw materials used in its manufacture: lignin, hemicelluloses, pectins, waxes, tannins, proteins and mineral constituents. In fact, paper can be manufactured from textiles or from wood through various and complex operations. The content of these components varies according to the papermaking process, the type of paper and the period of production. In the middle Ages, for example, the quality of paper was particularly good; it was manufactured from carefully selected cotton rags and contained a great amount of cellulose and only a few impurities. Subsequently, the quality of paper changed for the worse modern papers produced by industrial manufacturing processes since the end of the seventeenth century. These are derived from the stem of wood or wood pulp and contain a great amount of polymers and non-fibrous material other than cellulose, as well as impurities. Modern papers are thus more vulnerable to micro organism attack than older ones |180|.

Paper is a good source of nourishment for heterotrophic micro organisms and organisms. The indispensable condition for a microbial attack is a high water content, and the hygroscopicity of paper makes it more sensible to the biodegradation.

The main biodeteriogens micro organisms for paper are bacteria, micro-fungi and actinomycetes, either cellulolytic strains damaging the chemical structure of paper or noncellulolitic ones with non-specific action. But, the most common are fungi because they show a great tolerance to environmental conditions and can live with lower water content than bacteria and actinomycetes.

Among cellulolytic strains of micro-fungi, many species of Deuteromycetes (e.g., *Alternaria* spp*., Aspergillus* spp*., Fusarium* spp., *Humicola grisea, Myrothecìlim verrucaria, Penicillium* spp*., Stachybotrys atra, Stemphylium* spp., *Trichoderma* spp*., Ulocladium* spp., etc.) and Ascomycetes (c.g., *Chaetomium* spp.) are frequently isolated from books, documents and prints |181,182|. Certain cellulolytic species of *Aspergillus* and *Penicillium* are particularly harmful to paper because they are able to grow on substrates having a 7-8% moisture content, which can be reached in some types of paper at RH levels as low as 62-65%; such conditions are not rare in many places where paper is kept |182|.

The binding is the first part of a books to uptake moisture from the air, thus fungal growth spreads more quickly on bindings than on sheets of paper |208|. Some species of *Chaetomium, Trichoderma viride* and *Stackybotrys atra* cause a deep decay of cellulosic material, which then lose its mechanical properties.

In general, fungi cause alterations on paper producing various kinds of stains, either round or irregular in shape and coloured in red, violet, yellow, brown, black, etc. These stains are due either to the presence of pigment mycelium or to the release of coloured metabolites |182|. The colour of pigments can change depending on the conditions of growth and the properties of paper (e.g., pH, presence of starch or gelatine sizing, presence of metal, etc.). A particular kind of alteration due to fungi is the discoloration of inks due to tannase, an enzyme that catalyses the hydrolysis of gallotannate, produced by some strains *of Aspergillus* and *Penicillium.*

Bacteria attack paper less frequently than fungi, but several bacteria have been isolated from papers maintained in environments with RH higher than 85% |180,184,185|. The cellulolytic species are obviously more harmful than non-cellulolytic ones; the genera *Cytophaga, Cellvibrio* and *Cellfalcicula* belong to the first group, the latter one is rarely found.

During metabolism, all micro organisms produce different organic acids (oxalic, fumaric, succinic, citric, etc.), which reduce the pH of paper, conditioning the dynamics of bacterial and fungal growth in secondary attacks. Frequently, bacterial and fungal attack makes the paper feltish and fragile.

One particular and very common chromatic alteration of paper is *foxing;* it appears as rust-coloured marks of different shapes, frequently as spots. The causes of foxing have not yet been clarified, many authors consider fungi as possible cause, but others believe that the presence of heavy metal, most commonly iron, might be among the factors favouring this kind of alteration |186-188|.

A particular type of damage “Consolidation” can affects books that have been flooded or heavily moistened. The alteration is brought about by growth of bacteria and fungi. This is related to the production, during cellulose degradation, of oligosaccharides with mucous properties and, on substrates particularly rich in sugar, to the formation of secondary metabolic products of a slimy nature. It is clear that the composition of paper (fibre content and type of glue) affects the intensity of the degradation |189|.

Insects are frequently involved in the deterioration of paper. Many insects are able to attack cellulose with processes like those used on wood. Others also cause damage by utilizing fillers, glues, boards, textile fibres, leather or other constituent elements of paper materials. Based on frequency, there are customary and occasional insects. The former are using this material for nourishment, while the latter (e.g., wood or textile borers) sometimes damage the paper only when feed different materials present on it, such as leather, starch and wood. The morphology of insect damage ranges from tiny superficial abrasion and surface erosion to holes and sometimes tunnels.

In Table 2 the main orders and families of insects responsible for deterioration of paper works of art are listed |182,190|

Table 2 - Insects frequently found on paper materials

Orders	Families	Common names	Type of damage
Thysanura	*Lepismatidae*	silverfish	Small surface erosion with irregular outline
Isoptera	*Kalotermitidae* *Rhinotermitidae* *Termitidae*	termites	Deep crater-shaped holes and erosion; destruction of the interior of object while the outside remains intact
Coleoptera	*Anobfidae*	furniture beetles	Winding, circular tunnels
	Lyctidae	powder post beetles	Tunnel with oval section
	Dermestidae	skin beetles	Short blind tunnels with circular section and irregular perforation
Corrodentia	*Liposcelidae*	book lice	Tiny surface abrasion
Blattoidea	*Blattidae* *Blattelidae*	cockroaches	Surface erosion

Anobiidae, *Lyctidae* and *Dermestidae* complete their life cycle inside books. Dermestid beetles mainly eat leather bindings, but they burrow into books, making tunnels where they pupate. *Blattidae*, *Blattelidae*, *Lepismatidae* and *Termitidae* live in environments where books are kept, and paper, cardboard and glue of vegetable or animal origin represent their source of nourishment. *Liposcelida*e are the smallest insects in book biodeterioration (1-2 mm), and are very common. They feed on paper, glue, etc., and live especially on bindings. These insects also feed microfungi developed on deteriorated surfaces, so they appear frequently under high-humidity conditions.

4.3 Textiles

Textiles can be of either vegetable or animal origin. Those of vegetable origin are cotton, flax, hemp, jute and sisal. They are mainly composed of cellulose derived from plant fibres; for example, flax and jute are phloem fibres, sisal and manila are leaf fibres and cotton is made from seed fibres. Like other materials of vegetable origin, the susceptibility or resistance of textiles to biodeterioration depends on the content of cellulose, lignin and other organic constituents. The presence of non-cellulosic components such as lignin and waxes decreases susceptibility; in contrast, pentosans and pectins increase susceptibility. Natural cotton contains about 5% of non-cellulosic products, flax 15%, so cotton is less susceptible to microorganisms than flax.

Fibres with a high amount of lignin are more resistant to microbiological attack than purified fibres, while textiles contain sizing (starch and dextrin) are more susceptible. The following structural features of fibres and textiles also affect biodegradation: degree of polymerisation, length of cellulose chain, fibre crystallinity and orientation. Furthermore, mechanical, and photochemical degradation increase susceptibility to biodeterioration. Loosely woven fabrics are less resistant than tightly woven fabrics because they hold more dirt and biological pollutants between fibres, creating favourable conditions for biological attacks|191|.

The most frequent biodeteriogens of cellulosic textiles are *micro-fungi* (Ascomycetes and Deuteromycetes) and *bacteria* of cellulolytic and non-cellulolytic species, many of which have been mentioned for the wood and paper biodeterioration.

The most active agents of textile deterioration are fungi. Among Deuteromycetes, we find several species of *Alternaria, Aspergillus, Fusarium, Memnoniella, Myrothecium, Neurospora, Penicillium, Scopulariopsis, Stachybotrys* and *Stemphylium,* etc. Quite frequent and particularly harmful because of its high cellulolytic activity is the genus *Chaetomium* of Ascomycetes |181,191,192|. Zygomycetes such as *Mucor* and *Rhizopus* sometimes occur on textiles.

Bacteria and *actinomycetes* need high water content in fabrics, and their occurrence in museums should be rare. But in damp environments or when textiles are buried in the soil (e.g. archaeological textiles), the most serious decomposition of cellulose is caused by bacteria belonging to the species of *Sporocytophagamyxococcoides* and then by *Cellvibrio, Cellfalcicula, Microspora* and anaerobic *Clostridium* |206|. Degradation of fabrics exposed outdoors has also been associated with species of Myxomycetes and actinomycetes |191|.

Under conditions of damp, heat, lack of ventilation and contact with decaying matter, the microbiological attack can occur in a few days. The biodeterioration of textiles can take several forms, such as discoloration, staining and loss of strength.

The metal fibres, used for embroidery in the manufacture of high cost textiles objects such as religious furniture, noble dressing and ancient costumes, can inhibit microbial growth, particularly when copper or other heavy metals are present.

Cellulosic textiles are also susceptible to attack by insects such as silverfish (*Lepisma saccharina*) and cockroaches *(Blattelidae)*. The probability of attack is increased when this material contain glues made of starch or dextrin. The main insect families involved in textile biodeterioration are *Blattidae, Lepismatidae* and *Mastotermitidae*, *Hodotermitidae*, *Rhinotermitida*e (termites), but many others may occur occasionally |193|. In general, their attack ranges from surface erosion to destruction of parts of the object.

4.4 Parchment and leather

Parchment as a writing support was produced from sheep or goatskin. It is composed of collagen, some amount of keratin and elastin, and a minimal amount of albumin and globulin. Collagen is one of the proteins most resistant to micro organisms attack it can only be hydrolysed by specific enzymes, *collagenases,* produced by some anaerobic bacteria of the genus *Clostridium* |180,181|. The mechanical disintegration of skin during the manufacture of parchment, depolymerise the collagen. Non-specific proteolytic enzymes produced by many bacteria and some microfungi in aerobic conditions can decompose this denatured collagen. Other factors such as temperature (increases), humidity (violent change), pH and exposure to UV rays can also influence the stability of collagen in parchment; these factors cause changes in its structure, and decrease the resistance to the biodeterioration. In addition to collagen, some other components (other proteins, lipids, carbohydrates, mineral constituents and impurities) either derived from the original skin or are added during manufacture, are involved in the decay of parchment.

The parchment's susceptibility to biodeterioration depends: by the raw material, its method of production and its conditions of preservation. Under aerobic conditions, only bacteria can attack partially decomposed collagen, among which are strains of *Bacillus* (e.g., *B. mesenthericus), Pseudomonas, Bacteroides, and Sarcina.* Ancient parchment materials can also be attacked by certain species of fungi of the genera *Cladosporium, Fusarium, Ophiostoma, Scopulariopsis, Aspergillus, Penicillium, Trichodernia, etc.,* |194|. As a result of biodeterioration, parchment loses its original properties and becomes hard and brittle, often with deformation of the structure. The microbial attack also causes variegated spots, white films and fading of the texts.

Leather has a chemical composition similar to parchment and its susceptibility to biodeterioration and the species involved are almost the same. To produce leather, skins are cured by subjecting them to tanning, which causes chemical and physical changes. The attack of micro organisms varies depending on whether the skins were tanned or not. Bacteria under conditions of high humidity attack untanned skins. Tanned leathers are not readily subject to bacterial attack, but are degraded by fungi. Usually, after tanning, leather has a pH of about 3 to 5, which is more suitable for fungal growth. Vegetable-tanned leathers are more susceptible to fungal growth than chrome-tanned types because they contain some amount of glycosides. Chrome-tanned leather seems to afford some fungistatic activity and protection against microbial activity but sometimes this type of leather shows the growth of fungi of the genera *Penicillium* and *Paecilomyces,* which are tolerant to the chromium compounds. Fungi that attack tanned leather often belong to lipolytic species and utilize the fats present in leather as a source of carbon. In this case the proteins are not directly affected, but can be damaged by organic acids released as a metabolic end products and the artefact becomes stained and stiff |195,196|. The principal effects of microbial deterioration on proteic materials are the presence of different stained spots, loss in tensile strength, and, if the proteins are attacked, hydrolysis of the leather. Proteinaceous materials like parchment and leather are also susceptible to attack by insects: Dermestidae and Tineidae are the main families that can selectively attack either collagenous or keratinous materials |195,196|. Occasionally, cellulose and proteinaceous feeders cause damage to parchment and leather materials. The insect damage appears as superficial erosion, deep erosion, holes or loss of material.

4.5 Wool and silk

The wool is produced from the hair of different mammals (e.g., sheep, alpaca, vicuna, etc.), consists mainly of keratin, a highly insoluble protein containing sulphur linkages in the molecule.

The commercial silk is a proteinaceous filament secreted by the domesticated "silkworm" *Bombyx mori* for making its cocoon. A caterpillar of the Antheridae family produces wild silk. The silk fibre is composed of two filaments made of a protein (60-70%), *fibroin*, linked together by another protein (25-30%), *sericin*. Fibroin is a highly crystalline scleroprotein, which is very resistant to chemical agents and highly insoluble. Sericin is an albuminoid substance. In some manufacture of silk fibres, it is removed by treatment with warm water and soaps (degumming process); this treatment increases silk's resistance to microbial attack.

The main agents of deterioration are micro organisms (bacteria, actinomycetes and fungi) and insects, but insects are considered the most serious pests for cultural objects kept in museums. Although protein fibres, such as silk and wool, are not as susceptible to micro organism deterioration as cellulosics, they can be attacked if they contain a high degree of impurities (such as sericin for silk or sizing and suint for wool) and if they are stored under warm and humid conditions |191|.

In general, wool fibres are more subject to attack by bacteria than cotton fibres and less subject to fungal attack. An indispensable condition to start a bacterial attack is a high water content; this condition is favoured by the high hygroscopicity of wool. Bacteria may be keratinophiles, able to produce enzymes that break down keratin, such as some dermatophytic fungi *(Trichophyton* and *Microsporum), which* can cause disease in human beings.

The micro organisms most frequently mentioned in degradation of wool and other protein fibres are bacteria of the genus *Bacillus (B. mesenthericus* and *B. subtilis), Proteus (P. vulgaris)* and some species of Actinomycetes *(Streptomyces albus* and *Streptomyces fradiae)* |181,191|. *Pseudomonas aeruginosa* frequently causes green, red or other coloured stains according to the pH of the substrate.

Micro-fungi are not very frequently involved in biodeterioration of protein fibres but species of *Aspergillus, Fusarium* and *Trichoderma* are sometimes reported |181,191|.

Silk is fairly resistant to micro organisms if impurities and sericin are removed in manufacturing processes (degumming). The strains degrading silk include the species that attack other protein fibres, but specific deteriogen micro organisms for silk are not identified. The microbiological damage appears as discolorations, stains of different shapes, colour and diffusion. Tensile strength may be reduced: for example, silk becomes brittle.

The most serious damage of animal-derived textiles, in museums in moderate climates, is related to insects, as microbial attacks on protein fibres rarely occurs. The insects most frequently found are some species within the families Dermestidae, Oecophoridae (brown house moth) and Tineidae (clothes moth). The better-known species of moths are *Tinea pellionella, T bisselliella* and *Hofmannophila pseudospretella*, which degrade extensively wool and rarely unscoured silk. Larvae, which use wool as food and to build a protective sheath, do the damage. Carpet beetles, too, cause damage to textiles. Among most frequent species of Dermestidae are *Anthrenus verbasci, A. museorum, Attagenus pellio,* which cause damage only through their larvae because the adults usually spend their life elsewhere.

Infestation by these insects is rather widespread and not confined to textiles. Susceptibility is increased and the range of deteriogen insects can become larger if these materials contain sizing, composed of starch or dextrin, and other organic components. The type of damage involves the production of holes of irregular shape, superficial abrasion or deep erosion.

5. COMPOSITE MATERIALS

Works of art are often made of a combination of different organic and inorganic materials, rather than just one. It is impossible to list all the types of cultural heritage assets and to give information about their susceptibility to biodeterioration. In general, the risk of biological attack is linked to the most susceptible component. The biodeterioration phenomena on composite materials are similar in their character and effect to those of the single component.

5.1 Paintings

Paintings are composed of a support (canvas, wood, paper or parchment), a preparation layer and a paint layer, the chemical composition of which varies according to the mode of painting, the kind of paints used (oil paints, distemper or watercolour), the historical period and the author.

In canvas paintings, the preparation is usually made with lime or gypsum with addition of animal or vegetal glue. On this smooth surface several layers of colour are present, which consist of pigments mixed with binders of oil or distemper (egg or glue). The surfaces are usually covered with a thin, translucent protective varnish. In paintings on wooden supports, a similar multilayer structure is observed.

Paintings on paper can be watercolours, gouaches or pastels. The paint is laid directly on paper and the state of preservation of the paper determines the durability of the whole painting. In watercolours, the transparent paint layer also contains a small amount of binder, usually gum arabic. Pastels are made with crayons consisting of pigment without binder and are, therefore, extremely difficult to store and preserve.

In painted works of art, the biodeterioration processes can involve either a portion of the painting or all of its components. Thus, paintings may show traces of a biological attack on the reverse side, the support, or on the painted side, and a part or all components may be damaged.

The organic components in paintings represent a good source of nutrition for a wide range of heterotrophic micro organisms and organisms. But, biological attack occurs only when there are favourable environmental conditions, and such conditions are often found in museum rooms, old churches or in deposits without any control of the humidity and temperature.

The micro flora attacking paintings include virtually all species of micro-fungi because the variety of organic components of these works of art can represent a carbon source for practically all species. In addition, they show a great tolerance for environmental conditions and can use condensation moisture. In contrast, the moisture content of these objects is rarely so high as to favour development of bacteria, which are unable to use condensation water |184|.

Generally, the first part subjected to the microbial deterioration is the support. In fact, in paintings on canvas, the microbial attack usually starts from the reverse side, because the glue sizing increases the natural susceptibility of textiles. Then the biodeteriogens penetrate inside canvas reaching the back side of the paint layer, causing cracks and detachment, while the cellulose hydrolysis creates differences of adhesion between the paint layer and the canvas itself |197|. In paintings on wooden board, the decay of the support can be different from the paint layer.

The micro organisms involved in biodeterioration processes are those mentioned for biodeterioration of materials of vegetable and animal origin. Sometimes the presence of substances, such as sizing glue or lining paste used for different treatments of the support, can increase susceptibility |184,198|.

Biological attack on the paint layer is less frequent than on the support and depends on the nature of pigments. The most susceptible to biological attack are casein and egg distemper, emulsion distemper and linseed oil in this order. In contrast, the presence of heavy metals in some pigments, such as lead, zinc or chromium, can increase the resistance of the paint layer. Watercolours contain only a small amount of organic binder and are, therefore, as susceptible to microbial deterioration as pastels |184|.

The growth of a microfungal mycelium by a microscopical germinating spora is quite rapid, with radial development, and it become macroscopically visible in few days. Among the species of micro-fungi most frequently involved in deterioration of the paint layer, species of Penicillium, Aspergillus, Trichoderina and Phomapigmentovora disintegrate distemper and oil binders, Aureobasidium decompose oil binders, Geotrichum develop on casein binders, Mucor and Rhizopus attack glue |183,184,199|.

The development of micro-fungi on the surface of paintings induces aesthetical, mechanical and biochemical decay. In fact, the growing mycelium spread over the paints, masking design and colour, while the growth of hyphae and fruiting bodies inside the support can cause, friability and loss of the paint layer. Exoenzyme activities can cause more serious damage by decomposition of some polymers both of the paint layer and of the support, whereas the presence of coloured fruiting bodies and the production of coloured metabolites provokes the formation of permanent stained patches while organic acids produces irreparably modifications in the structure.

In biodeterioration of painted materials, insects are also often reported but they only occasionally damage the paint layer, preferentially attacking the wood of the frame or support and the vegetable glue.

5.2 Restoration Materials

The material used during the restoration procedures (animal and vegetal glues, re-lining paste, varnishes, temperas and other materials used for cleaning and soaking) can contribute to the biological risk being these fresh and with high water content.

6. METHODS OF INTERVENTION

In order to control biodeterioration processes the most suitable methods and products must be used. With regard to the principles and the nature of the means employed, control methods can be classified as mechanicals, physicals, biologicals, or biochemicals, even if those based on active principles in solution (chemicals) are the more frequently applied (either as wide-spectrum active principles, or more specific fungicides, algaecides, herbicides, insecticides and repellents for birds).

6.1 Direct Methods

6.1.1 Mechanical Methods

All the techniques described as "mechanical" have in common a method of displacing the biodeteriogens. Traditional mechanical methods involve the physical removal of biodeteriogens either by hand or with tools such as scalpels, spatulas, scrapers, air abrasive or vacuum cleaners. Although frequently used in the past, these methods do not produce lasting results and the eradication of vegetation could do not completely arrest its vegetative activity. Moreover, mechanical methods can damage the substrate, even if they have the advantage to not add on it any substance that might cause further deterioration. In some cases the mechanical methods alone can be sufficient to eradicate some of these biodeteriogens such as, mosses, foliose lichens and annual herbaceous plants. Nevertheless a preliminary biocide treatment is surely advantageous to facilitate the removal of the biological growth and to hinder the diffusion of the colonisation of lichens, reproducing by means of soredia, through their dispersal due to mechanical cleaning methods |200|. The mechanical removal of lichens on stone can be facilitated by applying beforehand some alkaline solutions (e.g., 5% of ammonia) in order to swell and soften the thalli

6.1.2 Physical Methods

The methods used experimentally are: ultraviolet rays (UV), gamma rays, low frequency electrical current systems, heat, deep-freeze temperatures and ultrasonic.

Ultraviolet rays have been used especially against bacteria, algae and fungi in the treatment of renders and plasters |201|. The part of the UV spectrum with germicidal activity is between 300-200 μm, with a maximum of activity between 275-230 μm. Micro organisms vary in sensitivity depending on their growth phase (greater during the logarithmic phase) and the nature of the substrate on which they are found. UV radiation is more effective at low RH values (less than 50-60%). It has a poor penetration power and can modify some materials (e.g., cellulose, proteins) and the colours (natural or dyes) of surfaces.

Gamma rays are a form of electromagnetic radiation. They are extensively used for sterilizing micro flora and killing insects, especially on organic materials such as paper, parchment and wood |202,203|. A dose of 500 Gy is required to kill larvae and to prevent the emergence of adult insects. Moulds are less sensitive to ionising radiation than insects, and different strains show different levels of sensitivity; generally most fungi are killed by a total dose of 10 kilo Grays (kGy) |204|. Despite its power, gamma irradiation does not induce any secondary radioactivity, does not damage oil and tempera polychromy, leaves no hazardous residues and penetrates completely into the objects. Moreover, a large quantity of materials can be treated at one time. Paper is much more sensitive than wood to the effects of gamma radiation. A possible negative effect of gamma rays on musical instruments has been verified in the modification of the “sound” of a violin after treatment. Repeated treatments are not recommended because the effects are cumulative. Gamma rays have recently been employed to cause the polymerisation of resins used in the impregnation of decayed wooden objects; in this case, wood-boring insects are exterminated at the same time.

High-frequency current can be used to kill wood-destroying insects (Anobiidae), but only in the absence of metals |202|.

Low-frequency electrical current systems have recently been used to keep birds from roosting on monuments |205|. This method is completely harmless for animals and humans.

Heat (dry or wet) is used in the disinfestations and disinfections of organic materials. The application of moist heat for disinfections of books is still one of the most widely used techniques |206|. A temperature of 95°C and 40% RH for 4 hours is recommended.

Other methods used to eliminate infestations of books are exposure to blast-freeze temperatures or to substitute the atmosphere in close container with N_2 or with O_2 scavengers (concentration below 0,05 %) |207|. Reduction in pressure, using vacuum dryers, causes cell’s explosion.

Finally, we might mention the suggested use of light traps for controlling insects attracted by light, in museums, and the exclusion of light, using opaque plastic sheets, to prevent the photosynthesis by biodeteriogens present on statues in wooden environments.

6.1.3 Biological Methods

This application is based on the presence of parasitic or antagonistic organisms of the biodeteriogens. Bacteria, insects and phage might be used, but up to now this kind of intervention has been applied chiefly in the agricultural sector. Antagonistic or predators species have been introduced for pull away the birds from cities.

6.1.4 Biochemical Methods

In this group, we consider biodeterioration control systems that use chemical compounds of biological origin even if many of these are now synthetically produced.

Antibiotics are substances produced by micro organisms during their growth in order to avoid the competition of other species, which are inhibited or killed |208|. These compounds are active at very low doses, but can lose effectiveness after storage.

Enzymes are proteins that catalyse biochemical reactions. They have been used on rare occasions as biocides and are sometimes mentioned for cleaning of adhesives |209|.

The proteolytic enzyme trypsin has been used for the removal of lichenic crusts, but reports have indicated disadvantages in the practical difficulty of maintaining a good enzymatic activity, as a function of temperature and pH |210|.

Pherormones are volatile substances produced by one individual, which have a specific action on the opposite sex of the same species. Some experiments have been made with one of this for controlling insects in museums with sexual attractants traps. These substances could be very useful as preventive, taking into account the difficult for liquid biocides to deeply penetrate inside the object attacked by boring insects.

6.1.5 Chemical Methods

Many organic and inorganic compounds have been used, as biocide agents, to eliminate the biodeteriogens from cultural objects (see Table 3).

Pesticides are chemicals used for killing undesirable biological growth. They have a biocide action with a specific toxicity for the species to be eliminated (it is also possible to use the term biocides). These are classified in different ways depending on their chemical nature (e.g., organic or inorganic) or on the target pest species. It is also possible to classify pesticides according to their chemical groups or their mode of action. In addition to the pesticide, other ingredients, such as carriers or additives to improve the effectiveness of the product or to facilitate its application, are present in the chemical formulation. These are called coformulants and could have negative effects on the objects to be treated.

Disinfectants are chemicals that destroy vegetative forms but are not always effective against survival resistant or quiescent phase structures (bacterial spores, fungal conidia and insect eggs).

A side problem with the use of pesticides is the persistence of the product in the soil or water. This problem is especially prevalent with herbicides that are applied or dispersed in external environments, with high risk for soil and water contamination.

6.1.6 Inorganic Substrata

The biodeterioration of monumental stones, rock and archaeological remains is a worldwide problem. In fact, the growth and development of many organisms, belonging to different systematic groups, acting alone, together or in ecological succession, surely enhance the stone decay and makes their conservation more difficult. For these reasons adequate interventions must be taken to stop or at least slow down the biodeterioration process.

Once assessed the active presence of organisms on stone monumental surfaces and before to adopt any remedial measures, is necessary to properly evaluate the entity of damages induced by this presence. In fact, when this is very slights and a regular maintenance is impossible, any biocide treatment is useless. The same when the biological patina (lichens) could act as protective layer against major injuries due to physical-chemical factors. Otherwise is necessary to intervene in order to eliminate or control the biodeteriogens.

Many authors have used several products having biocide characteristics, in order to eliminate the biodeteriogens developing on stone monuments or rock sites. These products are commercially available both as active principle or formulates and cover a wide range of chemical classes, from very simple inorganic compounds such as Na and Ca hypochlorite to very complex organic ones such as the Quaternary Ammonium Compounds (Preventol R50, Neo-Desogen). Furthermore, they can possess a strictly specific mode of action such as the urea derivatives (Diuron, Karmex) that block the photosynthetic process, or a broad toxic spectrum like the Organotin compounds (TBTO). A list of the most frequently applied products and the different methodologies used for their application are reported in some articles |17,19,78,211-216| and in a recently published book, in which are reported detailed technical forms for 31 of these biocides |217|. Any biocide intended for use on historic monuments and rock sites must be not only effective against biological growths but at the same time cause no damage to the stone material either by direct action or by leaving deposits on it which may result in successive damage |17,111|. Furthermore, must be underlined the necessity of testing these products under similar “real case” conditions as the interactions among type of substrates, biocides and organisms can give a more realistic indication of how a biocide will perform in situ |213|.

The treatments of weeds and higher plants can be made either using pre or post-emergence products. In the first case the treatment (Simazine, Monuron) must be done in winter in order to block the seed’s germination; in the second case the treatment is made in summer by spraying the product (Picloram, Secbumenton) on leaves |215|.

The problem represented by the birds roosting on monuments can be easily solved by placing on these either low current wires or simple plastic needles (unaestethical). The more general problem of the invasion of the urban areas by this fauna has been fronted either with the use of feeding materials mixed with anti pregnant products together with their periodic capture and release in other sites.

The following steps must be assessed before the application of any treatment in this field of intervention:

a) Effectiveness of action of biocides

The product should be chosen on the basis both of the organisms to be eliminated and of its highest efficacy at lowest dosage |218|. Normally the data reported in bibliography are very accurate on the type, concentration and mode of application of the biocide used, while rarely is mentioned the effective amount of biocide applied for square meter of surface. Thus, it is very difficult to extrapolate the results obtained with the same biocide in different applications. Besides, a general lack of a common test organism |219| to be used as internal standard for lab tests contribute to the impossibility to correlate the real efficiency of different biocides used in various laboratories. Normally the results of positive biocide treatments are clearly visible on the "in situ" biological growth with the exception of lichens which can remains macroscopically unchanged for a long time even if the treatment was effective |217|. The lasting of a treatment in outdoor conditions is ranging from 1 to 3 years depending both on type of the chemical used and environmental conditions to which the treated stone is exposed. Moreover, if the biocide must be wash off, after its action, or leave as preventive on the surface is a matter of discussion |217,220,221|, included the extreme suggestion made for the conservation of the Acropolis of Athens, where the preventive biocide treatments of the critical spots, perhaps even the whole buildings, seem to be as unavoidable as daily tooth brushing for the biological status of human teeth |222|.

b) Interaction biocide/stone/organisms

Possible negative effects on stone surface treated with biocides have been detected either with SEM observations or determined by colorimetric measurements |223-225|. It was ascertained the influence (positive or negative) that a lithotype exerts on the efficacy of a biocide |26,226|. In addition, the same biocide can be more or less active on different strains present in the consortium to be eliminated |227|. Hence the selected biocide should always be tested on test specimen made with the same material of the monument, possibly colonized with the same biocoenosis developing on it. More recently some applications have been made using protective or consolidating polymers alone or mixed with biocides in order to enhance their efficiency |228-230|, while simple cleaning procedures with water-jet have been tentatively used for eradicate biological growth |231|. The ultimate approach is to increase the susceptibility of organisms to biocides prior to treatment, using product as EDTA |232| or ionising radiation |233| which could lead to the use of even lower concentrations of biocides with low environmental impact |232|. Some attempt have been made using enzymes such as protease instead of biocides for the elimination of lichens |19,217,234|.

6.1.7 Organic Substrata

For organic materials even more care should be followed when biocide treatments are required and these must be applied only as last resource. Most of these objects are maintained in closed environment and the development of biodeteriogens, especially microfungi, is always due to unexpected increasing of the water content of the air or of the material itself. Thus the first steps is to individuate the source of the humidity and to block it, then the material could be dried in a ventilated environment, and successively the biological infestation can be gently brushed without any chemical treatments. The application of biocides is foreseen in very special cases, when a material is heavily attacked and for different reason the drying is very difficult or too slow.

For the elimination of bacteria, microfungi and actinomycetes have been used either specific products such as antibiotics or broad-spectrum biocides, in the first group, *streptomycin pimafucin, kanamycin, echonazole* and *nystatin* |208|, together with *pimaricin,* for the treatment of textiles with good results |235|. In the second group have been used the same products used for inorganic substrata (*TBTO* and *Benzalkonium chloride*) together with some chlorine derivatives (*Sodium Pentaclorophenate, O-Phenyl-Phenol, P-cloro-m-cresol*) very active against fungi but with possible drawbacks due to the liberation of chlorine in the substrate.

For the elimination of insects developing inside wooden structures the best solution is the use of poisonous gases, highly penetrating and effective also against eggs, but this is practically very difficult to do. This treatment needs special precaution and permission as for the gamma irradiation system. Furthermore, only small dimensions and mobile objects can be treated. Normally these biodeteriogens are eliminated with application of insecticides, either of synthesis (Dichlorovos and Dieldrin) or natural derivatives (Resmetrine and Permetrine), in organic solvents. The efficiency of the treatment is linked to a complete absorption of the product by the wooden structure because its action (neural block) is effective only after the ingestion of treated wood by the larvae.

In deposit and unsafe confined environment there exist the possibility that small roditors can deteriorate the objects made with organic materials. In this case specific products mixed with food (rice or flour) can be leave inside.

6.1.8 Application Procedures

Spraying and *brushing* of diluted biocide solutions are the most common systems of treatment. The spraying method is preferred for paintings with a very deteriorated surface layer and, generally, when the component material is in poor condition. In the case of organic materials, such as paintings on paper, prints, parchment or old books, spraying or brushing biocides is not always possible because some liquid solutions can dissolve pigments and inks.

Application of *poultices* is carried out especially in the case of hard encrustations in order to increase the contact time and use the dissolving action of water itself. These compresses are made of carboxymethyl cellulose, paper pulp or inert materials that are soaked in the biocide solution. They are covered with sheets of polyethylene or something similar in order to reduce dehydration of the compress itself. The length of application ranges from one day to several days. Sometimes the chosen biocide can be added to a gelatinous solvent paste used for cleaning stone and mural paintings. In some cases, the removal of biological or chemical incrustations on stone is obtained through non-selective absorbent clays (e.g., sepiolite or attapulgite).

Injection of pesticides, using the larvae's tunnels, can be used to increase their diffusion for infested wooden structures. When trees or bushes have high root system and their eradication can be dangerous the injection of concentrated antigerminative substances inside stumps, is needed to completely block new emergencies. In the case of subterranean termites, injection is performed under pressure into the soil.

Fumigation methods are widely employed for organic materials. The treatment consists of distributing the fumigant (gas) in the air and through materials. This method has rapid effectiveness and reach deep penetration inside the object. Due to the high toxicity of fumigants, they must be applied in airtight chambers or in perfectly sealed spaces (sometimes created with polyethylene sheets), where the pressure can be modified to improve the penetration of the gas. Fumigants require highly trained applicators and have no residual effects after aeration. As an alternative to conventional fumigation techniques, researchers have recently investigated the possibility of employing inert gases, such as nitrogen, together with low RH for controlling museum pests |236,237|. The advantage of this system is that it is safe, inexpensive and not invasive, but more tests are necessary to assess its effect against the various species of micro organisms and insects. Treatments with substances active as vapour have similar advantages and, moreover, do not require complicated equipment because the products generally used in vapour form are not very toxic. The object can be treated in a polyethylene bag or airtight cabinet to permit the concentration of vapours.

When either spraying and brushing treatments or poultices are used, it is sometimes advisable to wash off the biocide residues in order to avoid possible secondary reactions (due to the degradation of products) or toxicological difficulties (high-persistence products on premises open to the public or for operators).

The time necessary for a substratum to be reinfested by biodeteriogens is depending on the preventive measures taken for its conservation. This can be very long (several years) if the restored objects are maintain in conditioned and safe confined spaces Otherwise, it is surely very short (1-2 years) when these objects can not be moved inside and are left exposed in outdoor conditions, even if a biocide treatment is left as preventive.

6.2 INDIRECT METHODS

These should control the environment surrounding the objects. The control of weather variables is practically inapplicable for monumental stones, while for rock sites the construction of protective structures, preventing the water run-off of surfaces, can effectively reduce the biological growth |238|. An effective and simple good practice for limit the biodeterioration of exposed stones is their maintenance. In fact, the periodical simple cleaning of exposed surfaces eliminate the "soiling" effect due to the deposit of environmental particles which can favour the development of reproductive bodies. Furthermore, after stone cleaning interventions some chemical substances (protective coatings or consolidants) can be applied to the object in order to increase its water repellence and cohesion. These products may prevent or retard the recolonization of the substrate decreasing its porosity, roughness and water content, but in critical environmental conditions, they could favour the development of specialised micro flora.

In order to reduce the biological risk, for the organic objects maintained in confined environments, taking into account that in our climates the temperature and humidity are for long periods of time favourable to the development of the widespread biological reproductive bodies (conidia, spores and eggs), these should be conditioned at values of temperature ranging between 16° e 18°C and at relative humidity level below the 60%. This humidity value can be a limiting factor for the development of fungi while the temperature for the insects' eggs.

Preventive measures against insects attack are the conservation of the objects inside cardboards, boxes or glass cases treated with insecticides, further the circulation of flying insects can be reduced with air filter and thin wires screens.

7. BIOREMEDIATION

Chemical and physical techniques have been widely used for the cleaning of cultural objects but in order to decrease any possible risk for the materials treated, innovative systems related with biological methods have been introduced in this sector starting from the biological pack |239|.

Enzymes, such as lipase, have been successfully used to remove aged acrylic resin coatings in paintings |240|, while for the removal of sulphates, nitrates and organic matter from artistic stone works, carefully selected microbial cultures have been used |241,242|. A similar methodology has been applied for the elimination of insoluble calcium oxalate patinas from monuments. In fact, some bacteria and actinomycetes living in soils use the oxalate salts as sole source of carbon and energy |243|.

The biomineralization process has been investigated in order to develop a new conservative method for monuments restoration. The precipitation of new calcite crystals inside stone samples was primarily tested utilising the mineralisation process mediated by the organic matrix extracted from mineralised hard parts of marine organisms. Preadsorption of soluble acidic matrix glycoproteins extracted from the shell of the mollusk *Mytilus californianus* was found to increase the amount and depth of penetration of calcite crystals formed inside the stone structure with a significant decrease of its porosity |244|. Furthermore, such a treatment seems to improve the stone superficial mechanical strength |245|.

Also the precipitation of calcium carbonate crystals produced in nature by bacteria has been applied using specific bacterial strains |246| (*Bacillus cereus*), even if the use of heterotrophic viable organisms does not seem appropriate for this purpose. In fact, chemical reactions with stone minerals due to metabolic by-products and the growth of fungi, due to the application of organic nutrients for bacterial development, can have negative effects on monumental stone materials. The results obtained using *Bacillus subtilis* and *Micrococcus sp.*, seem chiefly influenced by the presence of a big microbial mass, due to bacterial growth, rather than to the presence of new crystals due to calcite precipitation |247|. For these reasons the study of the genetic mechanism that controls bacterial calcite precipitation has been started. The *Bacillus subtilis* strain has been mutagenized and not-precipitating mutants isolated. This mutation has been successfully transferred, by transformation, to another *B. subtilis* strain, showing that it affected one gene only. The isolation of this mutant suggests the presence of a genetic control for crystal formation. Characterization of this mutation, and of possible other mutations, should allow the identification of the protein(s) involved in the process |248|.

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8. BIBLIOGRAPHY

INORGANIC SUBSTRATA

Physical and Chemical factors

[1] Schaffer, R.,J. (1967). Causes of deterioration of building materials. I Chemical and physical causes. Chemistry and Industry, 23, 1584-1586.

[2] Winkler, E. M. (1975). Weathering rates of stone in urban atmosphere. In The Conservation of Stone I. R.Rossi-Manaresi Ed., Bologna 27-35.

[3] Jedrzejewska, H. (1981). Physical-chemical factors in the conservation of stone. In The Conservation of Stone II, Part A, R.Rossi-Manaresi Ed., Bologna, 195-204.

[4] Furlan, V., Girardet, F. (1983). Considerations on the rate of accumulation and distribution of sulphurous pollutants in exposed stones. In Material Science and Restauration, Lack und Chimie Eds., Esslingen, 285-290.

[5] Amoroso, G.,G., Fassina, V. (1983). Stone decay and conservation. Material Science Monographs n° 11, Elsevier Ed., New York, USA.

[6] D. Camuffo, (1955). Physical weathering of stones. The Science of the Total Environment, vol 167, 1-14

Microbial weathering of stone materials

[7] Webley, D.M., Henderson, M.E.K. And Taylor, I.F. (1963). The microbiology of rocks and weathered stones. J. Soil Sci. 14 n° 1, 102-112.

[8] Henderson, M.E.K. and Duff, R.B. (1963). The release of metallic and silicate ions from minerals, rocks, and soil by fungal activity. J. Soil. Sci. 14, nø 2, 236-246.

[9] Stambolov, T. and Asperen De Boer, J.R.J. (1967). The deterioration and conservation of porous building materials in monuments. A preliminary review. In ICOM Committe for Museum Laboratories, Brussels.

[10] Hueck Van Der Plas, H.E. (1968). The microbiological deterioration of porous building materials. International Biodeterioration Bullettin 4, nø1, 11-28.

[11] Pochon, J., Jaton, C. (1968). Facteurs biologiques de l'alteration des pierres. In Biodeterioration of materials, H. Walters and J.J: Elphick Eds., Elsevier Publishing Co., 258-268.

[12] Krumbein, W.E. (1972). Role des microrganismes dans la génese, la diagenése et la dégradation des roches en place. Rev. Ecol. Biol. Sol. 9, 283-319.

[13] Berthelin, J. (1983). Microbial weathering processes. In Microbial Geochemistry. Blackwell Scientific Publication, Oxford, 223-262.

[14] Eckhardt, F.E.W. (1985). Solubilization, transport, and deposition of mineral cations by microorganisms-efficient rock weathering agents. In The Chemistry of Weathering. Drever, J.I. Ed., D. Reidel Publishing Co., Dordrecht, 161-173.

[15] Tiano P. (1986). Biological deterioration of exposed works of art made of stone. In Biodeterioration of Constructional Materials. L.G.H. Morton Ed., the Biodeterioration Society Occasional Publication n° 3, 37-44.

[16] AA.VV.(1990) Microflora autotrofa ed eterotrofa tecniche di isolamento in coltura. Raccomandazioni NORMAL 9/88, CNR - ICR Eds. Roma, 1-26.

[17] May, E., Lewis, F.J., Pereira, S., Tayler, S., Seward, M.R.D. and Allsopp D. (1993) Microbial deterioration of building stone - a review. Biodeterioration Abstracts, Cab International, vol 7, n 2, 109-123

[18] Urzì, C., Krumbein, W.E. (1994) Microbiological Impacts on the Cultural Heritage. In Durability and Change: The Science, Responsability and Cost of Sustaining Cultural Heritage. W.E. Krumbein, P. Brimblecombe, D.E. Cosgrove and S. Stainforth Eds., J. Wiley & Son 107-135.

[19] Caneva, G., Nugari, M.P., Salvadori, O. (1991) Biology in the conservation of Works of Arts. ICCROM Ed., Rome, 87-102

[20] Tiano, P. (1994) Biodeterioration of Stone Monuments. A Critical review, in Biodeterioration of Cultural Heritage vol I, K.L. Garg, N. Garg & K.G. Mukerji Eds., Naya Prokash Publ. Calcutta, , 301-321.

[21] Guillitte, O. (1995) Bioreceptivity: a new concept for building ecology studies. The Science of the Total Environment, vol 167, 215-220

[22] Warscheid, T., Petersen, K. & Krumbein, W.e:, (1988) Effect of cleaning on the distribution of microorganisms on rock surfaces. In Biodeterioration 7, D.R.Houghton, R.N. Smith and H.O.W.Eggings Eds., Elsevier Applied Science Publ. 455-460.

[23] Nimis, P.L., Pinna, D., Salvadori O. (1992) Licheni e conservazione dei monumenti C.L.U.E. Ed., Bologna.

[24] Guillitte, O., Dreesen, R. (1995) Laboratory chamber studies and petrographical analysis as bioreceptivity assessement tool of building materials. The Science of the Total Environment, vol 167, 365-374.

[25] Urzì, C., Realini, M., (1996) Bioreceptivity of Rock Surfaces and Its implication in Colour Changes and Alteration of Monuments, Study Case of Noto's Calcareous Sandstones. Dechema Monographs vol 133, VCH Ed.,151-159.

[26] Tiano, P., Accolla, P. and Tomaselli. L., (1995) Phototrophic Biodeteriogens on lithoid surfaces: an ecological study. Microbial Ecology, vol 29 n° 3, 299-309

Bacteria general

[27] Pochon, J., Coppier, C., Tchan, Y.T. (1951). Role des bactéries dans certaines altérations des pierres des monuments. Chimie et Industrie 65, 496-500.

[28] Barcellona Vero, L., Bianchi, R., Monte Sila, M., Tiano P. (1976). Proposal of a method of investigation for the study of the presence of bacteria in exposed works of art in stone. In The Conservation of Stone I, R. Rossi-Manares Ed., Bologna, 257-266.

[29] Krumbein, W.E. (1987). Microbial interaction with mineral materials. In Biodeterioration 7. D.R. Houghton, R.N. Smith and H.O.W. Eggins Eds., Elsevier Applied Science, 78-100.

[30] Lewis, F.J., May, E. And Bravery, A.F. (1985). Isolation and enumeration of autotrophic and heterotropich bacteria from decayed stone. In Vth International Congress on Deterioration and Conservation of Stone, Press Polytechniques Romandes, Lausanne, vol 2, 633-642.

[31] Warscheid, T., Petersen, K., Krumbein, W.E. (1988). Physiological characterization of chemoorganotrophic bacteria isolated from sandstones. VIth International Congress on Deterioration and Conservation of Stone, Suppl. Vol., Torun, 26-32.

[32] Eckhardt, F.E.W. (1988). Influence of culture media in studying microbial weathering of building stones and monuments by heterotrophic bacteria and fungi. VIth International Congress on Deterioration and Conservation of Stone, Suppl. Vol., Torun, 71-81.

[33] Lewis, F.J., May, E. Greenwood, R. (1988). A laboratory method for assessing the potential of bacteria to cause decay of building stone. VIth International Congress on Deterioration and Conservation of Stone, Supplement Vol., Torun, 48-58.

Sulphur-cycle bacteria

[34] Pochon, J., Tchan, Y.T. (1948). Role de bacteries du cicle du soufre dans l'alteration des pierres des monuments. Comptes Rendus Acad. Sci., Paris 226, 2188-2189.

[35] Jaton, C. (1972). Aspects microbiologiques des alterations des pierres de monuments. 1st International Symposium on the Deterioration of Building Stones, La Rochelle, 149-154.

[36] Barcellona Vero, L., Monte Sila, M., Silveri A. (1973). Influenza dell'azione dei solfobatteri nei processi di alterazione dei materiali lapidei. In Problemi di Conservazione. Compositori Ed., Bologna, 439-451.

[37] Tiano, P., Bianchi, R., Gargani, G., Vannucci, S. (1975). Research on the presence of sulphur-cycle bacteria in the stone of some historical buildings in Florence. Plant and Soil 43, 211-217.

[38] Ferrari, R., Passarelli, F., Spano, M., Starace, G. (1979). Utilizzazione dell'anidride solforosa da parte di un ceppo di batteri solfoossidanti in relazione all'alterazione dei materiali calcarei. In 3° Congresso Internazionale sul Deterioramento e Conservazione della Pietra, Venezia, 281-288.

[39] Monte Sila, M., Tarantino, G. (1981). The metabolic state of microorganisms of the genus Thiobacillus on stone monuments. In The Conservation of Stone II - Part A, R.Rossi-Manaresi Ed., Bologna, 117-138.

[40] Guagliandolo, C., Maugeri, T.L. (1988). Isolation of Thiobacillus spp. from stone. In VIth International Congress on Deterioration and Conservation of Stone, Supp. Vol., Torun, 92-101.

[41] Sramek, J. (1980). Determination of the source of surface deterioration on tombstones at the Old Jewish Cemetery in Prague. Studies in Conservation 25, 47-52.

[42] Skoulikidis, T., Poziotis, P. (1981). The mechanism of sulfation by atmospheric SO_2 of limestone and marble of ancient monuments and statues. British Corrosion Journal 16, - part I: 63-69 and part II: 70-77.

[43] Parker, C.D. (1945). The corrosion of concrete. 1 The isolation of a species of bacterium associated with the corrosion of concrete exposed to atmospheres containing hydrogen sulphide. Aust. J. Exptl. Biol. Med. Sc. 23, 81-98.

[44] Forrester, J.A. (1959). Concrete corrosion induced by sulphur bacteria in a sewer. The Surveyor 118, 881-884.

[45] Sand, E., Bock, E. (1986). Simulation of biogenic sulphur acid corrosion of concrete. Importance of hydrogen sulphide, thiosulphate and methyl mercaptane. In Biodeterioration of Constructional materials. L.G.H. Morton Ed., The Biodeterioration Society Occasional Publication n° 3, 29-36.

Nitrogen cycle bacteria

[46] Kauffmann, M. J. (1952). Role des bacteries nitrifiantes dans l'alteration des pierres calcaires des monuments. Comptes Rendus Acad. Sci.,Paris 234, 2395-2397.

[47] Lebedeva, E.V., Lyalikova, N.N. & Bugeliskii, Y.Y. (1978). Partecipation of nitrifying bacteria in the weathering of serpentinized ultra-basic rocks. Microbiol. 47, 898-904.

[48] Meincke, M., Ahlers, B., Krause-Kupsch, T., Krieg, E., Meyer, C., Sameluck, F., Sand, W., Wolters, B. And Bock, E. (1988). Isolation and characterization of endolithic nitrifiers. VIth International Congress on Deterioration and Conservation of Stone, Torun, 15-23.

[49] Bock, E., Sand, W., Meincke, M., Wolters, B., Ahlers, B., Meyer, C. & Sameluck, F. (1987). Biologically induced corrosion of natural stones - Strong contamination of monuments with Nitrifying organisms. In Biodeterioration 7. D.R. Houghton, R.N. Smith and H.O.W. Eggins Eds., Elsevier Applied Science, 436-440.

[50] Livingston, R.A. (1985). The role of nitrogen oxides in the deterioration of carbonate stone. In Vth International Congress on Deterioration and Conservation of Stone, Press Polytechniques Romandes, Lausanne, vol 1, 509-516.

Heterotropic bacteria

[51] Duff, R.B., Webley, D.M., Scott, R.O. (1963). Solubilization of minerals and related materials by ketogluconic acid-producing bacteria. Soil Sci. 95, 17-39.

[52] Deveze, L., Le Petit, J., Matheron, R. (1966). Note préliminarire sur la présence dans le eaux et les sediments marins de bactéries solubilisant certains sels minéraux insolubles (carbonates, phosphates et silicates). Bull. Inst. Oceanogr. Monaco, 1, 1-13.

[53] Ahmad, N. and Jha, K.K. (1968). Solubilization of rock phosphate by micro organisms isolated from Bilbar soils. J. Gen. Microbiol., Tokio , 14, 89-95.

[54] Huang, G.H., Keller, W.D. (1970). Dissolution of rock-forming silicates minerals in organic acids: simulated first stage weathering of fresh mineral surfaces. Amer. Mineral. 55, 2076-2094.

[55] Wood, P.A. And Macrae, I.C. (1972). Microbial activity in sandstone deterioration. International Biodeterioration Bulettin. 8, 397-406.

[56] Bruni, V. (1979). Presenza di batteri solubilizzanti i carbonati su monumenti marmorei calcarei. In 3° Congresso Internazionale sul Deterioramento e Conservazione della Pietra, Venezia, 301-304.

[57] Tiano, P., (1955) Biological Hazard on exposed marble surfaces In the Proceedings of the Congress LCP 95 "Conservation and restoration of cultural heritage", Montreux 25-29/9/95, 723-72758 -

[58] Lewis, F.J., May, E. & Bravery, A.F. (1987). Metabolic activities of bacteria isolated from building stone and their ralationship to stone decay. In Biodeterioration 7. D.R. Houghton, R.N. Smith and H.O.W. Eggins Eds., Elsevier Applied Science, 107-112.

[59] Curri , S.B. (1978). Biocide testing and enzymological studies on damaged stone and frescoes surfaces : preparation of antibiograms. In UNESCO-RILEM International Symposium on Deterioration and Protection of Stone Monuments, Paris, 4.2

[60] Lewis, F.J., May, E., Daley, B., Bravery, A.F. (1986). The role of heterotrophic bacteria in the decay of sandstone from ancient monuments. In Biodeterioration of Constructional materials. L.G.H. Morton Ed., the Biodeterioration Society Occasional Publication n° 3, 45-54

[61] Realini, M., Sorlini, C., Bassi, M. (1985). The Certosa of Pavia: a case of biodeterioration. In Vth International Congress on Deterioration and Conservation of Stone, Press Polytechniques Romandes, Lausanne, vol 2, 627-629.

[62] Tiano, P, Tomaselli, L. (1989). Un caso di biodeterioramento del marmo. ARKOS, notizie GOR n° 6, 12-18.

Instrumental methods

[63] May, E. Lewis, F.J., (1988). Strategies and techniques for the study of bacterial populations on decaying stonework. VIth International Congress on Deterioration and Conservation of Stone, Suppl. Vol., Torun, 59-70.

[64] Tayler, S., & May, E., (1995) Detection of Specific Bacteria on Stone using an Enzime-linked Immunosorbent Assay. International Biodeterioration & Biodegradation 1994, 155-167.

[65] Curri, S.B., Paleni, A. (1981). Histochemical procedures for the evaluation of organic components in deteriorated stone materials. In The Conservation of Stone II - Part A, R.Rossi-Manaresi Ed., Bologna, 445-454.

[66] Curri, S.B., Paleni, A. (1976). Some aspects of the growth of chemolithotrophic microorganisms on the Karnak temple. In The Conservation of Stone I, R.Rossi-Manaresi Ed., Bologna, 267-272.

[67] Warscheid, T., Petersen, K., Krumbein, W.E. (1990). A rapid method to demonstrate and evaluate microbial activity on decaying sandstone. Studies in Conservation 35, 137-147.

[68] Gomez-Alarcon, G., Munoz, M., Arino, X., Ortega-Calvo, J.J. (1995) Microbial communities in weathered sandstones: the case of Carrascosa del Campo church, Spain. The Science of the Total Environment, vol 167, 249-254.

[69] Tayler, S., & May, E., (1995) Comparison of methods for the measurement of microbial activity on stone. Studies in Conservation 40, 163-170.

[70] Tiano P., Tomaselli, L., Orlando, C. (1989). The ATP-bioluminescence method for a rapid evaluation on the microbial activity in the stone materials of monuments. J. Bioluminescence and Chemiluminescence 3, 213-216.

[71] Praderio, G., Schiraldi, A., Sorlini, C., Stassi, A. and Zanardini E. (1993) Microbiological and calorimetric investigation on degraded marbles from the Cà d'Oro facde (Venice). Thermochimica Acta, 227, 205-213.

[72] Swings, J, Descheemaeker, P. (1995) The Role of Heterotrophic Bacteria in the Degradation of Stone. In Environment/Protection and Conservation of the European Cultural Heritage. Research Report n° 2. M De Cleene Ed., Science Information Office University of Ghent Publ., 123-139.

[73] Koestler, R.J., Charola, A.E., Wypyski, M., Lee, J.J. (1985). Microbiologically induced deterioration of dolomitic and calcitic stone as viewed by scanning electron microscopy. In Vth International Congress on Deterioration and Conservation of Stone, Press Polytechniques Romandes, Lausanne, vol 2, 617-626.

[74] Bassi, M., Giacobini, C. (1973). Scanning electron microscopy: a new technique in the study of the microbiology of works of art. International Biodeterioration Bulletin 9, 57-68.

[75] Koestler, R.J., Charola, A.E. And Wheeler, G.E. (1983). Electron microscopy in conservation: the abydos reliefs. In proceedings of the Vth International Seminar on Application of Science in Examination of Works of Art. Boston.

[76] Giacobini, C., Roccardi A., Bassi, M., Favali, M.A. (1986). The use of electron microscope in research on biodeterioration of works of art. In proceedings of the symposium on Scientific Methodologies Applied to Works of art, Firenze, P.L.Parrini Ed., 71-75.

Algae and Cyanobacteria

[77] Giaccone, G. Velloccia Rinaldi, M.L., Giacobini, C. (1976). Forme biologiche delle alghe esistenti sulle sculture all'aperto. In The Conservation of Stone I, R.Rossi-Manaresi Ed., Bologna, 245-266.

[78] Richardson, B.A. (1976). Control of moss, lichen and algae on stone. In The Conservation of Stone I, R.Rossi-Manaresi Ed., Bologna, 225- 231.

[79] Witton, B.A., Donaldson A., Potts, M. (1979). Nitrogen fixation by Nostoc colonies in terrestrial environment of Aldabra atoll. Indian Ocean. Phycologia 18, 278-287.

[80] Ortega-Calvo, J.J., Arino, X., Hernandez-Marine, M., Saiz-Jimenez, C. (1995). Factors affecting the weathering and colonisation of monuments by phototrophic microorganisms. The Science of the Total Environment, vol 167, 329-341

[81] Grossin, F., Dupuy, P. (1978). Méthode simplifiée de détermination des constituants des salissures. In UNESCO-RILEM International Symposium on Deterioration and Protection of Stone Monuments, Paris, 4.4.

[82] Ahmadjian, V. (1976) A guide to the algae occurring as lichen symbionts: isolation, culture, physiology and identification. Phycology 6, 127-160.

[83] Tomaselli, L., Margheri, M.C., Florenzano, G. (1979). Indagine sperimentale sul ruolo dei cianobatteri e delle microalghe nel deterioramento di monumenti e affreschi. In 3° Congresso Internazionale sul Deterioramento e Conservazione della Pietra, Venezia, 313- 325.

[84] Agnostidis, K., Economou-Amilli, A. & Roussomoustakaki, M (1983) Ephilitic and Chasmolitic Microflora (Cyanophyta, Bacillariophyta) from Marbles of the Partenon (Acropolis-Athens, Grece) Nova Hedwigia, Band XXXVIII - Braunschweig, 227-325.

[85] Ortega-Calvo, J.J., Hernandez-Marine, M., Saiz-Jimenez, C. (1991) Biodeterioration of Building Materials by Cyanobacteria and Algae. International Biodeterioration 28, 165-185.

[86] Ortega-Calvo, J.J., Hernandez-Marine, M., Saiz-Jimenez, C. (1993) Cyanobacteria and Algae on Historic Building and Monuments. In Biodeterioration of Cultural Heritage vol I, K.L. Garg, N. Garg & K.G. Mukerji Eds., Naya Prokash Publ. Calcutta, 173-203.

[87] Trotet, G., Dupuy, P. et Grossin, F. (1972). Sur une nuisance biologique provoquée par le cyanophycées. 1st International Symposium on the Deterioration of Building Stones, La Rochelle, 167.

[88] Giacobini, C., Andreoli, C., Casadoro, G., Fumanti, B., Lanzara, P., Rascio, N. (1979) Una caratteristica alterazione delle murature e degli intonaci. In 3° Congresso Internazionale sul Deterioramento e Conservazione della Pietra, Venezia, 289-299.

[89] Danin, A., Gerson, R., Marton, K. And Garty, J. (1982). Patterns of limestone and dolomite weathering by lichens and blue-green algae and their paleoclimatic significance. Paleogeogr. Paleoclimatol. 37, 221-233.

[90] Pietrini, A.M., Ricci, S., Bartolini, M. and Giuliani, M.R.A. (1985). A reddish colour alteration caused by algae on stoneworks. Preliminary study. In Vth International Congress on Deterioration and Conservation of Stone, Press Polytechniques Romandes, Lausanne, vol 2, 653-662.

[91] Frediani, P, Manganelli Del Fa', C., Matteoli, U., Tiano, P., Galli, G. (1978). A methodological approach to the study of the deterioration of the stone in Boboli Garden. In UNESCO-RILEM International Symposium on Deterioration and Protection of Stone Monuments, Paris, 7.4.

[92] Dupuy, P., Trotet, G. et Grossin, F. (1976). Protection des monuments contre les cyanophycées en milieu abrite et humide. In The Conservation of Stone I, R.Rossi-Manaresi Ed., Bologna, 205-219.

[93] Lamenti G., Bosco M., Tomaselli L., Tiano P.,(1996) PCR-MPN: un nuovo approccio per il monitoraggio rapido, senza isolamento, dei microrganismi colonizzanti substrati lapidei. XV Conv. Sci. SIMGBM, Abbadia S. Salvatore (SI). Abstract 103

[94] G.Cecchi, L.Pantani, V.Raimondi, D.Tirelli, L.Tomaselli, G.Lamenti, M..Bosco, P.Tiano, (1996) Fluorescence lidar technique for the monitoring of biodeteriogens on the cultural heritage in Remote Sensing for Geography, Geology, Land Planning and Cultural Heritage. D.Arroyo-Bishop et al. Edts., SPIE, Bellingham, 2960, 137-147.

Lichens

[95] Schatz, A. (1963). The importance of metal-binding phenomena in the chemistry and microbiology of the soil. part I : The chelating properties of lichens and lichens acids. Advancing Frontiers Pl. Sci., 6, 113-134.

[96] Schatz, A. (1963). Soil microorganisms and Soil Chelation. The pedogenic Action of lichens and Lichen Acids. Agric. Food Chem. 11, 112-118.

[97] Lounamaa, K.J. (1965). Studies on the content of iron, manganese and zinc in macrolichens. Ann. Bot. Fenn., 2, 127-137.

[98] Syers, J.K. (1969). Chelating Ability of Fumarprotocetraric Acid and Parmelia Conspersa. Plant Soil 31, 205-208.

[99] Iskandar, I.K. and Syers, J.K. (1972). Metal-complex formation by lichen compounds. J.Soil. Sci. 23, 255-265.

[100] Williams, M.E. and Rudolph, E.D. (1974). The Role of Lichens and Associated Fungi in the Chemical Weathering of Rock. Mycologia 66, 648-660.

[101] Ascaso, C. and Galvan, J. (1976). Studies on the pedogenic action of lichen acids. Pedobiologia 16, 321-331.

[102] Ascaso, C., Galvan, J., and Ortega C. (1976). The pedogenic action of Parmelia conspersa, Rhizocarpon geographicum and Umbilicaria pustulata. Lichenologist 19, 151-171.

[103] Jones, D., Wilson, M.J. and Tait, J.M. (1980). Weathering of a Basalt by Pertusaria corallina. Lichenologist 12, 277-289.

[104] Galvan, J., Rodriguez, C. and Ascaso C. (1981). The Pedogenic Action of Lichen in Metamorphic Rocks. Pedobiologia 21, 60-73.

[105] Ascaso, C., Galvan, J. and Rodriguez, C. (1982). The Weathering of Calcareous Rocks by Lichens. Pedobiologia 24, 219-229.

[106] Wilson, M.J. And Jones, D. (1983). Lichen weathering of minerals: implications for pedogenesis. In Wilson, R.C. Ed., Blackwell, London, 5-12.

[107] Jones, D. and Wilson, M.J. (1985). Chemical Activity of Lichens on Mineral Surfaces - A Review. International Biodeterioration Bulletin. 21, 99-105.

[108] Jones, D., Wilson, M.J. And Mc Hardy, W.J. (1987). Effects of lichens on mineral surfaces. In Biodeterioration 7. D.R. Houghton, R.N. Smith and H.O.W. Eggins Eds., Elsevier Applied Science, 129-134.

[109] Fry, E.J. (1927). The mechanical action of crustaceous lichens on substrata of shale, schist, gneiss, limestone and obsidian. Ann. of Botany 41, 437-460.

[110] Gehrmann, C.K., Petersen, K., Krumbein, W.E. (1989). Silicole and calcicole lichens on jewish tombstones - Interactions with environment and biocorrosion, VIth International Congress on Deterioration and Conservation of Stone, Torun, Suppl. Vol., 33-38.

[111] Ciarallo, A. Festa, L., Piccioli, C., Raniello, M. (1985). Microflora action in the decay of stone monuments. In Vth International Congress on Deterioration and Conservation of Stone, Press Polytechniques Romandes, Lausanne, vol 2, 607-616.

[112] Richardson, B.A. (1973). Control of Biological growths. Stone Industries vol 8, 22-26.

[113] Fry, E.J. (1922). Some Types of Endolithic Limestone Lichens. Ann. Bot. 36, 541-562.

[114] Pallecchi, P., Pinna, D. (1988). Alteration of stone caused by lichen growth in the roman theatre of Fiesole (Firenze). VIth International Congress on Deterioration and Conservation of Stone, Suppl. Vol., Torun, 39-47.

[115] Monte M. (1993) The influence of environmental conditions on the reproduction and distribution of ephilitic lichens. Aereobiologia vol 9, n° 2, 169-180.

[116] Piervittori, R., Laccisaglia, A. (1993) Lichens as biodeterioration agents and biomonitors. Aereobiologia vol 9, n° 2, 181-186.

[117] Nimis, P.L., Monte, M., Tretiach, M. (1987). Flora e vegetazione lichenica di aree archeologiche del Lazio. Studia Geobotanica 7, 3-161.

[118] Garcia-Rowe, J. & Saiz-Jimenez, C. (1991) Lichens and Bryophytes as Agents of Deterioration of Building Materials in Spanish Cathedrals. International Biodeterioration 28, 151-163.

[119] Jaton, C., Orial, G., Brunet, F.A. (1985). Action des vegetaux sur les materiaux pierreux. In Vth International Congress on Deterioration and Conservation of Stone, Press Polytechniques Romandes, Lausanne, vol 2, 577-586.

[120] Seaward, M.R.D., Giacobini, C., Giuliani, M.R., Roccardi, A. (1989). The role of lichens in the biodeterioration of ancient monuments with particular reference to central Italy. International Biodeterioration. 25, 49-55.

[121] Lallement, R. and Deruelle, S. (1978). Presence de lichens sur le monuments en pierre: nuisance ou protection. In UNESCO-RILEM International Symposium on Deterioration and Protection of Stone Monuments, Paris, 4.6.

[122] Salvadori, O., Zittelli, A. (1981) Monoydrate and dihydrate calcium oxalate in living lichen incrustations biodeteriorating marble columns of the basilica of Santa Maria Assunta on the island of Torcello (Venice). In The Conservation of Stone II, Part A, R.Rossi-Manaresi Ed., Bologna, 379-390.

[123] Syers, J.K., Birnie, A.C., Mitchell, B.B. (1967). The calcium oxalate content of some lichens growing on limestone. Lichenologist 3, 409-414.

[124] Wadsten, T. and Moberry, R. (1985). Calcium oxalate hydrates on the surface of lichens. Lichenologist 17, 239-245.

[125] Bech-Andersen, J. (1987). Oxalic acid production by lichens causing deterioration of natural and artificial stones. In Biodeterioration of Constructional materials. L.G.H. Morton Ed., the Biodeterioration Society occasional publication n° 3, 9-13.

[126] Del Monte, M., Sabbioni, C., Zappia, G. (1987). A study of the patina called "scialbatura" on imperial roman marbles. Studies in Conservation 32, 114-121.

[127] Del Monte, M., Sabbioni, C., Zappia, G. (1987). The origin of calcium oxalate on historical buildings monuments and natural outcrops. Science Total Environm. 67, 11-39.

[128] Lazzarini, L., Salvadori, O. (1989). A reassessment of the formation of the patina called Scialbatura. Studies in Conservation 34, 20-26.

[129] AA. VV. (1989) The oxalate films: origin and significance in the conservation of works of arts. Proceedings of the International Symposium, G. Alessandrini Ed., CNR Milano.

[130] AA.VV (1995) The oxalate films in the conservation of works of art. Proceedings of the II International Symposium, G. Alessandrini Ed., CNR Milano.

[131] Jones, D., Wilson, M.J. and Mc Hardy, W.J. (1981). Lichen weathering of rock-forming minerals: application of scanning electron microscopy and microprobe analysis. J. Microscopy 124, 95-104.

[132] Bech-Andersen, J. and Christensen, P. (1983). Studies of lichen growth and deterioration of rocks and building materials using optical methods. In Biodeterioration 5, Oxley, T.A. and Barry, S. Eds., J.Wiley & Sons, Chichester, 568-572.

Actinomycetes and Fungi

[133] Agarossi, G., Ferrari, R., Monte, M. (1984). The Basilica of S. Clemente in Rome Studies on Biodeterioration. In Proceedings of the symposium on Scientific Methodologies Applied to Works of art, Firenze, P.L.Parrini Ed., 52,56.

[134] Giacobini, C., De Cicco, M.A., Tiglie I. & Accardo, G. (1987). Actinomycetes and Biodeterioration in the Field of Fine Art. In Biodeterioration 7. D.R. Houghton, R.N. Smith and H.O.W. Eggins Eds., Elsevier Applied Science, 418-427.

[135] Agarossi, G., Ferrari, R., Monte, M., Gugliandolo, C., Maugeri, M. (1988). Changes of microbial system in an etruscan tomb after biocidal treatments. VIth International Congress on Deterioration and Conservation of Stone, Torun, Supp. Vol., 82-88.

[136] Henderson, M.E.K. and Duff, R.B. (1963). The release of metallic and silicate ions from minerals, rocks and soils by fungal activity. J. Soil Sci. 14, 236-246.

[137] Silverman M.P. and Munoz, E.F. (1970). Fungal attack on rock: solubilization and altered infrared spectra. Science 169, 985-987.

[138] Silverman, M.P. (1979). Biological and organic chemical decomposition of silicates. In Studies in Environmental Science. Trudinger, P.A. and Swaine, D.J., Eds., Elsevier, New York.

[139] Eckhardt, F.E.W. (1980). Microbial degradation of silicates.- The release of cations from aluminosilicates minerals by yeasts and filamentous fungi. In Biodeterioration. Oxley, T.A., Allsopp, D. and Becker, G. Eds., Pitman Publ. Ltd, London, 107-116.

[140] Petersen, K., Kuroczkin, J., Strzelczyk, A.B. and Krumbein, W.E. (1987). Distribution and effects of fungi on and in sandstones. In Biodeterioration 7. D.R. Houghton, R.N. Smith and H.O.W. Eggins Eds., Elsevier Applied Science, 123-128.

[141] De la Torre, M.A., Gomez-Alarcon, G., Melgarejo, P. and Saiz-Jimenez C. (1991) Fungi in weathered sandstone from salamanca cathedral, Spain. The Science of the Total Environment, 107, 159-168.

[142] Wollenien, U., de Hoog, G.S., Krumbein, W.E., Urzì C. (1995) On the isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. The Science of the Total Environment, 167, 287-294

[143] Krumbein, W.E., Grote, G. and Petersen, K. (1986). Metal biotransfer and biogenic crust formation in building stones. In Biodeterioration of Constructional materials. L.G.H. Morton Ed., the Biodeterioration Society Occasional Publication nø 3, 15-27.

[144] Krumbein, W.E. and Jens, K. (1981). Biogenic rock varnishes of the Negev Desert (Israel). An ecological study of iron and manganese transformation by cyanobacteria and fungi. Oecologia 50, 25-38.

[145] Petersen, K., Grote, G., Krumbein, W.E. (1988). Biotransfer of metals by fungi isolated from rocks. VIth International Congress on Deterioration and Conservation of Stone, Supp. Vol., Torun, 111-119.

[146] Leznicka, S., Strzelczyk, A., Wandrychowska, D. (1988). Removing fungal stains from stone works. VIth International Congress on Deterioration and Conservation of Stone, Torun, Suppl. Vol., 102-110.

[147] Saiz-Jimenez, C. (1995) Microbial melanins in stone monuments. The Science of the Total Environment, 167, 273-286.

Lower plants and weeds

[148] Lewis, C.C., Eisenmenger, W.S. (1948). Relationship of plant development to the capacity to utilize potassium in orthoclase feldspar. Soil Sci. 65, 495-500.

[149] Keller, N.D., Frederickson, A.F.,(1952). The role of plants and colloid acids in the mechanisms of weathering. Am. Journ. Sci. 250, 594-608.

[150] Altieri, A., Ricci, S. (1994) Il ruolo delle briofite nel biodeterioramento di materia lapidei. In Proceedings of the 3rd International Symposium "The Conservation of Monuments in the Mediterranean Basin", Venezia, V. Fassina, H. Off, F. Zezza, Eds. 329-333.

[151] Tiano, P. (1986). Problemi biologici nella conservazione del materiale lapido. La Prefabbricazione 22, 261-272.

[152] Bettini, C., Villa, A. (1976). Il problema della vegetazione infestante nelle aree archeologiche. In The Conservation of Stone I, R.Rossi-Manaresi Ed., Bologna, 191-204.

[153] Caneva, G., Galotta, G. (1994) Floristic and structural changes of plant communities of the Domus Aurea (Rome) related to a different weed control. In Proceedings of the 3rd International Symposium "The Conservation of Monuments in the Mediterranean Basin", Venezia, V. Fassina, H. Off, F. Zezza, Eds. 317-322.

[154] Winkler, E.M. (1975). Stone decay by plants and animals. Stone properties, durabilities in man's environment. Springer Verlag Ed., 154-163.

[155] Caneva, G., Salvadori, O. (1989). Biodeterioration of stone. Studies and Documents on the Cultural Heritage n°16, UNESCO, 182-234.

[156] Keller, N.D., Coleman, N.T. (1952). Cation exchange properties of plant roots surfaces. Plant and Soil 11, 243-256.

[157] Rovira, A.D. (1969). Plant root exudates. Bot. Rev. 35, 35-57.

[158] Caneva G., Altieri, A. (1988). Biochemical mechanisms of stone weathering induced by plant growth. VIth International Congress on Deterioration and Conservation of Stone, Torun, 32 -44.

[159] Caneva, G. (1985). Ruolo della vegetazione nella degradazione di murature ed intonaci. Atti del Convegno Scienza e Beni Culturali. L'intonaco: storia, cultura e tecnologia. Bressanone, 199-209.

[160] Tiano, P., Caneva, G. (1987). Procedure for the elimination of vegetals biodeteriogens from stone monuments. In Preprints of 8th Triennial Meeting ICOM, Sydney, vol. III, 1201-1205

[161] Fusey, P. and Hyvert, G. (1966). Biological deterioration of stone monuments in Cambodia. Soc. Chem. Ind., Monograph 23, 125-129.

[162] Rieder, J. (1984). The restoration of archeological monuments in the tropical climates. In Preprints of 7th Triennial Meeting of ICOM, Copenhagen, 84.10.21.

[163] Fajrusina, S.A. (1974). Capparis spinosa L., destroyer of architectural monuments in Uzbechistan. Ac. Sc. Rep. Soc. Sov. Uzb. Journ. Biol. Uzbechistan 22, 134-139.

[164] Fisher, G.G. (1972). Weed damage to materials and structures. International Biodeterioration Bullettin 8, 101-103.

[165] Allsopp, D., Drayton, D.R. (1975). The higher plants as deteriogens. Proc. III Int. Biod. Symp., Kingstone, USA, 357-364.

[166] Bech-Anderson, J. (1984). Biodeterioration of natural and artificial stone caused by algae, lichens, mosses and higher plants. Proc. VIth Int. Biodetn. Symp., Washington, 126-131.

[167] Lewin, S.Z., Charola, A.E. (1981). Plant life of stone surfaces and its relation to stone conservation. Scanning Electron Microscopy 1, 563-568.

Animals

[168] Lloyd, A.O. (1976). Progress in studies of deteriogenic lichens. In Proceedings of the Third International Biodegradation Symposium, J.M.Sharpley and A.M. Kaplan Eds. Applied Science Publishers Ltd, London, 395-402.

[169] De Silva, R.H. (1975). Rock painting in Sri Lanka. In Preprint of IIC Congress on Conservation in Archeological and the Applied Arts. Stockholm, 69-74.

[170] Agrawal, O.P., Tei Singh, B.V., Kharbade Kamal, K., Jain and Toshi, J.P. (1987). Discolouration of Taj Mahal Marble. A Case Study. In Preprints of 8th Triennial Meeting ICOM, Sydney, vol II, 447-451.

[171] Clapp, W.F., Kenk, R. (1963). Marine borers. An annotated bibliography.. Office of Naval Research. Dpt. of the Navy, Washington D.C., 10+1134..

[172] P.Tiano " Problematiche biologiche nella conservazione e restauro degli intonaci dipinti". Le pitture murali. Centro Di Ed., Firenze 1990, 189-194.

ORGANIC SUBSTRATA

Woods

[173] Kirk, K.T., and M. Shimada (1985). "Lignin biodegradation: the micro organisms involved, and the physiology and biochemistry of degradation by white-rot fungi," *Biosynthesis and Biodegradation of Wood Components.* Ed. T. Higuchi. London: Academic Press. 579-605.

[174] Allsopp, D., and K.J. Seal (1986). *Introduction to Biodeterioration.* London: Edward Arnold.

[175] Bravery, A.F., R.W. Berry, L. Carey and D.E. Cooper (1987). "Recording wood rot and insect damage in buildings," *Building Research Establishment Report.*

[176] Garg, K.L., and S. Dhawan (1985-1987). "Microbial deterioration of wooden cultural property," *Conservation of Cultural Property in India,* ed. T. Singh. Vol XVI11-XX. New Delhi: Indian Association for the Study and Conservation of Cultural Property. 4-85.

[177] Florian, M. (1988). "Deterioration of organic materials other than wood," *Conservation of Marine Archaeological Objects,* ed. Colin Pearson. London: Butterworths. 21-54.

[178] Abbate Edlmann, M.L., A. Gambetta, G. Giachi and E. Orlandi (1989). "Studio del deterioramento di alcune specie legnose appartenenti ad un relitto navale del VII secolo. A.C., effettuato con il microscopio elettronico a scansione," Il *restauro del legno. Atti del* 21 *Congresso Nazionale Restauro del Legno.* Florence: Nardini Editore. 121- 127.

[179] Edlmann Abbate, M.L. (1967). "Primo contributo allo studio delle alterazioni da Teredini in vari legni immersi nel Mare Ligure," *Contributi Scientifico-Pratici per una Migliore Conoscenza e Utilizzazione del Legno. VII,* No. 10. Rome: CNR. 9-35.

[180] Feilden, B.M. (1982). "Insects and other pests as causes of decay," *Conservation of Historic Buildings.* London: Butterworth Scientific. 131-151.

Paper

[181] Kowalik, R. (1980a). "Microbio deterioration of library materials. Part 1," *Restaurator,* 4. 99-114.

[182] Kowalik, R. (1980b). "Microbio decomposition of basic organic library materials. Microbio deterioration of library materials. Part 2," *Restaurator,* 4 (34). 135-219.

[183] Gallo, F. (1985). *Biological Factors in Deterioration of Paper.* Rome: ICCROM.

[184] Dhawan, S, and O.P. Agrawal (1986). "Fungal flora of miniature paintings and lithographs," *Int. Biodet. Bull.,* 22 (2) 95-99

[185] Strzelczyk, A.B. (1981). "Painting and sculpture," *Microbial Biodeterioration. Economic Microbiology,* vol. 6. Ed. A.H. Rose. London: Academic Press. 203-234.

[186] Dhawan, S. (1986). *Microbial deterioration of paper material - A literature review.* Government of India, Department of Culture, National Research Laboratory for Conservation of Cultural Property. Ed. M.M. Khan. Lucknow, India. 1-18.

[187] Arai, H. (1987). "On the foxing-causing fungi," ICOM *Committee for Conservation, 8th Triennial Meeting, Sydney, Sept. 1987,* ed, K. Grimstad, U.S.A.: The Getty Conservation Institute. 1165-1167.

[188] Arai, H, N. Matsui And H. Murakita (1988). "Biochemical investigations on the formation mechanisms of foxing," *The Conservation of far Eastern Art. Preprints of the contributions to the Kyoto Congress, 19-23 September 1988,* eds. S. Mills, P. Smith and K. Yamasaki. London: *11C. 11-14.*

[189] Hey, M., G. Pasquariello, F. Gallo, G. Guidi and F. Pierdominici (1988). "Paper analysis in relation to foxing," III *Conferenza Internazionale sulle Prove non Distruttive, 17-20 Aprile, 1988. Perugia.* Preprints. Rome: ICR, A1Pn13. 11/9.1-9. 10.

[190] Strzelczyk, A.B and S. Leznicka (198 1). "The role of fungi and bacteria in the consolidation of books," *Int. Biodet. Bull.,* 17 (2) 57-67.

[191] Menier, J. (1988). "Sur quelques insectes deprédateur des archives," *Patrimoine culturel et alterations biologiques. Actes des journees d'etudes de la S.F.I.I.C., Poitiers, 17-18November 1988.* 45-52.

Textiles

[192] Vigo, T.L., (1980). "Protection of textiles from biodeterioration," *Conservazione e Restauro dei Tessili. Convegno Internazionale, Como.* 18-26.

[193] Mahomed, R.S. (1971). "Antibacterial and antifungal finishes," *Chemical After treatment of Textiles,* eds. H. Mark, N.S. Wooding and S.M. Atlas. New York, London: Wiley Interscience. 507-552.

[194] Hueck, H.J. (1972). 'Textiles pests and their control," *Textiles Conservation.* London: J. E. Leene. 76-97.

Leather

[195] Varonina, L.L., O.N Nazarova, U.P. Petushkova and N.L. Rebrikova (1981). "Damage of parchment and leather caused by microbes," *ICOM Committee for Conservation, 6th Triennial Meeting. Ottawa, 21-25 September 1981.* Preprints. Paris: ICOM. 1913.1-19/3.11.

[196] Von Endt, D.W., and W.C. Jessup (1986). "The deterioration of protein materials in museums," *Biodeterioration* 6, *Proceedings of the Sixth International Biodeterioration Symposium,.* eds. S. Barry and D.R. Houghton. Great Britain: Cab International. 332-337.

[197] Strzelczyk, A.B, J. Kuroczkin and W.E. Krumbein (1987). "Studies on microbial degradation of ancient leather bookbindings: part I," *Int. Biodet. Bull.,* 23, (1) 3-27.

Paintings

[198] Makies, F. (1981). "Enzymatic consolidation of paintings," ICOM *Committee for Conservation. 6th Triennal Meeting. Ottawa, 21-25 September 1981.* Preprints. Paris: ICOM. 81/2/7- 1 - 7n.

[199] Makies, F., (1984). 'Enzymatic removal of lining paste from painting," *ICOM Committee for Conservation. 7th Triennal Meeting. Copenhagen, 10-14 September 1984.* Preprints. Paris: ICOM. 84.2.26-2.30.

[200] Ionita, 1. (1971). "Contribution to the study of the biodeterioration of the works of art and of historic monuments. 11. Species of fungi isolated from oil and tempera paintings," *Rev. Rourn. Biol. Botanique,* 16 (5) 377-381.

METHODS OF CONTROL

[201] De Cleene, M. (1994) Summary of the results, General conclusion and Recommendation. The Role of Heterotrophic Bacteria in the Degradation of Stone. In Environment/Protection and Conservation of the European Cultural Heritage. Research Report n° 2. M De Cleene Ed., Science Information Office University of Ghent Publ., 261

[202] Van Der Molen, L, J. Garty, B.W. Aardema and W. Krumbein (1980). "Growth control of algae and cyanobacteria on historical monuments by a mobile UV unit (MUVU)," *Studies in Conservation,* 25 (2) 71-77.

[203] Hickin N., *"Wood-destroying insects and works of art",* in *IIC New York Conference* ,2nd Ed., vol II, 1971, 75-85.

[204] *Conservation of Wood in painting and Decorative Arts*. Contributions to the Oxford Congress, London IIC, 1978.

[205] Horakova, H., and F. Martinek (1984). "Disinfection of archive documents by ionizing radiation," *Restaurator,* 6. 205-216.

[206] Zuffi, S. (1988). "Ferrari da Passano: così! ho sfrattato i piccioni dal Duomo di Milano," *Rassegna dei Beni Culturali,* 2. 44-45.

[207] Szent-Ivany J.S., "L'identification des insectes nuisibles et le maniéres de les combattre", in La Préservation des Biens Culturels, Musées et Monuments, Unesco, 1976, 55-75.

[208] Smith R.D., *"Background, use and benefits of blast freezers in the prevention and extermination of insects"* , in *Biodeterioration* 6, C.C. Llewellyn & C.E. O'Rear Eds., Cab International Mycological Institute, England, 1986, 374-379.

[209] Gargani, G., (1968) Fungus contamination of Florence art-masterpieces before and after the 1966 disaster. Bodeterioration of Materials 1. Amsterdam, Elsevier, 252-257.

[210] Sorlini, C. (1984). *L'azione degli Agenti Microbiologici sulle Opere d'Arte,* ENAIP, Ed. del Laboratorio, Botticino (Brescia). 1-48.

[211] Capponi, G. and Meucci, C. (1987). Il restauro del paramento lapideo della facciata della chiesa di S. Croce a Lecce," *Bollettino d'Arte, Suppl.* 41 vol. II. 163-182.

[212] Richardson, B.A. (1988) Control of microbial growth on stone and concrete. In Biodeterioration 7. D.R. Houghton, R.N. Smith and H.O.W. Eggins Eds., Elsevier Applied Science, 101-107.

[213] Allsopp, C. & Allsopp D., (1983) An updated survey of commercial products used to protect materials against biodeterioration. Biodet. Bull., vol 19, 99-146.

[214] Grant, C., Bravery, A. F. (1981) Laboratory evaluation of algicidal biocides for construction materials, 1. An assessment of some current test methods. International Biodeterioration Bulletin, 17, 113-123.

[215] Martin, A.K., Johnson, G. C. (1992) Chemical control of lichen growths established on building material: a compilation of the published literature. Biodeterioration Abstracts 6, 101-117.

[216] Tiano, P., Caneva, G. (1987) Procedures for the elimination of vegetal biodeteriogens from stone monuments. In "Preprint of 8th Triennial Meeting of ICOM" Sidney 6-11/9/1987, 1201-1205.

[217] Mirsha, A.K., Jain, Kamal, K., Garg, K.L. (1995) Role of higher plants in the deterioration of historic buildings. The Science of the Total Environment, 167, 375-392.

[218] Caneva, G., Nugari, M.P., Pinna, D., Salvadori, O. (1996) Il controllo del degrado biologico. Nardini Ed., Fiesole (FI). 200 pp, 20 ills.

[219] Caneva, G., D'Urbano, M.S., Salvadori, O. (1993) Test methods for comparative evaluation of biocide treatments. In Proceeding of the International Congress on "The Conservation of Stone and other Materials", Paris, 29th June -1st July 1993. M. J. Thiel Ed., E & FN Spon Publis., London, vol 2, 565-572.

[220] Tiano, P.(1979) Antialgal effect of some chemicals on exposed stoneworks. 3rd International Congress on 'The Deterioration and Preservation of Stones', Venezia 24-27/10/1979, 253-260.

[221] Frey, T., von Reis, J., Barov, Z. (1993) An evaluation of biocides for control of the biodeterioration of artifacts at Hearst Castle. In Preprints 10th Triennial Meeting ICOM, Washington, D.C., Usa, 875-881.

[222] Tiano, P. Accolla, P. Tomaselli, L. (1994) Biocidal treatments on algal biocoenosis. Control of the lasting activity "Science and Technology for Cultural Heritage, vol 3, 45-55

[223] Anagnosidis, K., Gehrmann, C.K., Gross, M., Krumbein, W.E., Lisi, S., Pantazidou, A., Urzi, C. & Zagari, M. (1995) Biodeterioration of marbles of the Parthenon and Propylaea, Acropolis, Athens - associated organisms, decay and treatment suggestions. In Proceedings of the 2nd International Symposium on "The Conservation of Monuments in the Mediterranean Basin". Geneve (CH), Decrouez, D, Chamay J. And Zezza F. Eds., 305-321.

[224] Pasetti, A., Massa, V., Cozzi, F. (1992) Studio dell'impatto del diserbo chimico su paramenti in laterizio a vista. Il caso delle mura di Ferrara. Atti del Convegno di stdi "Le superfici dell'architettura, il cotto. Caratterizzazione e trattamenti", Bressanone (IT), 743-751.

[225] Nugari, M.P., Pallecchi, P., Pinna, D. (1993) Methodological evaluation of biocidal interference with stone materials. Preliminary laboratory tests. In proceedings of the International RILEM/UNESCO Congress "Conservation of Stone and other Materials", M.J. Thiel Ed., E &FN Spoon, Publ, London, 295-302.

[226] Altieri A., Coladonato, M., Lonati, G. Malagodi, M., Nugari, M.P., Salvadori, O. (1997) Effects of biocidal treatments on some Italian lithotypes samples. In Proceedings of IV International Symposium on the Conservation of Monuments in the Mediterranean Basin , Rodhes, 355 - Controllare.

[227] Young, M. E., Wakefield, R., Urquhart, D.M.C, Nicholson, K., Tonge, K. (1995) Assessment in a field setting of the efficacy of various biocides on sandstones. In International Colloquium on Methods of evaluating products for the conservation of porous building materials in monuments, Rome, 19/21/June/1995, ICCROM Ed.,93-99.

[228] Tiano, P. Accolla, P. Tomaselli, L.. (1995) The effectiveness of some biocides against algal biodeterioration. In Proceeding of the International Congress on "The Conservation of Stone and other Materials", Paris, 29th June -1st July 1993. M. J. Thiel Ed., E & FN Spon Publis., London, vol 2, 573-580.

[229] Tiano, P. Camaiti, M., Accolla. P. (1995) Methods for the evaluation of products against algal biocoenosis of monumental fountains. In International Colloquium on Methods of evaluating products for the conservation of porous building materials in monuments, Rome, 19/21/June/1995, ICCROM Ed., 75-86

[230] Grant, C., Bravery, A.F. (1985) Laboratory evaluation of algicidal biocides for use on contructional materials. 3 Use of the vermiculite bed technique to evaluate toxic washes, surface coatings and surface treatments. International Biodeterioration 21 (4), 285-293.

[231] Leznicka, S. (1992) Antimicrobial protection of stone monuments with p-hydroxybenzoic acid esters and silicone resins. In Proceedings of the 7th International Congress on "Deterioration and Conservation of Stone", Lisbon, Delgado Rodriguez, J., Henriques, F. and Telmo F.J. Eds., 481-490.

[232] Pantazidou A. and Theoulakis, P. (1997) Cyanophythes and associated flora at the neoclassical place of STs George and Michael in Corfu (Greece). Aspect of cleaning procedures. In Proceedings

of IV International Symposium on the Conservation of Monuments in the Mediterranean Basin , Rodhes, vol 2, 355-367.

[233] Tayler, S. May E., (1994) The investigation of biocides against bacteria isolated from stone and their effectiveness against in situ population. Material und Organismes 28, 265-278.

[234] Petushkova, J.P., Lyalikova, N. N. and Nichiporov, F.G. (1988) Effect of ionizing radiation on monument deteriorating organisms. J. Radioanalytical an Nuclear Chem. Articles, 125 (2), 367-371.

[235] Hill, E.C., (1990) Biocide for the future. International Biodeterioration 26, 281-285.

[236] Nugari, M.P., G.F. Priori, D. Mate' and F. Scala (1987). "Fungicides for use on textiles employed during the restoration of works of art," *Int. Biodet. Bull.,* 23. 295-306.

[237] Gilbert, M., (1989). Inert atmosphere fumigation of museum objects.)," *Studies in Conservation* 34*,* 80-84.

[238] Valentin, N., M. Lidstrom and F. Preusser (1990). "Microbial control by low oxygen and low relative humidity environment," *Studies in Conservation,* 35. 222-230.

[239] Young, G.S., Wainwright , N.M. (1995) The control of algal biodeterioration of a marble petroglyph site. Studies in Conservation 40, 82-95.

BIOREMEDIATION

[240] Moncrieff, A., Hempel, K., “Biological Pack” (1970) in *Conservation of stone and wooden objects*, New York Conference, 2nd Ed. ICC, Ed., London, vol 1, 103-114.

[241] Bellucci, R., Cremonesi, P., and Pignagnoli G. (1999) A preliminary Note on the use of enzymes in conservation: the removal of aged acrylic resin coatings with lipase. *Studies in Conservation* 44, 278-281.

[242] Heselmeyer, K., Fischer, U., Krumbein, K.E., Warscheid, T. (1991) Application of *Desulfovibrio vulgaris* for the bioconversion of rock gypsum crusts into calcite. BIOforum 1/2: 89

[243] Ranalli, G., Matteini, M., Tosini, I. Zanardini, E. ans Sorlini C., (2000) Bioremediation of Cultural Heritage: Removal of Sulphates, Nitrates and Organic Substances, *Of Microbes and Art - The role of Microbial Communities in the Degradation and Protection of Cultural Heritage*, Kluwer Academic-Plenum Publisher, O. Ciferri, P. Tiano, G. Mastromei Eds., 231-245.

[244] Tiano, P., Tosini, I., Rizzi, M., Tsakona. N., (1996) Calcium oxalate decomposing microorganisms: a biological approach to the oxalate patinas elimination. II Inter. Symp. “*The oxalate films in the conservation of works of arts”*, Milan, March 25-27/1996, Editeam 489-493 (539).

[245] Tiano P., Addadi L. & Weiner S. (1992)."Stone reinforcement by induction of calcite crystals using organic matrix macromolecules, feasibility study". Proceeding of 7th International Congress on *Deterioration and Conservation of Stone*, Lisbon, vol. **2**, 1317-1326

[246] Tiano, P., (1995) "Stone reinforcement by calcite crystals precipitation induced by organic matrix macromolecules." *Studies in Conservation*, Vol 40 n° 3, 171-176.

[247] Orial G., Castanier S., Le Métayer G., and Loubiere J.F. (1993). The biomineralisation: a new process to protect calcareous stone applied to historic monuments. *Proceedings of 2nd International Conference on Biodeterioration of Cultural Property,* Yokohama, 98-116,. K.Toishi, H. Arai, T.Kenjo, K. Yamano Eds. Japan.

[248] Tiano P., Biagiotti L., Mastromei G. 1999 " Bacterial Bio-mediated Calcite Precipitation for monumental Stone Conservation: Methods of Evaluation". *Journal of Microbiological Methods* 36, 139-145.

[249] Perito S., Biagiotti L., Daly S., Galizzi A., Tiano P., Mastromei G. (2000)"Bacterial genes involved in calcite crystal precipitation" *Of Microbes and Art - The role of Microbial Communities in the Degradation and Protection of Cultural Heritage*, Kluwer Academic-Plenum Publisher, O. Ciferri, P. Tiano, G. Mastromei Eds., 217-228