Welcome

Welcome to the Metropolitan Museum of Art. We are pleased to be able to host this symposium, the first ever held at the Met and devoted to art, science, and conservation. Over the next three days you will be enthralled as almost 40 of the top scientists and conservators in this field present their latest research on the interactions of microbes and art, and offer approaches to counter the effects of biodeterioration.

No work of art, whether a del Sarto or a Picasso, is immune from microbial attack. For microbes are cosmopolitan, and are a threat to art collections worldwide, as they are always present in the environment, lying dormant, and waiting for the right conditions to occur so they can flourish. A major event such as a water leak, or even a temporary increase in moisture level, can foster an infestation within just hours. Whether this leads to serious damage depends on many factors, including the material composition of the art, the amount of water present, the species of microbe on the art, and how long conditions are favorable for growth.

Of course, every effort is made to prevent such mishaps, but when catastrophic failure occurs, the work of scientists and conservators comes into play.

We are pleased to bring together this select group of biologists and conservators to address these concerns. Many of these professionals have met before—in Florence in June 1999—where they discussed the causes and mechanisms of biodeterioration, and ways to control it. This second symposium will be building upon the work of that one. Recent advances in biotechnology, including genetic engineering, have contributed to our understanding of this problem.

Among our missions is the preservation of things most dear to us—works of beauty, and those with historical significance. We value the contributions that science makes in the service of art.

I hope that you have a very successful meeting.

Philippe de Montebello, Director

Acknowledgements

Many individuals assisted in making possible this first ever art, science, and conservation symposium at The Metropolitan Museum of Art. The Director Philippe de Montebello gave his approval and encouragement to this endeavor and mobilized the efforts of our Development Office under Christine Scornavacca to find the necessary funding to host the event. He also guided Education, under Kent Lydecker, to provide the essential structural support for the meeting. Elizabeth Hammer and Tara Diamond ably handled all of the many arrangements necessary for this program to happen; this included accommodations for the speakers, contracts, registration, and all aspects of the social program. Many other members of Education gave vital assistance: Masha Turchinsky and Lena Howansky helped with many details of the poster and program brochure, which were designed by Kevin Park and expertly edited by Meratine Hens. Thanks also go to Karen Ohland and Nathaniel Wilcox for their financial expertise and to Stella Paul, head of General Programs.

I want to give a special thanks to my department head, James H. Frantz, Conservator-in-Charge of the Sherman Fairchild Center for Objects Conservation, for his many years of support for a strong science program in conservation. It was his behind-the-scenes efforts and encouragement that ensured that this symposium became a reality. Without his support we would not have been able to have this meeting.

There are many other people who assisted in various ways: A. Elena Charola was an excellent sounding-board and an able organizer, helping me develop the details of the program. Ann Baldwin was always there to give advice on the poster, the cover, and many other facets of this project. Bruce Schwarz produced a wonderful photograph of a fungal-damaged Tiffany drawing that we used for the poster art and abstract booklet. Rebecca Rushfield spent many hours promoting the program to the conservation community.

To each and every one involved with this program, thank you.

Finally, this symposium is made possible in part with the generous support of an anonymous donor and the Samuel H. Kress Foundation.

Robert J. Koestler, Symposium Chair

Program

Art, Biology, and Conservation 2002:
Biodeterioration of
Works of Art

A Symposium
June 13–15, 2002

The Metropolitan Museum of Art

All sessions are presented in the Uris Center Auditorium.
Attendance is by reservation only. Please call 212-570-3710 for more information.

Thursday, June 13

9:30 a.m. Registration, Uris Center for Education

10:30 a.m. *Welcome and Introduction*
Philippe de Montebello, Director, The Metropolitan Museum of Art

James H. Frantz, Conservator in Charge, Sherman Fairchild Center for Objects Conservation, The Metropolitan Museum of Art

Robert J. Koestler, Research Scientist, Sherman Fairchild Center for Objects Conservation, The Metropolitan Museum of Art, symposium chair

11:00 a.m. *Session 1: Special Topics*
A. Elena Charola, independent scholar, and Robert J. Koestler, chairs

- *The Evaluation of Biodeterioration Processes on Cultural Objects and Respective Approaches for Their Effective Control*. Thomas Warscheid, Institute for Material Sciences (IWT/MPA), Germany
- *Passive and Active Conservation of the Hull Timbers of the Tudor Warship* Mary Rose. Mark Jones, The Mary Rose Foundation, United Kingdom
- *Biodeterioration Studies on Pastels and Oil-Based Paintings*. Marin Berovič, University of Ljubljana, Slovenia
- *Chemical and Microbiological Causes of the Deterioration of Toothbrushes—The Prisoners' Memoirs in Auschwitz-Birkenau: Methods of Their Conservation*. Alicja Strzelczyk* and Halina Rosa*, Nicolas Copernicus University, Poland
- *Collateral Damage: Anthrax, Gas, and Radiation*. David Erhardt*, Charles Tumosa, and David von Endt, Smithsonian Center for Materials Research and Education (SCMRE), Smithsonian Institution, Maryland

12:30 p.m. Lunch

1:15 p.m. *Session 2: Paper Conservation*
Douglas E. Eveleigh, Cook College, Rutgers University, New Jersey, and Ann Baldwin, The Metropolitan Museum of Art, chairs

- *Microbiologically Caused Foxing on Paper: Twenty-Five Years of Studies*. Hideo Arai, International Council of Biodeterioration of Cultural Property (ICBCP), Japan
- *Introduction to Tiffany Drawings Studies*. Ann Baldwin
- *Studies of Fungal Infestations of Tiffany's Drawings: Limits and Advantages of Classical and Molecular Techniques*. Maria Pia Di Bonaventura*, The Metropolitan Museum of Art, Rob DeSalle, American Museum of Natural History, New York, Douglas E. Eveleigh, Ann Baldwin, and Robert J. Koestler
- *Enzyme Treatments for Fungal Stain Removal on Paper*. Fernando Nieto-Fernandez*, Old Westbury College, New York, and Ann Baldwin, Maria Pia Di Bonaventura, Silvia Centeno, Mark T. Wypyski, and Robert J. Koestler, The Metropolitan Museum of Art
- *Artworks, Drawings, Prints, and Documents–Fungi Eat Them All!* Hanna Szczepanowska*, Maryland Archives, Maryland, and Ralph Cavaliere, Gettysburg College, Pennsylvania
- *Practical Applications of Enzymes in Paper Conservation*. Yana Van Dyke, The Metropolitan Museum of Art

2:45 p.m. Coffee break

3:15 p.m. *Session 3: Paper and Textile Conservation*
Orio Ciferri, University of Pavia, Italy, and Nancy Britton, The Metropolitan Museum of Art, chairs

- *A Review: Fungal Problem Assessment, Monitoring Methods, and Interpretation of Results Pertaining to Air Quality and Potential Contamination of Collections*. Mary-Lou Florian, Royal British Columbia Museum, Canada
- *Characterization of Bacteria Isolated from Naturally Aged Silk Fibroin*. Edda De Rossi, University of Pavia, Italy, Mary B. Becker, Fukui University, Japan, and Orio Ciferri*
- *Microbial Growth on Textiles: Conflicting Conventions*. Mary Ballard, Smithsonian Center for Materials Research and Education (SCMRE), Maryland
- *Biodegradation of Water-Degraded Archaeological Textiles with Implications for Their Conservation*. Elizabeth E. Peacock, The Norwegian University of Science and Technology, Norway

Friday, June 14

9:30 a.m. Registration, Uris Center for Education

9:30 a.m. *Session 4: Stone and Mural Paintings*
Eric May, University of Portsmouth, United Kingdom, and Wolfgang Krumbein, University of Oldenburg, Germany, chairs

- *Microbial Communities in Rock Art Caves*: Ecology, Physiology, and Affects on Rock Paintings. Leonila Laiz, Juan M. Gonzalez*, and Cesareo Saiz-Jimenez, Instituto de Recursos Naturales y Agrobiologia, Spain
- *Red Stains on Carrara Marble: A Case Study of Certosa of Pavia, Italy*. Elisabetta Zanardini*, Pamela Abbruscato, Laura Scaramelli, Elisabetta Onelli, Marco Realini, Giuseppe Patrignani, and Claudia Sorlini, University of Milan, Italy
- *Lichens and Deterioration of Stone: Progress and Problems*. Ornella Salvadori, Soprintendenza per il Patrimonio Storico Artistico e Demoetnoantropologico di Venezia, Italy
- *Microbial Processes in Deterioration of Mayan Archaeological Buildings in Southern Mexico*. Ralph Mitchell, Harvard University, Massachusetts
- *Microbiodeterioration of Mural Paintings: A Review.* Joanna Karbowska-Berent, Nicolas Copernicus University, Poland

11:00 a.m. Coffee break

11:30 a.m. *Session 5: Biotreatments and Biocides*
A. Elena Charola, and David P. Wessel, Architectural Resources Group, California, chairs

- *Consequences of Microbe-Biofilm-Salt Interactions for Stone Integrity in Monuments*. Eric May*, Sophia Papida, University of Portsmouth, United Kingdom, and Hesham Abdulla, Suez Canal University, Egypt
- *Mechanisms of Microbial Calcium Carbonate Precipitation*. Giorgio Mastromei*, Chiara Barabesi, and Brunella Perito, University of Florence, Italy
- *Biomediated Calcite Precipitation for Reinforcement of Monumental Stones*. Piero Tiano*, Susanna Bracci, and Silvia Rescic, Consiglio Nazionale delle Richerche, CNR, C.s. "Opere d'Arte," Italy, and Brunella Perito
- *Biological Mortars as a Solution for Stone Sculpture Conservation*. Geneviève Orial*, Laboratoire de Recherche des Monuments Historiques (LRMH), France, Thomas Vieweger, Groux SA, France, and Jean-François Loubiere, LRMH, France
- *Biocides and Treatment of Stone: Limitations and Future Prospects*. Maria Pia Nugari*, Instituto Centrale per il Restauro, Rome, and Ornella Salvadori
- *The Use of Metallic Oxides in Control of Biological Growth on Outdoor Monuments*. David P. Wessel
- *Conservator as Product Developer*. Norman Weiss, Columbia University, New York

1:30 p.m. Lunch

2:30 p.m. *Session 6: Analytical Methods*
Fernando Nieto-Fernandez and Robert J. Koestler, chairs

- ♦*Art, Genes, and Microbes*. Sabine Rölleke, Genalysis GmbH, Germany
- ♦*How Can Cyanobacterial Biofilms Alter Stone in Hypogeum Environments?* Patrizia Albertano, University of Rome, Italy
- ♦*Non-Destructive Versus Destructive Sampling Methods: Limits and Advantages for Studying Microbial Communities Colonizing Monument Surfaces*. Clara Urzì*, Filomena De Leo, Paolo Donato, and Violetta La Cono, University of Messina, Italy

3:30 p.m. Coffee break

4:00 p.m. *Session 7: Treatment and Prevention*
Clara Urzì and Diana Harvey, The Metropolitan Museum of Art, chairs

- ♦*Visual Assessment of Biocide Effects on Japanese Paint Materials*. Jun Suzuki*, Museum of Fine Arts, Boston, and Robert J. Koestler
- ♦*Detection of Life in Art and Discussion of Anoxia Eradication of Insects and Fungi*. Franc Pohleven*, Crtomir Tavzes*, and Jure Pohleven, University of Ljubljana, Slovenia, and Robert J. Koestler*
- ♦*Results of a Novel Germicidal Lamp System for Reduction of Airborne Microbial Spores in Museum Collections*. Harold W. Rossmoore* and Katalin Rossmoore, Biosan, Inc., Michigan, Mohammed Sondossi, Weber State University, Utah, and Robert J. Koestler
- ♦*How to Prevent Microbial Damage on Glass and Glazed Art Objects*. Anna Andrejevna Gorbushina, Wolfgang Elisabeth Krumbein*, and Ktarzyna Aldona Palinska, University of Oldenburg, Germany

Saturday, June 15

9:30 a.m. Registration, Uris Center for Education

9:30 a.m. *Session 8: Wood and Archaeological Materials*
Robert A. Blanchette, University of Minnesota, and Per Hoffmann, Deutsches Schiffahrtsmuseum, Germany, chairs

- ♦*Deterioration in Historic and Archaeological Woods from Terrestrial Sites*. Robert A. Blanchette
- ♦*Degradation Patterns in Waterlogged Wood and the Two-Step PEG Treatment for Archaeological Finds: The Case of the* Bremen Cog. Per Hoffmann

- ♦ *The Conservation of Wooden Objects from Gordion, Turkey: Methods for the Treatment of Dry Archaeological Wood*. Elizabeth Simpson, The Bard Graduate Center for Studies in the Decorative Arts, New York
- ♦ *Deterioration and Conservation Issues Associated with Antarctica's Historic Huts.* Benjamin W. Held*, Robert A. Blanchette, Robert L. Farrell, University of Minnesota, and Shona Duncan, University of Waikato, New Zealand
- ♦ *Evaluating the Wooden Remnants of the* Tektas Burnu *Shipwreck.* Joel A. Jurgens* and Robert A. Blanchette, University of Minnesota, and Deborah N. Carlson, Institute of Nautical Archaeology, Texas

11:30 a.m. Coffee break

12:00 p.m. *Conclusions and Recommendations*
Robert J. Koestler, Orio Ciferri, Norbert Baer, New York University, Institute of Fine Arts, Conservation Center, New York, summaries

1:30 p.m. Lunch

Closing Reception

*denotes speaker

A poster session will be on display during the symposium.

This symposium is made possible in part through the generous support of the Samuel H. Kress Foundation.

Advance registration is required to attend this symposium. The standard registration fee for the full three days is US$300 per person (US$130 per student). The fee includes a copy of the abstracts booklet, refreshments, and lunch on the days of the symposium, and one evening reception, as well as a copy of the symposium papers to be published at a later date. To obtain a registration form, please email ABC2002@metmuseum.org or call (212) 570-3710.

Session 1: Special Topics

Paper 1-1: The Evaluation of Biodeterioration Processes on Cultural Objects and Respective Approaches for their Effective Control. Thomas Warscheid, IWT- Institute for Material Science (MPA Bremen-Microbiology), Germany

1. Introduction

The obvious importance of biodeterioration processes on historical objects of art has reached a growing attention of people in charge with the restoration and conservation of cultural heritage. Microbial impacts within the entire deterioration process are already acknowledged and their analysis and evaluation can be widely found integrated in anamnesis programs within the conservation practice. In order to meet the resulting requirements of conservators and restorers to handle biodeterioration problems adequately with regard to their respective importance and to develop practically reliable strategies for their prevention, it will be necessary to focus on these growing demands in future research and case studies. In the following some of these furtherleading aspects for an evaluation process and their relevance for an effective control of biodeterioration processes on historical objects of art will be discussed.

2. Biodeterioration = Damage and /or Health risk?

The importance of microbial impacts on materials and their relevance for the entire deterioration process has to be evaluated very carefully. “Biofouling” (e.g. presence of colloidal microbial biofilms on or inside of materials) leads to an aesthetical impairment of original surfaces and potentially causes alterations of physico-chemical characteristics of the materials matrix, while due to “Biocorrosion” (e.g. microbial induced or influenced corrosion of materials) alterations in the structure and stability of materials might be consequently affected. Nevertheless all potential deteriorating properties of the microflora and possibly related biodeterioration phenomena have to be proven to take real qualitative and (more important) quantitative effect in complementary changes of the material properties (Warscheid, 2000).

While on organic materials (e.g. paper, parchment, leather, textile, binders and polymers) the proof of microbially induced destruction is generally easy to establish due to mostly known enzymatic and metabolic pathways, it is quite difficult to discriminate the microbial impact on inorganic materials (e.g. stone, plaster, glass) from further abiotic damage factors and to verify its specific importance, directly or indirectly, in the respective case of damage (Koestler et al., 1997). Thus, furtherleading and interdisciplinary studies on the effects of microbial biofilms to changes in the thermal-hygric behaviour (Dornieden et al., 2000), the alteration in the moisture transport (Warscheid, 1996a), the microbial desintegration (Mitchell, this volume) the enhanced deposition of aerosols and pollutants (Warscheid, 1991) as well as in a modified crystallization of harmful masonary salts (May, this volume) on mineral materials are desperately needed in order to establish a critical evaluation and respectation of biodeterioration processes within the conservation community (Warscheid, 1996b).

More over that, hazardeous risks of microbial cells and metabolites (e.g. allergenic spores, toxins, microbial volatile compounds, pathogenic microorganisms) to human health are increasingly claimed by conservators and restorers, especially in the field of archeaology and archive conservation. The potential risks are known and standardised analytical set-ups and evaluation guidelines were actually formulated (Gabrio, 2002), but no reliable information exists so far on the real endargement to the respective employees in conservation laboratories and archives. In order to establish here a profound analytical assessment of respective case studies it will be of a great advantage to apply especially modern molecularbiological techniques in order to target even non-culturable, but possibly allergenic, toxic or even pathogenic microbial strains (Roellecke, this volume) as well as their hazardeous metabolites, only detectable by high resolution analytical techniques (Gabrio, 2002). Nevertheless, the evaluation of data should always consider, whether the possible microbial endargement is only theoretically or really given under the prevailing exposure conditions (Warscheid, 2000).

3. Approaches for an Effective Control of Biodeterioration

Cultural objects, whether derive from dirt and anoxic excavations, kept in dark and humid archives or being openly exposed to corrosive and nutritive atmospheric pollutants, need in the first instance a profound anamnesis, constant care and conservative maintainance.

In the regard of biodeterioration more detailed information is needed on the real growth conditions and nutrient requirements of the respective biodeteriorating microflora: whether it is alive and active or whether is simply present without any measurable deteriorating impact. This will allow to define more clearly the necessity and intensity of cleaning processes and whether they need to be essentially combined with consequent desinfection treatments. Over and above that the knowledge on the microbial growth patterns will help to formulate guidelines for an effective “good-house-keeping” and adequate hygiene control and monitoring. This way, the use of biocides could be restricted to a minimum with respect to ecological and health implications, while their long-term effectiveness itself, in cases they are essentially needed, might be improved in future by synergistic formulations in special designed hygienic coatings. Bioremediation techniques in the conservation of materials, such as biocalcification, biodesalination or enzymatic removal of biofilm and stains, will open in addition new chapters on possibly bioprotective impacts of microorganisms vs. the (bio)-deterioration processes on cultural objects in future.

The above mentioned considerations and first approaches in their practical realization on hand of recent case studies, will be presented and discussed.

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Paper 1-2: **Passive and Active Conservation of the Hull Timbers of the Mary Rose.**

A.Mark Jones, Head of Collections, The Mary Rose Trust, HM Naval Base, Portsmouth, UK

Abstract

The Tudor warship Mary Rose was built in Portsmouth, England between 1509 and 1511 and served in Henry VIII's navy until she sank on 19 July 1545. Over the many years that followed, the hull and her contents were covered by a deposition of sediments. Within this environment, the hull and objects remained protected from further deterioration. Upon recovery from the protective sediments and re-exposure to aerobic conditions, chemical, physical and biological degradation ensued. Experiments were undertaken to evaluate the effectiveness of passive conservation methods with the view of controlling the activity of wood decay organisms in hull timbers. Storage in CO_2 atmosphere, cold storage, reburial and gamma irradiation were evaluated for their effectiveness in controlling the bacterial, fungal and insect activity in Mary Rose archaeological timbers. Of these methods, cold storage was shown to reduce microbial activity, although soft rot fungi were identified growing on hull timbers. The suitability of reburial within anoxic sediments was assessed. Physico-chemical conditions were characterised by a near neutral pH, low Eh and absence of dissolved oxygen. However, cellulolytic activity in recent and Tudor sediments was demonstrated. Gamma irradiation was found to be highly effective passive conservation method against bacteria, fungi and insects. A dose of 15KGy is required for inactivation of these organisms. At doses in excess of 100KGy radiolytic damage resulted in increased hygroscopicity, increased cracking and warping, loss of surface texture, reduction in compression and bending strength, and chemical alteration and degradation to cell wall components. This work concluded that cold temperature and gamma irradiation are suitable methods of storing waterlogged archaeological wood for long periods of time.

Following long-term storage of the hull (1982-1994), an active conservation technique involving a two stage polyethylene glycol treatment method has been employed to retain the structural integrity of the waterlogged hull timbers. Active conservation of the hull commenced in 1994 and will be completed by 2008. The hull timbers will then be dried by controlled air drying.

Paper 1-3: Biodeterioration Studies on Pastels and Oil Based Paintings.
Marin Berovič, University of Ljubljana, Slovenia

Biodeterioration of art monuments is defined as a natural phenomenon where microorganism in his life circle is deteriorating various organic substances as well as natural resins or cellulose fibres. In this process organic compounds, as carbohydrate fibres, proteins or the other kinds of natural polymers are decomposed by microbial enzymes into various metabolites including organic acids and amino acids. This products together with cellulolythic enzymes released from various filamentous fungi, accelerates further hydrolysis of cellulose fibres from paper or canvas ground. pH from 5 to 7, high relative humidity jointly with temperature from 20 to 30°C significantly promotes this process. This phenomena on one side represents a life circle of various microorganisms present on art monument but on the other side it could represent a serious problem for a restorer. In many cases mixed fungal infection is just cleaned or mechanically removed from the infected surface without any denaturation of the rest of fungal spores and mycelia, sanifying and further preservation of the ground.

Methods

Biodeterioration of pastel ground and flaxen canvas base on two different mechanisms. Pastel ground is often represented by cardboard or starch pasted rough paper on cardboard ground. In this case fungal infection is developing on the pigment covered porous painted surface. The main source of fungal growth represent starch glue and some organic wastes on the painted surface proceeded from various fixatives. Dry atmosphere and air circulation in exhibition halls inhibits the growth of fungal micelle as the natural protector. On the other side over 50% relative humidity joined with temperature from 20 to 30°C strongly promote development of fungal infection. The presence of some pigments based on copper, tin and lead oxides and salts usually block local infection, while the other pigments plays a neutral role.
Fungal mycelia on the pastel surface is exploiting a glucose hydrolysed from a starch glue or tragacant or the other polysaccharide parts rested as a resin of pastel pigment. In the presence of the micro amounts of water content in a solid matrix microbial growth is developing as a function of time and temperature. In very dry climate and after the exhausting all substrate and water sources microbial growth stops and fungal spores patiently wait for next suitable opportunity.

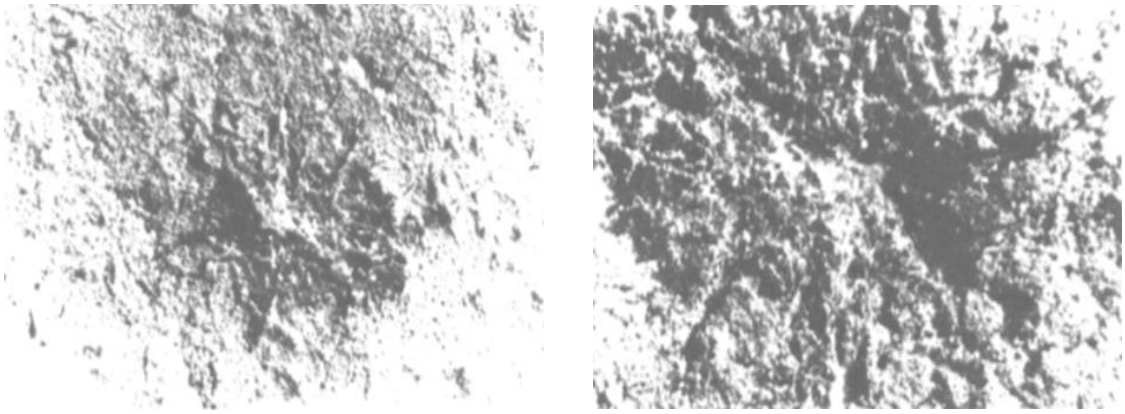

Fig.1 *Mucor michei* mycelia on the pastel surface
(Wild stereo microscope 12 and 25x magnification)

This process also includes the presence of various metabolic products and its acts that remain on the painted surface. Various organic acids, mostly citric, oxalic, malic of fumaric acids secreted in the metabolic TCA cycle could react with pastel pigment transferring it to its salts decolourising it and changing it optical impression. The influence of secreted organic acids on the paper fibres of various drawings or printed pages of graphics or books stored in humid and non aerated depositories often results as a brownish circles and spots of carbonised cellulose fibres. This effect on pastel painted surface results in formation of various funnel forms usually filled with fungal mycelia. If microbial infection is linked with wet starch under layers it further progress increase much rapidly.

To prevent this process fast measures has to be obtained. First essential step represent drying of the whole painted surface as well as the and its ground in the dry chamber. Further step represents mechanical cleaning of fungal mycelia from the painted surface followed by sterilisation of whole picture using gaseous formaldehyde for denaturation of fungal mycelia and spores.

Sterilisation with gases enables is the most effective method that enables entering the gas into painted surface and porous layer.

For conservation of pastel painted surface a solution of Sn-butilate in ethanol (1 : 4) was sprayed on the inner side of the protecting glass. Thin layer of this preservant enables indirect contact of its vapours and pastel surface in the distance of a few millimetres.

In the opposite of flaxen canvas bases fungal infection start from the background of oil picture. It actually starts as a result of water micro condense usually resulted as a result of microtransport of water from outlet walls towards inner side of exhibition halls.

With increased spring or autumn humidity and the temperatures from 20 to 30°C fungal infection of lime canvas impregnation in the back side of the oil picture starts. After consuming the available organic compounds from the surface of lime areas fungal growth is penetrating into a lime-chalk ground where fungal hyphae are growing from the backside towards the surface. The increasing of the amount of fungal biomass and the activity of its metabolic products, enzymes and organic acids, results in stretching of the painted surface. This problem finally results in cracking and hulling of the surface. This

action is followed by entrance of fungal biomass, from the back side towards the outlet painted surface, and growing of fungal mycelia in colonies on the painted surface.

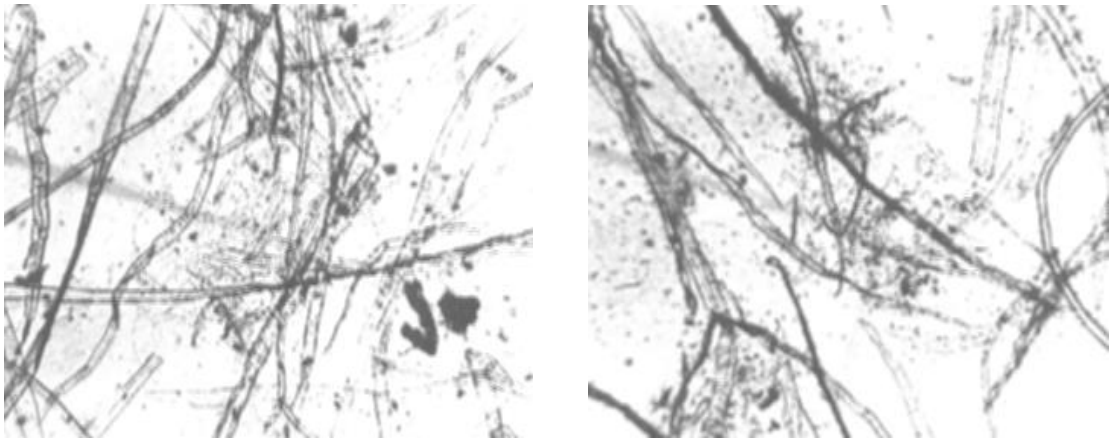

Fig.2 *Trichoderma viridae* (a) and *Aspergillus niger* (b) growth on cellulose fibres (Wild –20, optical microscope 100x magnification)

A part of the growth of fungal mycelia usually stops when organic compounds from lime are exhausted, but a part of infection that represents cellulose degradable fungi is still in progress. This part represents the most problematic and dangerous part of biodeterioration where this group of fungi could hydrolyse cellulose fibers from flaxen canvas. Under the attack of fungal xylanases and organic acids cellulose fibres start crumbling and the whole parts of the canvas background finally disappeared. The rest of painted surface and chalk-lime ground rest without a support so it breaks at first mechanical stroke and holes in the pictures finally appear.

Restoration and conservation of such a case starts with mechanical cleaning of fungal mycelia from the painted surface related to sterilisation of whole picture including frame, underframe and unframed picture with gaseous formaldehyde that enables denaturation of fungal mycelia and spores.
After this step a dubbing of the canvas on vacuum table and covering the holes with wax mass starts. Next step is related to the classical restoration procedures. For conservation of oil picture backside and the wooden parts of the frames a solution of Sn-butilate in ethanol (1 : 4) in spray form would be recommended.

Paper 1-4a: Chemical and Microbiological Causes of Deterioration of Toothbrushes Which Used to Belong to Prisoners of Auschwitz-Birkenau Konzentrazion Lager.
Alicja Strzelczyk, Nicolas Copernicus Univ. (Poland)

In the Auschwitz-Birkenau Konzentrazion Lager Museum are stored various objects, which used to belong to the prisoners of that camp. The sixty-year-long storage period has resulted in considerable changes in those objects, due mainly to natural ageing. Among those objects there is a large group of toothbrushes; their number at present is ca. 4776. They had been produced by various firms from all over Europe: Hungary, France, Germany, Italy, Britain etc., evidence of which are the firm signs on the handles. A certain percentage are small children's toothbrushes. The preservation condition of that collection varies widely. Part of the handles have suffered complete destruction and transformed into a shapeless granulate. The number of toothbrushes was evidenced by the heads with bristles, which were in a better condition.

The better preserved items show deep cracks, lack of transparency, the presence of a sticky deposit on the surface. It was found that the collection emitted vapour of strong acid.

Before starting conservation, the most badly destructed toothbrush handles were subjected to chemical and microbiological studies in order to find out the cause of their destruction. Use was also made of the publications in CCI Notes 15/1, 1998, CCI Notes 15/3 and the project of Eileen Blakenbaker et al. of USHMH (1995), designed for similar collections in the Holocaust Museum in Washington. It was found that the primary cause of destruction of the toothbrush handles had not been microbiological decomposition but chemical changes that had occurred in the principal material they were made of, i.e. celluloid. The development of microorganisms was a secondary factor, which may have accelerated the destruction. The development of microorganisms was made possible by the very strong hydrophilousness of decomposed celluloid (nitrocellulose). The isolated microorganisms belong to genera with very highly developed capability for enzymatic decomposition of cellulose.

Paper 1-4b: Conservation of Toothbrushes from Auschwitz-Birkenau Konzentrazion Lager. Halina Rosa, Nicolas Copernicus Univ. (Poland)

The whole collection was divided into 11 groups with relation to structure, appearance and preservation condition, which was widely varied. The handles of most of them were badly corroded, which sticky deposits on the surface. The final decomposition phase was a powder with coarser and finer grains. On many toothbrushes the firm signs could be identified.

The material of which most toothbrush handles were made was cellulose nitrate with an accessory of camphor as plasticizer. Some of them were made of a mixture of polymers of cellulose nitrate and cellulose acetate. The toothbrushes with handles made of cellulose nitrate and camphor were badly deteriorated. The cause of their destruction was partial loss of the plasticizer (camphor) through its sublimation and hydrolysis of the polymer. The loss of the plasticizer and the abridgement of polymer chains brought about changes in the structure of the material. As a result of those processes the material became brittle and cracked. In the toothbrushes made of a mixture of cellulose nitrate and acetate the cause of their deterioration was degradation of cellulose chains in the surface layer.

After studying relevant literature and close analysis of the object it was concluded that it was impossible to completely stop the processes of degradation of the polymer material the toothbrushes were made of. The objective of the conservatory treatment was slowing down the rate of those processes by neutralizing the acidic products of celluloid decomposition. Another objective was the reinforcement of the handles and bristles of the brushes.

The cleansed toothbrushes were deacidified in ammonia vapours. Deacidification was carried out in a vacuum device. The bristles of each toothbrush were reinforced before deacidification by saturating them with 2% Paraloid B 67 dissolved in White Spirit.

The toothbrush handles were twice saturated in a solution of acetyl cellulose in acetone. The acetyl cellulose solution, having high affinity to the cellulose material, easily penetrated into the structure of the material of the handles, thus improving their condition. Acetyl cellulose was also used for sticking together broken handles.

The recommended storing conditions for the collection are: 12ºC and 35% relative air humidity.

Paper 1-5: **Collateral Damage: Anthrax, Gas, and Radiation.** David Erhardt, Charles Tumosa, and David von Endt. Smithsonian Center for Materials Research and Education, Smithsonian Institution. Washington, D.C.

In the fall of 2001, a series of anthrax contaminated letters was sent to a number of public figures, including two Senators. This resulted in the contamination of the Hart Senate Office Building and the mail handling center serving it. Because of the scale of contamination, gaseous fumigation with chlorine dioxide was chosen as the method of decontamination for the Senate building. Because of the aggressive oxidizing nature of this gas, there was concern about the possible effects on artwork and personal items such as photographs in the building. Other protocols suggested the use of hypochlorites, hydrogen peroxide, or potassium peroxymonosulfate (Oxone®), each of which presents its own difficulties. While effective as palliatives for chemical and biological agents, the aggressive chemistries of these compounds will damage many materials used in cultural objects. This paper will cover some of the possible effects on such items and measures recommended to protect them.

Subsequently, it was decided to sterilize all mail that was present in the closed mail handling facility, as well as all subsequent mail that normally would have passed through it. Electron beam irradiation was chosen for this purpose. While this process is routinely used for food items with no apparent problems, the results for mail were quite drastic. The methods, dosage, and conditions chosen resulted in severe yellowing and embrittlement of papers, melting of plastics, and blocking of manuscripts. The effects produced by electron beam irradiation can be due either to reactions that occur as a direct result of electron ionization or to the high temperatures produced when the packets of mail absorb the large amounts of energy involved. Examples of the thermal effects, reportedly including fires, have appeared in the media.

Chemical analysis and measurements of changes in color and mechanical and physical properties verify the damage, and confirm reports that the irradiated mail reaches temperatures well over 100 C. L*a*b* color measurements of control and irradiated samples show definite yellowing. The color shift is not necessarily immediate and can occur over time. High temperatures are confirmed by the softening of slide mounts made from polystyrene, which has a softening temperature of about 110 C. The clear windows of some envelopes are also polystyrene, and these too have been found to exhibit softening and distortion, in some cases adhering to adjacent printed matter. Some printed materials are also adhered to each other, probably due to softening of the resins in the printing inks or photocopying toner.

Tensile measurements on irradiated paper show that there is a substantial loss in the ability of the paper to be deformed. This loss of extensibility is as high as 80%, resulting in sever brittleness. At this point, the paper does not survive simple folding. Analyses of the soluble material in irradiated and unirradiated samples of the same paper show an increase in the amounts of degradation products. The distribution of products is very different from that seen in naturally aged materials. The amount of glucose, especially, is not greatly increased. This shows that the degradation is due to reactions other than hydrolysis, which is the primary reaction during the natural aging of cellulose. The relatively small amounts of soluble degradation products probably do not account for the large loss of strength observed, indicating that the changes are most likely due to

radiation induced crosslinking. These effects have serious implications for museum objects and samples, and will affect decisions regarding the archival nature of irradiated documents.

Session 2: Paper Conservation

Paper 2-1: Foxing on Paper Caused Microbiologically: 25 Years of Studies. Hideo Arai, ICBCP-Japan Office, Japan

Foxing found in paintings, books and archives is a type of deterioration appearing on cultural property made of paper and related materials. Although curators and scientists all over the world have been interested in the phenomenon since 1919, several approaches could not agree on the reasons for foxing.

The author was requested to research the cause of foxing on paintings that were replicas of decorative paintings on hemp paper inside the Hō-ō-dō in Byōdōin Temple in 1982. When he incubated a piece of the foxed paper in a petri dish regulated at a water activity (Aw) of 0.94 and 25 ˚C, he discovered that fungi grew only on foxed areas of the samples after several weeks. He saw thus as a clue to supporting the microbiological studies on the foxing of paper at that time.

Firstly, fungi grown on foxed areas were identified taxonomically. As a result, the author concluded that fungi causing foxing belonged to the absolute tonophilic (= xerophilic) fungi like *Aspergillus penicilloides* and *Eurotium herbariorum*, which can grow in drier environments (75-85% RH) and impossible to germinate in totally wet environments (100% RH).

Secondly, when the author analyzed the components in foxed areas, a larger amount of malic acid was deposited in the foxed area than other organic acids. Also, since analyses on organic acids in the metabolites of absolute tonophilic fungi coincided with the results from foxed areas, it was considered that malic acid may be closely related to the formation of foxing. Moreover, thin layer chromatography (TLC) of saccharides in extracts from foxed areas detected cello-oligosaccharides composed of glucose, and analyses by liquid chromatography (LC) detected 16 amino acids. Of these amino acids, a remarkably large amount of γ-aminobutyric acid was detected.

Thirdly, glucose and amino acids, especially γ-aminobutyric acid, alanine, glycine, ornithine and serine were mixed and spotted on hemp paper, and then the papers were incubated for 40 days at 25 ˚C and 35 ˚C, each at the relative humidity of 75% RH (=Aw 0.75) and 84% RH (=Aw 0.84). As a result, these components reacted together on paper, causing brown spots: foxing was formed on hemp paper.

Additionally, the author showed that foxing can be formed not only on paper materials but also on the other materials. Examples are the fibers of raw silk, the mural paintings of the Tomb of Tutankhamen in Egypt and corners in the walls of exhibitions.

Finally the author proposed the following hypothesis as the formation mechanism of foxing based on microbiological studies on foxing: Airborne microorganisms and microdusts exist on/around paintings and books from the beginning. When these materials are kept in an environment of 75-85% RH (Aw 0.75-0.85) and 20-35 ˚C in temperature, conidia and ascospores of foxing-causing fungi germinate and make their colonies of about 3.0-5.0mm diameter around the microdusts. Growing fungi metabolize malic acid and other organic acids around their hyphae, and these organic acids deposit on cellulose fibers of the paper. The cellulose of the paper is attacked by acid gradually and produces cello-oligosaccharides and glucose, after it has been in contact with malic acid and other organic acids for a long time.

On the other hand, when foxing-causing fungi grow on paper, these fungi metabolize and deposit 16 amino acids in the colonies. Among them, the largest amount of amino acids is γ-aminobutyric acid. When cello-oligosaccharides, γ-aminobutyric acid and others exist together in limited areas of the fungal colonies, a browning reaction is accelerated between these components and browning materials are formed in fungal colonies. The author considers that brown spots are formed by the amino-carbonyl reaction (=Maillard reaction) and that the browning materials are melanoidines. This, appearing as brown spots, is foxing on paper.

The formation mechanism of foxing mentioned above to take place on paper materials can occur on the other materials like raw silk, mural paintings and walls in buildings. Therefore, the author concluded that when the optimum environment for growth of foxing-causing fungi exist, foxing can appear not only on paper but also on various materials.

Paper 2-2: An Introduction to Tiffany Studies. Ann M. Baldwin, The Metropolitan Museum of Art, New York

Introduction

Louis Comfort Tiffany (1848-1933), the American painter, designer, and colorist, is perhaps best known for his innovations in glass. From a young age, he was influenced by designers and artisans employed at his father's firm, Tiffany and Company. He studied informally with the American tonalist painter, George Inness (1825-1894), and his artistic sensibilities were shaped by travels to Europe, Northern Africa and Egypt.

In the early part of his career, Tiffany actively participated in arts organizations, and, at 22 years of age, he was elected an associate member of the National Academy of Design in New York City. Tiffany exhibited oil paintings with 'orientalist' themes such as *Snake Charmer at Tangier, Africa*, ca. 1872 (Koch 1966).

From 1879 to 1933, Tiffany served as a principal in business entities specializing in decorative arts, interior design, and glass. Tiffany established his reputation with projects for notable clients such as Mr. and Mrs. H.O. Havemeyer and President Chester A. Arthur, as well as with his renowned stained glass windows and blown vases. His distinctive work - parlor lamps, mantels, metalwork and vases - would make Tiffany a household name.

He revolutionized glass technology, with a special type of glass called *Favrile*, patented in 1890. This process created novel optical effects, such as opalescence and iridescence, through the mixture of metallic oxides and the manipulation of molten glass (Frelinghuysen 1998).

Over time, Tiffany expanded his staff and facilities, and he continued to employ outside artists specializing in fields such as stained glass. Before design schemes were selected and ultimately realized, proposals took form in graphite pencil and watercolor drawings on supports of commercial artist papers or illustration boards. Drafting devices such compasses were sometimes utilized, and photographic methods were also employed. Works, such as the finished drawings prepared by the Ecclesiastical Department of Tiffany Studios, were often presented in elaborately cut window mats, bearing pen and ink inscriptions.

In 1966, a collection of some 420 original preparatory sketches, renderings, and presentation drawings by Tiffany's studios was purchased by The Metropolitan Museum of Art. The collection offered a daunting challenge, for prior to acquisition, it had sustained considerable water damage for a long enough period of time to foster extensive fungal growth.

Project Overview

In the late 1990's, Alice Cooney Frelinghuysen, the Anthony and Lulu Wang Curator of American Decorative Arts at the Museum, established a preservation initiative so that these works, so obviously damaged, could be safely studied, exhibited, and published. Biodeterioration is evident in mold growth, insect damage, and biofilm residues. Examination revealed that some works were infected significantly, and others, negligibly

so, or not at all. Where water saturated the artwork, fungal growth and collateral loss and weakness were readily apparent.

The project brought together a team of conservators, biologists, microbiologists and chemists at the Metropolitan Museum of Art and local institutions to address these problems.

Conservation objectives included: 1) minimization of risks to the collection, as well as staff and scholars handling these works, 2) adoption of appropriate measures to reduce or eliminate fungal contamination, 3) consolidation of weakness and loss, 4) reduction of dark hyphal staining, and 5) post-treatment segregation of works affected by and treated for mold.

Scientific research was directed along the following avenues: 1) classical culturing methods and genetic sequencing (PCR) to identify fungi and rule out toxic species, 2) Raman spectroscopy and scanning electron microscopy to characterize pigmented fungi and their chemical components, and 3) testing of enzymatic treatments to reduce pigmented fungal hyphae and conidia.

Culturable fungi and occupational safety; Fungal species cultured and identified

Using transmitted light microscopy and SEM, the presence of the following cultured genera was determined: *Aspergillus*, *Cladosporium*, *Penicillium*, and *Fusarium*. These fungi are cosmopolitan, and are commonly found on cultural property (Florian 2000, Dicus 2000). They belong to an order of mitoscopic fungi, sometimes referred to as the *Deuteromycetes*, or 'Fungi imperfecti'. It is noted that not all spores could be cultured.

The risks of exposure

Spores produced by these genera are microscopic, measuring ~ 2-5 μm in diameter. Without filtration, they are readily inhaled. Owing to their size, morphology and chemical composition, they are known allergens and irritants. Some produce substances called mycotoxins, which can be carcinogenic, teratogenic, and deadly to both animals and humans.

Fungal infections

Fungal infections can take hold in persons with compromised immune systems. As of 1998, only ~6 anti-fungal drugs, which are not always effective, were available by prescription (Hatchfield, person. Com.)

Visual Appearance of Mold

In general, fungal contamination of the artwork took one of three forms: 1) dense superficial mold growth, 2) palpably weakened paper supports with a mottled appearance and a greyish cast, and 3) localized, dark, circular spots.

Superficial growth

Patches of powdery, matte, or wooly mold, highly colored, white or dark brown, are visible to the naked eye. At low magnifications, these masses have a bushy, or fuzzy appearance; and at high magnifications, vegetative and reproductive hyphae, and innumerable microscopic spores, are discernible.

Weakened paper supports with grey cast

Affected regions look and feel tangibly weakened, and indeed, there is a corresponding loss of paper constituents, indicating the presence of so-called 'second colonizer' fungi (Szczepanowska 1986).

At magnifications of 90-225 X, the amorphous grey staining is resolved into multiple twisting strands of black conidia entrenched within the fibrous paper.

Pigmented Hyphae

Pigmented hyphae appear as dark, nearly circular spots with dense centers and extending radially through their host material. At higher magnifications fungal filaments are distinguishable from paper fibers.

Remediation of superficial growth

Aseptic techniques for the removal of superficial growth found on cultural artifacts were adopted based on practices outlined in the literature (Price 1994, Nyberg 1998, Dicus 2000, Florian 2000).

Treatment of weakened supports with grey casts

Treatment of areas weakened by digestive fungal enzymes is problematic, as entrenched conidia and hyphae cannot be extracted without disturbing the paper matrix. Denaturing of spores, or their safe removal, and structural reintegration, are objectives of treatment.

Exploration of possible treatments for pigmented hyphae

A suitable conservation treatment for localized dark hyphal spots has yet to be developed.

Cell walls pigmented with melanin have proven impervious to conventional paper conservation reagents such as alkalized aqueous mixtures, organic solvents, oxidizing bleaches, and reducing agents.

The desire for specificity – for an agent or technique able to act only on fungal tissues, and not harm paper – has lead to the testing of microbial enzymes to degrade fungal cell walls (Florian and Purinton 1995). Chitinases have been found to break down unpigmented fungal cell walls, however, the presence of melanin appears to inhibit such penetration (Bloomfield and Alexander1967).

Polyphenols and Ligninases

Melanin

Melanin is a natural pigment distinguished by dark brown or black color. It consists of branched phenolic polymers derived from substances such as tyrosine, and catechol (Bell and Wheeler 1986).

Melanin takes different chemical forms; the type usually found in fungi is a polymer of 1,8-DHN, or dihydroxynaphthalene (Butler and Day 1998b).

Ligninases as a potential melaninase

Research has shown that oxido-reducto enzymes, such as the ligninases, manganese peroxidase, and laccase produced by the white rot fungi, *Phanerochaete chrysosporium*, act on melanized fungal walls and decolorize melanin (Butler and Day 1998a).

These findings have prompted experiments using ligninases as potential agents for the reduction of dark mold staining.

Conclusion

Developments in microbiology are helping art conservators address problems of biodeterioration. The combination of research, practical intervention, and proper storage will help to make the Tiffany drawings safe, structurally stable, and accessible to scholars and to the public.

Acknowledgements

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Paper 2-3: Studies of Fungal Infestations of Tiffany's Drawings: Limits and Advantages of Classical and Molecular Techniques. Maria Pia Di Bonaventura[1], Rob DeSalle[2], Douglas E. Eveleigh[3], Ann Baldwin[1], and Robert J. Koestler[1], [1]The Metropolitan Museum of Art, New York, [2]The American Museum of Natural History, New York, [3]Cook College, Rutgers University, New Jersey

Tiffany's collection is a group of about 400 original works by Tiffany Studios, dating from the mid-1880s to the early 1930s. The disposition of this collection was unknown for decades, until the mid-1960s when it was found in a 4th floor attic room of a marble supplier in Long Island. In 1967 the collection was accessioned by the Metropolitan Museum of Art and except for the cataloguing and photography, the group has remained largely undisturbed in museum storage boxes.

It has been supposed that sometime between 1930 to around 1960, that due to an accident, the collection sustained water damage for a period of time long enough to permit an extensive fungal growth, both on the surface and through the cellulosic substrate. Once mold growth on paper is established it may cause aesthetic and structural damage. Stains usually arise from colored fungal bodies and more commonly from organic metabolic products such as pigments, while the extracellular enzymes released by fungi can cause the digestion of the cellulose fibers modifying the stability of the paper. For instance, several species belonging to the genera of *Penicillium* sp., *Fusarium* sp., *Alternaria* sp. or *Cladosporium* sp. can cause stains on paper that range from light green to pinkish and brown to dark ones, whereas the presence of *Chaetomium globosum* or even *Stachybotris atra* on paper is not surprising considering the cellulolytic activity displayed by these species. Fungal-induced stains can sometimes be reversed through a mechanical stain removal or extracted with appropriate solvents. Laser treatments have been successfully applied to some of the fungal stains showing that their removal is dependent on the fungal species involved. Thus, to our knowledge, the fungal-induced stains on paper material still remain problematic and an understanding of their composition in terms of both single fungal species and/or mixed fungal communities involved can contribute to their removal.

Preliminary visual and stereomicroscopical investigations were made in order to select the fungal stains from infected drawings. In particular the brown, gray to dark fungal spots that are among the most difficult to remove were focused on. These works included: Mosaic Panel, Chancel Rail, Desk Items, Angel and Three Mary's, Four Seasons, Adoration, Palm Room, Vassar College, and Grisaille. Scraped fungal material, swabs, and where possible small pieces of contaminated paper were used as samples to be investigated with SEM, culturing tests, and molecular techniques. Morphological observations on the remains of the fungal contamination and an evaluation of the existing damage to the cellulose fibers were conducted under SEM. The fungal spots were characterized by the presence of fungal hyphae often collapsed and/or single groups or chains of fungal spores. In some cases these fungal spores showed the conidiophore fragments or the lack of aerial structures required to identify them at the genus level. Because of this no further work was undertaken to tentatively identify the fungal species using morphology. Through microscopical investigations selected spots were classified in fungal-induced stains caused by the presence of pigmented fungal hyphae, for instance, the green patina found in Mosaic Panel or the brown spots in Desk Items. In other cases,

the staining would still remain due to the organic metabolic products such as the pigments, as observed in Chancel Rail. The worst case in terms of conservation was shown in stains, like the gray to dark ones, caused by fungal spores associated with cellulose digestion, as shown in Angel and Three Mary's, Four Seasons, and Vassar College.

After this first screening, an attempt to grow and isolate the fungal species was carried out by direct incubation of scraped fungal material or contaminated paper in a humidity chamber and by culturing tests on selected media, e.g., MEA (Malt Extract Agar) and PDA (Potato Dextrose Agar). Both of these tests were conducted under high relative humidity in order to simulate the environmental conditions under which the drawings could have been contaminated. A few fungal cultures could be isolated and identified at genus or species level, e.g., *Cladosporium* sp., *F. oxysporium* and *Penicillium* sp. The isolates were identified by amplifying and sequencing the ITS (Internal Transcribed Spacer) region of the rDNA genes. As expected, many of the fungal species may be dead because of the age of the processed samples or not all the viable ones would grow under the selected laboratory conditions.

The limitations related to the culture-based methods may be overcome by the analysis of total DNA extracted directly from an environmental sample. The non-coding ITS region of the rDNA gene cluster has been widely used for studying fungal species profiles from uncultured fungal communities. The sequence variation of the ITS1 and ITS2 region has led to their use in systematic and phylogenetic analyses at lower taxonomic levels. Some species complexes have, however, not yet been resolved using the ITS region and many fungi may contain within-species variation in their ITS sequences. So far, molecular techniques have mainly been applied directly for detection and identification of mycorrhizal fungi, phytopathogenic fungi from within plant tissues, endophytic fungal communities, or wood-decaying fungi and microbial diversity in soil samples. There have been few studies that have used molecular techniques on uncultured fungal communities colonizing material such as paper.

Thus, DNA was extracted from the scraped material and/or contaminated paper in order to obtain the identification based on the sequence analyses of the fungal DNA. Dealing with an old fungal contamination, for instance the remains of the fungal hyphae on paper, often causes problems in extracting DNA and this may be related to the degrading processes that affect DNA. When samples are characterized by the presence of what can be identified as remains of the fungal mycelium, can DNA be found in it and what is the quality of the DNA? Further, the possibility to test different extraction methods is in many cases limited by the small amount of the starting material and generally the attempt to identify the fungal species is made on amplified DNA sequences of a few hundred base pairs. From old specimens yields may be tentatively increased by individual amplification of the two ITS regions, ITS1 and ITS2 region, with the primer pairs ITS1/2 or ITS 5/2 and ITS3/4. On the other hand, DNA extracted from thick-walled fungal spores, characterizing the gray to dark spots, as already mentioned above in Angel and Three Mary's, could be amplified and sequenced using the primer pairs ITS1/2, 3/4 and 1/4 that amplify the entire ITS region. In situations where drawings are infested by more than one species, other steps are required to obtain the fungal species profiles, such as cloning the PCR products, RFLP (Restriction fragment Length Polymorphism) or DGGE (Denaturing Gradient Gel Electrophoresis). In these experiments, the PCR

products were cloned and the sequences of the cloned fragments aligned to complete, or partial, ITS sequences available in the GenBank database. Although an identification of the fungal species may be achieved once the DNA has been extracted from fungal spores, it seems that similar results are unlikely to be obtained from old fungal contaminations characterized by the remains of fungal hyphae. The results of the present research will be discussed.

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Paper 2-4: An Enzymatic Approach to the Removal of Fungal Spots from TIFFANY Drawings. Fernando Nieto[1], Silvia Centeno[2], Ann Baldwin[2], Maria Pia Di Bonaventura[2], Mark T. Wypyski[2], and Robert J. Koestler[2]. [1]SUNY College at Old Westbury, Old Westbury, New York; [2]The Metropolitan Museum of Art, New York

A common side effect of microbial growth on surfaces is the appearance of pigments that were not originally on the substratum. In the case of autotrophic organisms these pigments, i.e., chlorophylls and carotenids are associated with photosynthesis. On the other hand, heterotrophic organisms such as fungi synthesize the pigments as a response to environmental stress and they appear to play a role in pathogenesis. These pigments, which range in coloration from brown to black (Bartnicki-Garcia and Reyes, 1964; Durrell, 1964; Lingappa et al., 1963) are found in the cell wall of vegetative mycelium and reproductive structures such as sclerotia and conidia, conferring protection on the fungus from physical-chemical degradation (Bloomfield and Alexander, 1967; Mirchink et al., 1968; Zhdanova et al., 1973; Zhdanova et al., 1981; Ursi et al., 1997; Wang and Casadevall, 1994; Zhdanova et al., 1994; Lockwood, 1960; Old and Robertson, 1970; Kohno et al. 1983; Butler, 1987; Butler and Day, 1998). In the past, enzymatic treatments have used proteases, chitinases, lipases, and glucanases in an attempt to breakdown the major components of the fungal cell wall (Bloomfield and Alexander, 1967; Zomer et al., 1985; Edmond and Horton-James, 1991). The presence of melanin in the cell wall protects its constituents from enzymatic digestion. Herein, an enzymatic approach to breakdown melanin using lignin-degrading enzymes is proposed. These enzymes are produced by white-rot fungi as part of their secondary metabolism, and have been shown to successfully breakdown melanin (Butler and Day, 1998). Assessment of the treatment using Raman spectroscopy and scanning electron microscopy will be presented.

Paper 2-5: Artworks, Drawings, Prints, and Documents- Fungi Eat Them All! Hanna Szczepanowska, Maryland State Archives, Annapolis, Maryland and A. Ralph Cavaliere, Gettysburg College, Gettysburg, Pennsylvannia

Our studies have focused on the examination of fungi infesting artworks and artifacts on paper. We have been concerned primarily with the mechanisms of deterioration by the fungi and the role played by the composition of the substrates upon which the fungi grow. Often the visual manifestation of molds is directly dependent on the materials that comprise the substrate. This report will review the current status of our understanding of these fungus-substrate parameters, discuss their impact upon the preservation of collections, and conclude by offering a number of suggestions for future practices to consider when choosing methods of conservation.

Part of our studies is aimed at the identification and numeration of the vast mycoflora that has been encountered on paper-based collections. Their growth is often linked to the materials upon which artwork and archival documents are rendered and the environment to which these works are exposed throughout their life. Moreover, fungus growth is often enhanced by the addition of the artists' pigments and the great variety of materials such as varnishes and glazes and additives applied later in the life of the work. The impact of the environment, specifically the time of the collections' exposure to moisture, greatly enhances both the number of species and the abundance of their growth. Several examples illustrate these relationships: infestation that occurred after exposure to periodic floods (18^{th} century Tilghman documents); exposure to water spills (19^{th} century watercolor and ink drawings); and growth occurring in microclimate of framed artworks exposed to fluctuating relative humidity (18^{th} and 20^{th} century pastel portraits).

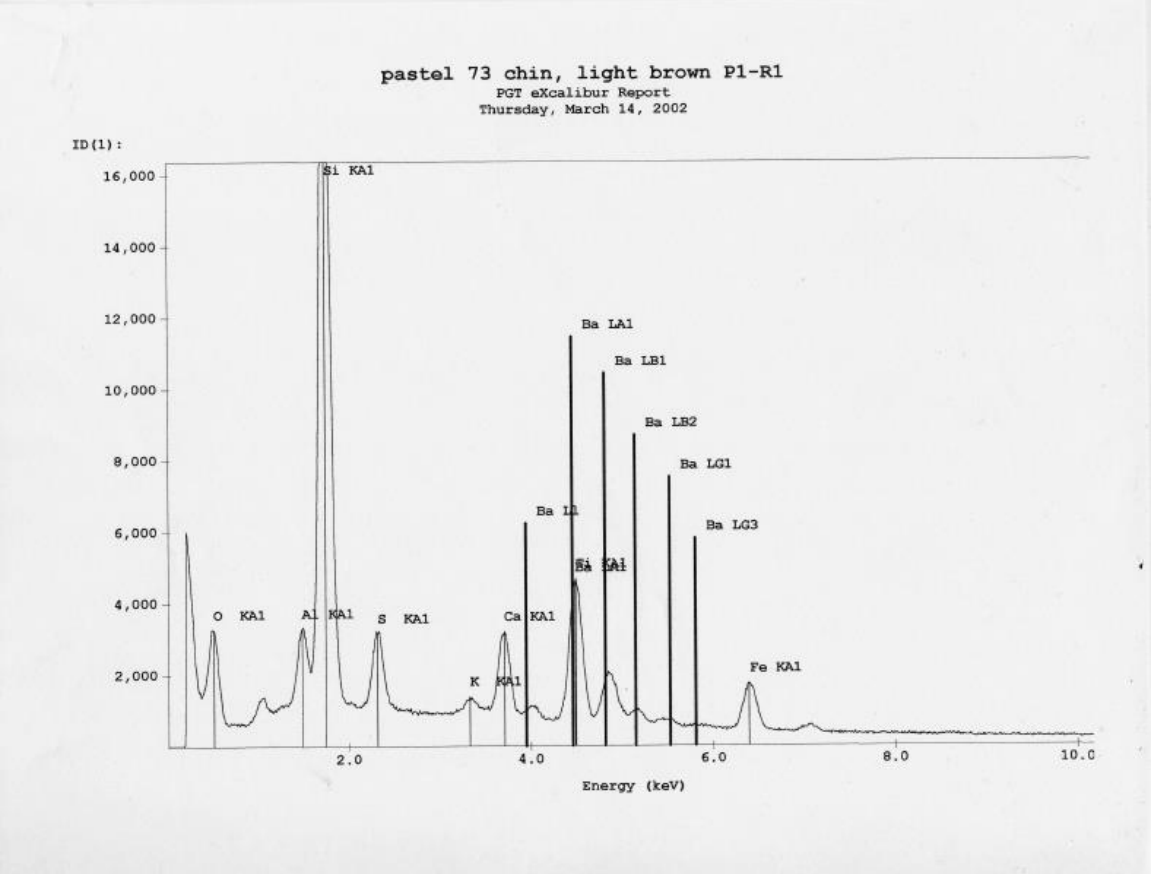
Understanding the interaction of all these elements and their implication on the longevity of collections must guide conservators in their choices of materials used in conservation practices.

Photo captions:

1. “Cecil” Pastel Portrait, 1973 American; pastel on prepared paper Ersta, Snuffingerpapier 012E made in Germany; detail illustrating fungus infestation.

2. *Penicillium* SEM 1000x. Fungus collected from the “Cecil” Pastel Portrait, 1973; 1000x

3. “Cecil” Pastel Portrait, 1973; Two spectra of pigment analysis from the area affected by the
fungus.



pastel 73shoulder P1-R2

PGT eXcalibur Report
Thursday, March 14, 2002

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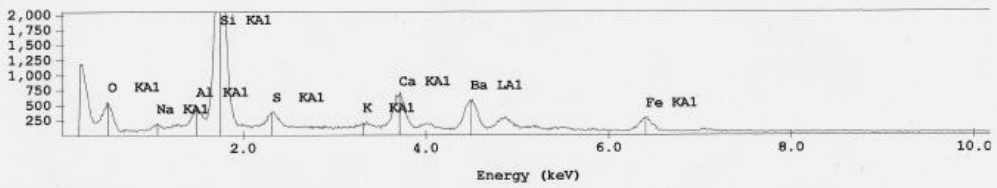
Beam Voltage: 25.00 Beam Current: 1.000 LT: 77.56 RT: 99.16
Takeoff Angle: 15.34



Z	Element	Line	Compound	Concentration wt%	at%	Cmpd wt%
8	O	KA1 @ 0.523		50.872	68.295	50.872
11	Na	KA1 @ 1.041		0.878	0.820	0.878
13	Al	KA1 @ 1.487		2.686	2.139	2.686
14	Si	KA1 @ 1.740		29.586	22.627	29.586
16	S	KA1 @ 2.307		2.429	1.627	2.429
19	K	KA1 @ 3.313		0.304	0.167	0.304
20	Ca	KA1 @ 3.691		4.095	2.194	4.095
26	Fe	KA1 @ 6.403		3.066	1.179	3.066
56	Ba	LA1 @ 4.465		6.084	0.951	6.084
			Total	100.000	100.000	100.000

Paper 2-6: Practical Applications of Enzymes in Paper Conservation. Yana Van Dyke. The Metropolitan Museum of Art, New York

Paper conservators have long recognized the benefits of enzymes in the conservation treatment of works of art. Most commonly, hydrolase-type enzymes are employed in paper conservation to assist in the breakdown of adhesive residues from previous restorations or to facilitate the removal of secondary supports such as linings or mounts. The principal advantages of these enzymes are their specificity and efficiency in catalyzing hydrolytic cleavage of polymers such as proteins, starches and fats. The author evaluates the effectiveness of two protease enzymes while taking into consideration cost effectiveness, optimal working conditions, and after treatment effects. Conservation treatment of an Indian miniature involving the use of an enzyme gel is also discussed.

Session 3: Paper and Textile Conservation

Paper 3-1: A Review: Fungal Problem Assessment, Monitoring Methods, and Interpretation of Results Pertaining to Air Quality and Potential Contamination of Collections. Mary-Lou E. Florian, Royal British Columbia Museum, B.C. Canada

Fungal infestations can range from a few visually observable isolated colonies to an infested room. Moldy odors suggest fungal activity.

A few isolated colonies on a heritage object are dealt with usually by isolating the object, cleaning it under a fume hood, and using a particulate mask and gloves. No monitoring is necessary but fungal species identification may be pursued for documentation. The infested room is dealt with the level of hazardous materials or asbestos abatement. Monitoring in the room is necessary to determine the level of contamination in the air and the success of the clean up. Monitoring of adjacent areas may be done to determine if the airborne fungal structures have spread. Species identification is usually done as a document and also to determine if a hyperallergenic or toxic fungal species are present. The cleanup usually does not wait for species identification. In the case of an odor, monitoring is done to determine if there are fungal structures in the air and to locate the source of the odor or amplifier.

We sample the settled fungal structures on objects and the airborne fungal structures -conidia and fungal fragments - and volatile fungal metabolic chemicals we smell, all of which make up the bioaerosol. There are many methods of sampling the bioaerosol; these are reviewed by Willeke and Macher (1999). There are biases to all the methods (Macher, 1999; Health Canada, 1995). Biases are due to the aerodynamics of the conidia, the air currents caused by air conditioning or people movement, etc. Today the recommended method is collection by air impaction and surface sampling, both are analyzed by microscopic examination for total numbers of fungal structures and genera. This may be combined with culturing to determining viable culturable species identification.

The health hazard is mainly a, dose level determined, antigenic response from antigenic fungal structures, conidia and hyphal fragments, dead or alive. The antigen is beta-glucans that is present; as a structural component in the cell wall structure of all conidia and hyphae; in extracellular mycofibrils (Celerin and Day, 1998; Larsen and Green, 1991; Locci, 1972) which encrust surfaces of hyphae; and in the fungal biofilm. The biofilm is present on the substrate where ever there is or has been fungal growth (Florian, 2000b). Beta-glucans is determined chemically and by immunological methods (Burge and Ammann, 1999). Determing the total of conidia and fungal fragments would appear to be just as meaningful but this would miss the small mycofibrils. The mycofibrillar contribution to the antigen level has been overlooked. The amount of beta - glucans may vary with species or physiological state of the fungus.

Beta-glucans is an antigen, but there are also mycotoxins, poisons, in the fungal structures that are a health hazard if eaten or inhaled. Mycotoxins (Burge and Ammann, 1999) are fungal secondary metabolic products in the fungal structures. This is the concern with *Stachybotrys*.

The odors and other microbial volatile organic chemicals MVOCs (Ammann, 1999) are secondary metabolic products, their monitoring and toxicity is still in the

research stage. Fungal MVOCs are the familiar mouldy, mildew, musty or dirty socks odors. The odor of the secondary fungal metabolite 1-octen-3-ol in *Aspergillus niger*, and *Penicillium roqueforti*, has different odors depending on its concentration. In high concentration it smells resinous and in low concentrations like mushrooms, old paper, or mildew-like mustiness. *A. flavus* produces both 2-octen-1ol as well as 1-octen-3-ol. *Chaetomiun* produces both 2-methyl-isoborneol and geosmin that cause its musty, earthy odor. *P. expansum* also produces geosmin. *P. casiecolum*, an *P. roqueforti* and *Botrytis cinerea* produce 2-methyl-isoborneol. All the above fungal species are common airborne surface contaminates. The mouldy odors in buildings have been shown to be caused by a combination of these secondary products.

These alcohols are terpenes. Terpenes are not unique to fungi they are common products in the higher plants i.e., pyrethrin, clove oil, lemon oil, etc, and all are toxic at a specific dose. Florian (1998) has reviewed this problem in reference to their suggested use as natural products in insect and fungal prevention.

MVOC samples from sorbent sampling and concentration canisters are analysed by gas and HPLC chromatography and mass spectroscopy. The present thought is that they are not at toxic levels but act only as an irritant or nuisance. To me all terpenes are suspect toxic chemicals.

In assessing bioaerosol samples for the dose level, whether it is the numbers of fungal structures or the concentration of beta-glucans or MVOCs, the main question is how much is too much? As yet we do not know what are the threshold levels (TLVs), that is levels at which a health hazard is present. The research has not been done mainly because of the major variables: the variable genetic make up of individual people; the variability of the fungal species (for example some 30% of *Stachybotrys* species do not produce the mycotoxin); and the variability of the environment in which the infestation grew. Thus the determination of health hazards is difficult.

It is also difficult to determine if the results of bioaerosol and surface analysis show a threat to heritage collections. The main weakness is that we do not have a base line reference. We use outdoor air as a base line reference because we do not have one for inside the buildings. From the analysis of the above, I am recommending to museums and archives that in their general environmental monitoring program that they have at least four seasonal bioaerosol and surface samplings of specific areas indoors of concern. This data bank would be available for logical comparisons. This data bank will replace the use of outdoor air analysis that is a meaningless reference. I also recommend that when dealing with any fungal problem, no matter what size, that all precautions are taken re personal protection and that stringent aseptic techniques are used to prevent an increase of airborne fungal structures and cross contamination of objects and to use sterile materials for conservation treatment (Florian, 1998).

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Paper 3-2: Characterization of Bacteria Isolated from Naturally-Aged Silk Fibroin.
Edda De Rossi[1], Mary Becker[2] and Orio Ciferri[1]. [1]University of Pavia, Italy; [2]Fukui University, Japan

Little is known about the microbiological degradation of silk proteins although a few bacteria and fungi have been isolated from raw and degummed silk (fibroin) exposed to soil (Ishiguro and Miyashita, 1996, Seves et al. 1998). Some of these microorganisms were capable of metabolizing fibroin in the laboratory and utilizing the protein as the sole source of carbon and nitrogen for growth. Furthermore, one such microorganism, *Variovorax paradoxus*, was found to produce an extracellular acidic protease that hydrolyzed fibroin as well as other proteins (Forlani et al., 2000).

We wish to report now the isolation and characterization of some bacterial strains present on samples of fibroin of different ages and provenance. Two samples of degummed Japanese silk were utilized, one attributed to the Taisho era was a pongee approximately 70 years old and the other a modern silk habutae ca. 20 years old. As a control in the microbiological experiments, samples of Italian degummed silk ca. 3 years old were utilized.

Determination of the tensile properties of the two Japanese samples were done according to the ASTM D5035-95 Test Method. Samples in both the warp and weft directions of the two silks were first cut into strips 35 mm wide and at least 150 mm long, then unraveled to a width of 25 mm. Prior to testing, all samples were equilibrated to standard test conditions, 21±1 °C and 65±2 % RH. Ideally one would test at least five specimens in the warp direction and eight in the weft. However, since these samples came from materials that had been in use and of limited size, this was not always possible.

The fabric from the 70 years silk is a pongee, or hand-spun silk. Because hand-spun yarns tend to be uneven in thickness along their length, the load can be redistributed to the thicker yarns during testing, thus allowing the fabric to continue to extend after reaching its maximum load before actually breaking. This behaviour is seen for the 70 years samples but not the 20 years samples. The 20 years modern fabrics, on the other hand, behave more like a fabric made from machine-spun yarn, which is more uniform. In this case, there are no thicker yarns to redistribute the load to, hence there is little extension once the maximum load has been reached. Overall, the 70 years silk is weaker than the 20 years silk. The warp-direction fabrics for both samples both extend to about 11 mm before breaking, but the load at this point is much lower for the 70 years samples. Also, although the 70 years fabrics tended to extend further, the load at break was much lower than for the 20 years samples. Unfortunately, whether this apparent weakness is truly due to age and deterioration and not, to some extent, fabric construction, cannot readily be determined from these data alone. Comparison with modern samples of the same fabric and yarn construction would be required.

Using standard microbiological techniques, bacterial strains were isolated from samples of the two Japanese and Italian fibroins. As expected, the number of colonies isolated on solid laboratory media from the three samples seemed to be positively correlated with the age of the fabric (the older the sample, the higher the bacterial colonization) as was the case also for the number of morphotypes identified by Gram staining, cell morphology, shape and colony colour. Of the 20 morphotypes isolated, 17

were found to be Gram-positive, 2 Gram-negative and one gave uncertain results. From the oldest sample 15 different morphotypes were isolated, 4 from the ~20 years old sample and only one from the contemporary Italian silk.

Since the isolation of a microorganism from a given substrate does not prove that the microorganism is capable of utilizing such a substrate, pure cultures of the 20 morphotypes were transferred to samples of sterile fibroin fabric and incubated under aseptic conditions (Seves et al., 1998). At different time intervals, colonization of fibroin was determined by assaying the number of bacterial cells that could be isolated on standard microbiological media from the fabric. At least 6 of the tested morphotypes (one, A, from the 3 years old sample and five, E, I, M, N and T, from the oldest sample) were found to adhere and grow onto fibroin. It must be emphasized that the assay determines only the bacterial cells that are released by shaking the fibroin suspended in a sterile saline solution. Thus it is quite likely that only a fraction of the cells in the biofilm adhering to the fabric (Seves et al., 1998) is released into the suspension. By sequencing approximately 800 base pairs of the 16S rRNA genes, the 6 isolates were identified as follows: A, *Micrococcus luteus*; E, *Bacillus licheniformis*; M, *B. pseudomegaterium*; N, *B. subtilis*. Strains I and T were both found to be members of the genus *Bacillus* but their identification at the species level was unsuccessful.

The *B. subtilis* isolate grew in a Davis minimal medium (Davis and Mingioli, 1950) in which fibroin replaced glucose and ammonium sulphate. Thus it is quite likely that this *Bacillus* metabolizes fibroin and utilizes the protein as a source of both carbon and nitrogen. A somewhat similar conclusion was drawn in the case of *M. luteus.* The bacterium grew quite well in a minimal medium in which fibroin represented the only source of carbon and nitrogen but it was unable to grow on the standard Davis' minimal medium indicating that the bacterium has a growth requirement that may be satisfied by one (or more) of the amino acids present in fibroin. When the capacity to hydrolize fibroin and other proteins such as those of skim milk was assayed on agar plates, cells of both isolates produced large halos of protein hydrolysis. However, an halo was evident only in the case of the cell-free supernatant from cultures of *B. subtilis* indicating that the proteolytic activity of the *M. luteus* isolate is mostly intracellular. In addition, hydrolysis of fibroin and skim milk proteins by the latter strain, but not by the *B. subtilis* isolate, was found to be strictly dependent on the presence of Ca^{++} ions.

The production of amino acids (and, possibly, peptides) when purified fibroin was incubated with the cell-free supernatant of pure cultures of *B. subtilis,* grown in a minimal medium in which fibroin represented the only source of carbon and nitrogen, confirmed the presence of protease(s) degrading purified silk fibroin. A preliminary characterization of the proteolytic activity revealed that the strain produces more than one protease active on fibroin and other proteins such as those of skim milk.

Work is under way to characterize the different proteases produced by the two bacteria and to ascertain if fibroin is degraded by a specific hydrolase or, rather, by enzymes whose main substrates are other proteins.

Acknowledgement

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Paper 3-3: Microbial Growth on Textiles: Conflicting Conventions. Mary W. Ballard, Smithsonian Center for Materials Research and Education. Washington, D.C.

Conditions suitable for microbial activity are often described in parameters that do not correspond to the terminology, data, or theories of moisture absorption and desorption in textile fibers and assemblies. Especially for hygroscopic fibers, a continuing confusion about the nature of hysteresis exists. The *equilibrium* moisture regains and moisture contents occur at vastly different rates. Mathematical formulae used to consider the multi-layer absorption and desorption are complex. Commercial regain rates have long been codified to deal with the economic ramifications of the inherent hygroscopicity of various fibers. Studies of the propensity of these fibers to support microbial growth show a much higher threshold of Relative Humidity than those articulated in museum literature, and textile testing laboratory conditions (65% RH) contradict museum caveats.

Complex changes in strength and dimension—swelling, hygral expansion, and shrinkage, also accompany changes in the moisture regain of fibers, yarns, and fabrics. Moreover, the current and historic wet processes of textile technology profoundly affect the chemical and physical properties of the textile products, including their potential as nutrient sources for microbial growth. Microbial growth can and does occur on textiles, but the actual condition for growth within museums and private collections may be less straightforward and less well defined than assumed.

Paper 3-4: Biodegradation of Water-Degraded Archaeological Textiles with Implications for Their Conservation. Elizabeth E. Peacock. The Norwegian University of Science and Technology, Norway

The natural biological disintegration and assimilation with the environment of organic matter demonstrates that the survival of natural fibre textiles in the archaeological record is the result of environmental conditions that reduce the rate and extent of microbial degradation. The preservation of textile materials in dry environments such as desert sites in Egypt, Peru and China is well known. However, with the advent in particular of urban archaeological excavations carried out in response to redevelopment in cities in many parts of the world, the corpus of textiles preserved in wet anoxic environments has grown exponentially. The state of preservation of textile materials recovered from wet anoxic environments varies widely. Common though is their wet, weakened and fragile state.

Scanning electron and light microscopy studies of fibres and textiles recovered from damp, wet and frozen archaeological contexts and fragments of mineral preserved textiles show alteration of the intrinsic morphology of natural fibres. Modern textiles degraded by actualistic and laboratory experimental soil burial provide the opportunity to monitor the developing morphological changes. Analysis of post burial modifications reveals recurring patterns of microbial damage such as tunnelling, surface etching, boring and fibrillation similar to that seen in the archaeological material. Decay patterns vary with fibre type and are most distinct between cellulose-based and protein-based fibres. Advanced stages of degradation lead to the physical disintegration of fibres, and in archaeological textiles the resulting loss of structural substance must be addressed when designing an appropriate conservation strategy.

Problems associated with the drying of ancient textiles following wet cleaning are widely recognised. For historic textiles, dry archaeological textiles already in museum collections, and recently excavated textiles from dry archaeological contexts, the decision can be made to not subject these fragile materials to a conservation treatment of wet cleaning. Textiles recovered from damp, wet and frozen archaeological contexts are already in a wet state and must be submitted to a drying treatment. For these materials, permanent cold and frozen storage are alternatives to drying, but not viable options. Such storage makes accessibility of these textiles to research, analysis and possible exhibition inconvenient, if not impossible.

The drying of water-degraded archaeological textiles is perhaps the most critical step in the conservation process. Drying stresses can bring about shrinkage, collapse, buckling, loss of surface fibres, inflexibility, and adherence of residual surface soil. A suite of suitable drying methods is desirable given the range of equipment and facilities available to conservators of archaeological textile materials. Research into the drying of water-degraded textile materials shows a correlation between the extent of morphological breakdown and the response to stresses that arise in the drying process. Air-drying, solvent exchange, and freeze-drying are drying methods that can be employed, but not all in all instances. Post-drying evaluation of experimentally degraded textiles by microscopic examination and of flexibility provides guidelines for method selection.

Paper 4-1: Microbial Communites in Rock Art Caves: Ecology, Physiology and Effects on Rock Paintings. Leonila Laiz, Juan M. Gonzalez and Cesareo Saiz-Jimenez
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Most karstic caves in northern Spain and southern France are characterized by permanent low temperatures which range from about 10-15°C. Some of the general features of these caves, in addition to low temperatures, are high relative humidity (90-100 %), low levels of organic matter and absence of light. However, extensive tourism affects the natural microenvironment of caves, producing the emission of heat through radiation via skin, increasing the content of carbon dioxide and water vapour, decreasing of oxygen through respiration and providing organic matter. In addition, lighting affects the natural cave microflora by promoting the growth of phototrophic microorganisms (cyanobacteria and algae), and, in some cases, mosses, ferns and even higher plants (Ariño and Saiz-Jimenez, 1996).

Caves are not uniform environments in terms of geological and geochemical characteristics, as they can vary from one to another. Show cave managements produce striking differences as well. Altamira and Lascaux caves, having valuable paintings, have been throughout visited in the past, but had to be closed due to the deterioration observed in the paintings. Nowadays, visitors are derived to cave reproductions. Cave management tends to reduce these anthropogenic impacts by controlling visitors and microclimate (Hoyos et al. 1998).

Interest in the microbiological study of many of these caves is derived from many facts, such as the presence of rock art paintings and the need to preserve this art from deterioration (Hoyos et al. 1998; Cañaveras et al. 1999), the finding of new species of bacteria (Groth et al. 1999a) or the search for new antibiotics (Schlegel et al. 2000).
In the last years several show caves were investigated in detail. These include Altamira, Tito Bustillo, La Garma and Llonin caves in northern Spain and Grotta dei Cervi in Porto Badisco, Italy (Groth and Saiz-Jimenez, 1999; Groth et al. 1999 a,b, 2001; Laiz et al. 1999, 2000). A considerable biodiversity was found among cultivable bacteria, the most abundant strains belonging to *Actinobacteria*. These bacteria were isolated using standard microbiological procedures and incubation at 28°C.

The almost constant low temperature throughout the year in most of the studied caves suggested the possibility of an indigenous psychrotrophic microflora adapted to low temperatures which could be overlooked using standard microbiological procedures and incubation at higher temperatures. To investigate this possibility, soil samples from three different northern Spain caves were collected and studied at four different temperatures: 5, 13, 20 and 28°C. Enumeration, characterization of isolated species and substrate utilization pattern analysis were carried out to investigate the influence of the different incubation temperatures on the isolates and their physiological versatility.

Microbes, are often harmful for paleolithic paintings, because they are related to constructive (mineral precipitation) and destructive (substrate dissolution) processes affecting different substrates (host-rock, speleothems, paintings, etc.) (Cañaveras et al., 1999, 2001). Therefore geomicrobiological studies were carried out to establish the role that microorganisms play in the microbial-mineral interactions that occur in hypogean

environments. A variety of crystals were found to be produced under culture conditions, e.g. calcium carbonate polymorphs.

The studies on cultivated microorganisms in caves most probably reveal just a very minor and not representative part of cave population. Barely no information is available on uncultivated microorganisms in these caves, except some not yet published papers (Schabereiter-Gurtner et al. 2002 a,b).

Paleolithic painting microbiology deserves attention since it has been reported that microorganisms can affect rock art pigments (Gonzalez et al. 1999). Research on cave biodiversity and linking of 16S rRNA information with bacterial function on the Paleolithic paintings is of interest. For instance, we have found that *Bacillus* spp. and *Arthrobacter viscosus*, isolated from rock art paintings, reduce hematite (iron oxide), a common rock art pigment, in laboratory cultures. In addition, closest phylogenetic relatives for about 10% of the clones from Tito Bustillo and La Garma caves were cultivated iron-oxidizing bacteria and uncultivated bacteria from ferromanganous micronodules (Schabereiter-Gurtner et al. 2002 a). This suggests that bacteria playing a significant role in both iron oxidation and reduction might represent a potential hazard for the conservation of the Paleolithic paintings.

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Paper 4-2: Red Stains on Carrara Marble: a Study Case of Certosa of Pavia (Italy).
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Introduction

Among the many stone alterations, of significant importance are the red stains that are observed on numerous Italian monuments including the façade of Orvieto and Siena Cathedral, the statues of Galatea fountain in Villa Litta and the Certosa of Pavia façade. With regards to the latter, the presence of the red stains was first observed in 1844, although exclusively on the Carrara marble and never on the other lithotypes (CNR Centre "Gino Bozza", 1988). These stains are progressively enlarged in the course of time.

The first studies in 1986 demonstrated the presence of red pigmented heterothrophic bacteria belonging to the *Micrococcus* and *Flavobacterium* genera and attributed the nature of the red coloration to the organic pigments such as carotenoids (Bassi *et al.*, 1986). These red pigmented micro-organisms were also subsequently found in the red stains present on the Orvieto Cathedral façade and on the statues of Galatea fountain in Villa Litta (Sorlini *et al.*, 1994; Zanardini *et al*, 1994).

More recent studies have not evidenced by resonance Raman and normal micro-Raman spectroscopic analyses the presence of organic pigments on altered marble samples, but showed bands perfectly corresponding in frequency and intensity ratio to those already observed for pure minium (Pb_3O_4). Similar results were also obtained studying red stained marble samples deriving from Orvieto Cathedral façade and from the statues of Galatea fountain in Villa Litta near Milan (Sorlini *et al.*, 1994; Zanardini *et al*, 1994 and 1997; Bruni *et al.*, 1995).

Different explanations on the origin of the lead were suggested by the authors, but the mechanism of the oxidation of lead was not clear. In the last two years the research has continued with the aim to extend the knowledge on such alterations and to study the microbial communities present in the stained and unstained areas both by traditional cultural and molecular methods. In particular, the aim was to confirm the presence of lead in the red areas of the marble, to investigate the chemical-physical decay and the possible relationship between the microbial colonisation and the alteration, and furthermore to perform more detailed studies on the lead resistant microflora already evidenced in our previous works.

Materials and methods

The microscopic observations of marble samples were carried out by Wild Makroskop M420, optical microscopy Leitz Ortholux and scanning electron microscope (SEM) Jeol 5910 LV equipped with the IXRF 2000 spectrometer (EDS).

For the microbial counts, cultural media as suggested by the Normal Committee (1988) were used. For the identification of some isolates API Staph, and API 20 NE, API 50 CH and ID 32 C were used.

The tests of lead resistance were performed in Plate Count Broth added with lead acetate at different concentrations (from 250 to 1500 mg litre-1). Microbial growth was

estimated by the turbidimetric measurements and ATP total content determinations (Hawronskyi and Holah, 1997).

Transmission electron microscope (TEM) observations were carried out to study the lead resistant bacteria ultrastructure and to investigate if the lead had accumulated in the cytoplasm using a Jeol 100 SX.

For the molecular analyses a Polymerase Chain Reaction (PCR) protocol, specific for the *Bacillus cereus* group (Daffonchio *et al.*, 1999), was used to confirm the affiliation to this group of one of the lead resistant isolates. Denaturing Gradient Gel Electrophoresis (DGGE) analyses were applied for the study of the microbial communities both on the stained and unstained samples following molecular protocols reported by Gurtner *et al.* (2000).

Microscopic observations

The red colouration is present on both the surface and on the inner layer of the samples (up to a depth of 1.5 mm) and in particular in correspondence with the intergranular microfracture of the calcite crystals. SEM observations showed that the stained samples are no more decayed than the unstained ones. Furthermore the stained surface evidenced the presence of a layer, with lamellar structure, in which lead is the only element detected by the EDS analysis.

The presence of microflora both on stained and unstained surfaces is very low: on occasions fungal hyphae and spores were observed while no bacteria were found, probably because located in the microfracture of the stone, where the colouration is concentrated.

Microbiological analyses

Chemolithotrophic and photosynthetic micro-organisms were not found in either of the stained and unstained samples. No significant differences between the microbial counts of the stained and unstained samples were found and only a low phenotypic biodiversity of bacterial colonies was observed. The exception was the presence of red-pink pigmented bacteria identified as *Micrococcus roseus* and *Micrococcus* sp. isolated only from stained samples. Among the other isolates identified were *Chriseomonas luteola, Bacillus* sp., *Staphilococcus* sp. and a yeast, *Rhodotorula minuta*: this confirms our previous results (Salvadori *et al.*, 1994; Sorlini *et al.*, 1994; Zanardini *et al*, 1994 and 1997; Bruni *et al.*, 1995).

From the enrichment cultures with increasing concentrations of lead acetate, mixed cultures and bacterial isolates resulted resistant up to a maximum concentration of 1500 mg litre-1. In particular, consortia from stained samples resulted more resistant than those from unstained samples and among the bacterial isolates found at the highest lead concentrations, were *Bacillus* sp. and *Staphylococcus* sp. as already observed by us in 1997 (Zanardini *et al*, 1997). None of the red pigmented bacteria were shown to be lead resistant. TEM observations showed that these resistant isolates do not accumulate lead in the cytoplasm.

Molecular analyses

The PCR procedure with specific primers confirmed the affiliation of the Bacillus sp. strain to the Bacillus cereus group. Its presence was also evidenced by PCR in the

lead enrichment cultures showing the expected band (750 bp) at the various concentrations that were tested up to 1500 mg litre-1. Our previous studies have already evidenced the presence of B. cereus in marble samples from Certosa of Pavia (Zanardini et al., 1997) and in other degraded stoneworks such as those from the Ca' d'Oro palace in Venice (Salvadori et al., 1994).

With regards to the study of the microbial diversity in stoneworks some authors have recently applied DGGE analysis evidencing the importance of molecular tools in the detection of uncultivable microorganisms (Rölleke et al., 1998; Gurtner et al., 2000). Our preliminary DGGE results evidenced differences among the profiles of the microbial communities in stained and unstained areas that had not been found with the traditional cultural techniques. In fact the microbial community of the unstained sample showed a wider biodiversity that was probably due to the selective pressure of the lead present in the stained areas.

In conclusion, in the stained marble areas the presence of lead and of lead resistant isolates were still confirmed and TEM analyses evidenced that these micro-organisms do not accumulate this element in the cytoplasm.

DGGE analyses evidenced the effect of the lead on the microbial communities showing a lower diversity in the stained areas compared to the unstained areas.

The study of the correlation between the presence of lead and the marble red colouration and how the oxidation of lead can takes place are still under investigations.

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Paper 4-3: Lichens and Deterioration of Stone: Progress and Problems. Ornella Salvadori. Soprintendenza per il Patrimonio Storico Artistico e Demoetnoantropologico di Venezia, Venice, Italy

Introduction

Lichens, and particularly crustose ones, are very useful for studying the biodegradation of minerals, as they adhere to stone permitting the analysis of organism-mineral interactions. The effects of lichen growth are well-known, they were attributed to biogeochemical (production of carbonic acid, oxalic acid and lichenic substances with complexing properties) and biogeophysical mechanisms (penetration of hyphae or rhyzines, expansion and contraction of thalli). Several species have been studied on different substrata with various techniques, and the results confirmed the active role of lichens in short-term biodeterioration processes. The identification of species is the preliminary essential step for the knowledge of the role lichen exert on monuments.

The ecological study of lichen vegetation on monuments permits to investigate the most important factors favouring their growth, and therefore it can be important for preservation. Furthermore, knowledge of the ecology of species supplies information about the best methods for preventing and/or controlling their presence. The growth of lichens is conditioned by several factors, such as exposure, substratum, eutrophication, etc., which can be detected by a floristic and vegetational study. The study of archaeological areas or single monuments is increasingly carried out following this approach. Anthropic factors are often more important than natural ones in conditioning lichen growth. Eutrophication is frequently the major cause of a rapid colonization of artworks in urban and rural environment. The proliferation of nitrophilous lichens on surfaces exposed to rain or to dominant wind can happen in a few years, and was often recorded.

Epilithic lichens

The effects of several foliose and crustose lichens on limestone, marble, sandstone, granite, schist, trachite, brick, mortar and mosaics have been studied. Thallus penetration in the rock is related to its texture and to the mineralogical-petrographical features. On several hard siliceous rocks poor in bases, but also on very compact limestone, the thalli apparently stick to the substratum being well-delimited from it. On granite and schist, fungal hyphae penetrate separately or more frequently arranged in bundles, generally along pre-existing fractures and cleavage planes of the minerals. The maximum penetration (up to 10 mm) was found on some natural (biocalcarenite, sandstone) and artificial stone (brick) probably as a consequence of the progressive dissolution of the calcareous matrix; a distinct boundary between the thalli and the rock is lacking, and a large part of lichen biomass occurs inside the substratum, thereby rendering the lichen-rock interface almost virtual (Pinna and Salvadori, 1992; Salvadori and Tretiach, 2002).

Very often several mineral fragments, biomineralogical products, and - near industrial and polluted areas - microsphaerical particles, are incorporated into the lichen. Mineral fragments (calcite, quartz, chert, micas, alkali feldspar, etc.) generally derive from the substratum, and show the signs of chemical attack on their faces; in the case of micas the crystal layers are often penetrated by algal cells and fungal hyphae and appear

depleted in K, which was probably extracted by oxalic acid. Moreover, some clasts may be particles of wind-born dust from the surrounding areas.

It is well known that calcium oxalate (in both forms of hydration, whewellite and weddellite) is the most common biomineralogical product in lichens. The amount and distribution of oxalate in the thalli and the rock (in the fissures of granitic rocks or among the calcareous matrix of limestone or sandstone) essentially depend on the species and on the kind of substratum. Less frequently, other metals can form different oxalates, such as Mn, Cu, Mg and Fe.

Sphaerical particles show different composition, some are composed of metals (Fe, V, Ti), others of high content of Si and Al, and minor concentration of other elements (K). These microsphaerules, generally with a smooth surface, are atmospheric particulate entrapped in the thalli by passive uptake mechanisms.

On the basis of many laboratory analyses and observations in the field, a scale of aggressivity of several species was proposed, ranging from *Dirina massiliensis*, the most aggressive species, irretrievably disfiguring frescos and stone surfaces (Seaward, 1997), to *Verrucaria nigrescens*, which seems to have almost no negative interaction with the substratum.

Endolithic lichens

These lichens have been studied less than epilithic ones, in part because often no core stone material or minimum samples can be taken from historic buildings or other artifacts.

It is well known that calcicolous endolithic lichens live practically immersed in the rock, they are very spread throughouth the calcareous areas of the world, but their number is not very high. The presence of endolithic lichens goes often unnoticed because their thalli may be coloured like the rock and are superficially impregnated by calcium carbonate. The ecophysiology of endolithic lichens has been investigated only recently, and differs in some aspects from that of epilithic lichens (Tretiach, 1995). The anatomy of some endolithic lichens was thoroughly investigated as well, and showed some common features, such as the depth of the photobiont layer also for species occurring in habitats with strongly different light regimes, but also several differences and peculiarities (Pinna et al., 1998; Tretiach and Modenesi, 1999; Pinna and Salvadori, 2000). The development of thalli inside the stone considerably differs among species, penetrating from 500 to 2.700 µm. Oil-hyphae, typical structures of endolithic lichens, may be of two kinds, and their amounts largely vary among species. Finally, some endolithic thalli (*Petractis clausa* and *Encephalographa elisae*) form hyphal clews (from 30 to 80 µm in diameter) arranged in more or less sphaerical voids produced by the dissolution of substratum. We do not have any idea about the percentage of lichens sharing this feature. From the conservation point of view, it is important to know how the presence of a zone parallel to surface and rich in holes can influence the behaviour of stone, e.g. with respect to water movements (maximum absorption, dynamic of absorption, evaporation etc.) and to damages connected with freeze-thaw cycles.

In spite of the widespread occurrence of endolithic lichens on stone in archaeological sites and on churches and other buildings, the knowledge of their effects on the substratum is still largely unknown. Many aspects of endolithic growth await further investigations, such as the mechanisms underlying the initial stages of rock

surface colonisation, and those of carbonate dissolution. The examined endolithic lichens do not produce calcium oxalate, which suggests an important physiological difference between epi- and endolithic lichens. A better anatomical and physiological knowledge of these lichens will provide the necessary information for a correct treatment of stone interested by their presence.

Biodeterioration vs. bioprotection

According to Wendler and Prasartet (1999) lichens could play a protective action against weathering of porous stone surfaces by reducing the intensity of hygric changes. Major diffusion of exfoliation, salts efflorescence, desquamation, pulverization, alveolization on surfaces where lichens are absent seems to corroborate this hypothesis. Howerer, one should also consider that lichens cannot establish and growth on highly unstable surfaces, such as stone subjected to salts crystallization and subsequent deterioration, because the rate of disappearance of the substratum is higher than the lichen growth rate. Nevertheless lichen thalli can exert a protective effect, especially on porous materials, mainly against atmospheric weathering factors, such as wind, raindrop impact and absorption, salt aerosol, pollutants (Ariño et al., 1995). Endolithic lichens seem to have a hydrophobic effect on stone surface, but when they die the stone loses these hydrophobic properties (P. Modenesi, pers. comm.).

In any case, the possible protective effects of lichens should be carefully evaluated case by case, and cannot be generalized, as the biodeteriorating capability of some lichen species and their disfigurement effects in a relatively short time have been demonstrated.

Among the protective action of lichens the production of ‘oxalate patinas’ by lichens is still mentioned in recent papers (Seaward, 1997; Chen et al., 2000). The origin of this ‘patina’ or ‘scialbatura’ or ‘rendering’, mainly composed by calcium oxalate, is exclusively attributed to lichens. This has often been questioned by other authors, and ‘patinas’ were considered remaining traces of surface treatment or decomposition products of organic material used to protect monuments in the past. A careful examination of the abundant literature on this topic (two dedicated International Symposia were carried out in Milan in 1989 and 1996) can clarify that (i) the so-called ‘scialbatura’ is not originated by lichens, (ii) previous lichen colonizations can leave encrustations of similar composition but of very different appearance (Lazzarini and Salvadori, 1989). A clear definition of terms employed for describing such different situations, together with an exhaustive analytical and photographic documentation are necessary to stop misunderstanding on this matter.

Treatments

The removal of lichens is generally undertaken because of the deterioration they cause to the substratum and/or of the aesthetic damage due to the presence of their variously coloured thalli. Very often lichens grow together with cyanobacteria, algae, fungi and mosses, and treatments aim to the total removal of the whole cryptogamic flora. The treatment of lichens is generally performed by using chemicals (biocides), together with mechanical methods; in the opinion of restorers, lichens are the most difficult organism to be taken away from stone.

The evaluation of biocide efficiency against lichens is generally tested *in situ*, as it is almost impossible to recreate the symbiosis in culture. Until ten years ago the modifications of lichen thalli were registered only with naked eyes: change of colour, of consistency and adherence to substrate. Observation of thalli sections under fluorescence microscope is a rapid method, which is now increasingly employed to check the loss of lichen vitality after a treatment, and to appreciate different sensitivity of species.

Unluckily, preventive measures such as roofing, reducing rising damp, windbreak barriers, etc. are very rarely taken, thereby causing a rapid recolonisation especially of nitrophilous species, while many areas could be thus maintained free of lichens for a long time.

Nevertheless, the presence of stone artifacts often determines the existence of substrata which would be normally absent in a certain area, on which a rich lichen flora can establish. Many Italian archaeological areas, with ancient stoneworks made of rare marble from Greece, Turkey, Africa etc., support a very interesting and peculiar lichen flora. Another example are Sardinian Nuraghes whose sub-cylindrical shape determines a variety of microclimatic conditions supporting a great number of rare lichen. In this cases, if the lichen growth does not heavily damage the substrata, the high biodiversity of many archaeological or monumental areas is in itself a cultural value added to the historic-artistic value of the site (Nimis at al., 1992). The biological importance of biodiversity is a relatively new concept, and before planning a reduction of living species from a certain site one should carry out a preliminary evaluation of their biological importance (Ariño and Saiz-Jimenez, 1996). According to Nimis and Zappa (1988) when endolithic lichens are present, biocidal treatments should be generally avoided. The degeneration of lichen thalli exposes the stone surface to a progressive exfoliation, followed by an intensified attack of weathering agents. Moreover, dead biomass remaining within the substratum may favour the development of a heterotrophic microflora.

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Paper 4-4: Microbial Processes in Deterioration of Mayan Archaeological Buildings in Southern Mexico. Ralph Mitchell, Harvard University, Massachusetts

Paper 4-5: Microbiodeterioration of Mural Paintings – A Review. Joanna Karbowska-Berent, Nicolas Copernicus Univ. (Poland)

Mural paintings are frequently subject to unfavourable changes – bleaching, coloured stains and deposits, associated with peeling and falling off of the painting layer as well as changes in colour of some susceptible pigments. Those alarming phenomena are observed worldwide. The results of a considerable number of studies point mostly to the participation of bacteria, hyphal fungi and algae ion those developments. Among bacteria both heterotrophic and autotrophic – thiobacilli and nitrifiers are observed. Also halophilic bacteria, capable of growing on salt affected mural paintings, actinomycetes and cyanobacteria are often isolated. Among the hyphal fungi the most frequently mentioned species are *Cladosporium herbarum*, *C. Sphaerospermum*, *Aspergillus versicolor, A. niger*, *Penicillium brevi-compactum*, *Acremonium spp.*, *Phoma glomerata*, *Chaetomium globosum.* The algae occuring on mural paintings are mostly one-celled green algae of the family *Chlorophyceae.*

On mural paintings prevail specific ecological conditions, characterized by a low number of carbon and energy sources, varying moistness level and mostly low temperature. The microorganisms occurring on the surface of the mural painting form a specific and complex ecosystem, called biofilm, consisted of populations of many species. The composition of microflora on a mural painting depend in the first place on the climatic conditions of its environment as well as on the materials used in the painting itself or for its conservation. For oligotrophic microorganisms, the mineral and organic components in the dust and soot settling on mural paintings over centuries may prove sufficient. The possible sources of carbon on mural paintings are binders, such as casein, meal paste, some pigments as well as synthetic polymers used for conservation. The consumption of the binder by microorganisms leads to its impairment and, consequently, to the falling off of pigment particles devoid of any binding factor. Another important factor leading to the destruction of mural paintings is organic acids excreted by some microorganisms growing on them. They reduce the pH on the painting and dissolve its mineral components, especially $CaCO_3$. Some of them can form complexes with metal ions taken from the mineral components of the painting, changing its qualitative composition. Also CO_2 arising as a result of microbial respiration contributes to the acidifying of the mural painting.

Over the course of time, quantitative and qualitative changes in the microflora on mural paintings occur under the influence of abiotic factors, e.g., cyclic diversification depending on the season of the year, or changes caused by the microorganisms themselves. The pioneer microorganisms, which are first to settle on the mural painting, through their development, the secretion of metabolites, slimes, the decomposition of the particular components and hindering water evaporation, change the properties of the habitat, making it more suitable for populations of more and more species. The destructive activity of the microorganisms in the biofilm on the painting is not limited to its surface; they penetrate deep inside the painting layer and the plaster, and their destructive effect can be even stronger in the deeper layers than on the surface.

Session 5: Biotreatments and Biocides

Paper 5-1: Consequences of Microbe-Biofilm-Salt Interactions for Stone Integrity in Monuments. Eric May, Sophia Papida and Hesham Abdulla. Univ. of Portsmouth, UK

Microbes and stone decay

Microorganisms may degrade stone mechanically, chemically and aesthetically through complementary metabolic activities and biomineralisation processes in biofilms (extracellular polymeric materials, EPS) and microbial mats (Sand, 1997). Microbial biofilms have a mechanical and chemical deterioration potential on stones and i*n situ* evidence suggests that bacteria deteriorate stones by biofilm production or through mechanical processes since bacterial acid production is too weak (Desheemaeker and Swings, 1995). However, an understanding of their contribution to stone decay by conservators is required. This paper is concerned with the environmental and microbiological factors that control the decay of stones in relation to interaction of water, salts, and microorganisms *in situ* (Portchester Castle, UK; Hania, Crete, Greece) and in laboratory challenge tests.

Stone decay and biofilms

The mineralogy and structure of the stone in relation to its capacity to collect water, organics and particles control its predisposition to biodeterioration, or bioreceptivity (Guillitte 1995). Bacteria produce EPS mucilages, sheaths and fibrous accumulations, consisting of polysaccharides, proteins and leaked intracellular polymers to facilitate irreversible adhesion to substrates and protect them against dehydration, temperature changes and toxic agents (Urzi *et al.*, 1991). This provides an anionic medium with high cation-exchange properties to trap aerosols, dust and nutrients, minerals, and organic compound complexes (Christensen and Characklis, 1990). EPS biofilms are non-homogeneous, thick and dense, largely composed of water, forming a very hydrophilic, hygroscopic gel (Decho, 1994). This takes up water from air and releases it under low RH conditions. Stone moisture is thereby increased while porosity, water-uptake capacity and evaporation are reduced (Warscheid *et al.*, 1993).

In situ observations

Notably rich and homogeneous biofilms, composed mostly of bacterial rods, were observed on substrates coming from sheltered areas of Portchester Castle. Heterogeneous biofilm structures and coagulated cells entangling stone particles were observed on the substrates mostly exposed to sea salt deposition by means of marine aerosols, often considered a primary factor for mechanical coastal stone decay. This was in agreement with Gerdes *et al.* (1994), whose findings determined that high salinity values might have caused flocculant and incoherent mats. NaCl does reduce the viscosity of charged polysaccharides (Christensen and Characklis, 1990) while inorganic salts and multivalent inorganic cations coagulate bacterial cells by neutralising the charge of individual cells (Daniels, 1980). No correlation was found between bacterial populations and the location of their substrates, as they were not clearly favoured on either sheltered or exposed areas.

Biofilms and salts

Salts acting on their own are very important decay agents and can attack stones, mainly mechanically via crystallisation, hydration and thermal expansion in pore spaces during RH and temperature changes (Rodriguez-Navarro and Doehne, 1999). Suspended particles and salts of (a)biotic origin present, serve as nucleation centres for mineral encrustation (Gerdes *et al.,* 1994) and significantly increase biofilm density and affect hydraulic properties (Christensen and Characklis, 1990). Their cohesiveness constantly depends on ionic concentration, temperature, interactions between exopolymer molecules, metal adsorption and enzymatic hydrolysis (Decho, 1994). In addition, salts formed as a result of microbial metabolism (e.g. nitrifying bacteria) may be stored in nearby biofilms (Wilimzig and Bock, 1995). Salts may also affect EPS properties and reduce the viscosity of charged polysaccharides so that chains are shielded against mutual electrostatic repulsion by contraction from an extended stiff form, to a smaller and more flexible one (Smidsrød and Haug, 1971; Christensen and Characklis, 1990).

Laboratory Challenge tests

In the laboratory, respectively, more homogeneous bacterial populations supported by rich biofilms were recovered on stones supporting MMP alone or with water. When salts were applied to stone, biofilm formation resembled more the heterogeneous, desiccated and filamentous-like types observed *in situ*. The destructive potential of salts, especially in collaboration with MMP, was shown in the laboratory. Salt accumulation provided some temporary consolidation, as indicated by increases in longitudinal (V_p) and shear (V_s) sound wave velocities. In general, when MMP were combined with salts, higher salt and biomass accumulation often caused an initial consolidation effect but eventually resulted in increased mechanical tension. This was reflected in a number of properties, i.e. porosity, dry density, weight, that were subjected to more acute changes than with salts alone. The clay content of stones may promote weathering by MMP but higher porosity may be protective, allowing space to deposit biofilm and crystallised salts. It is possible that, due to incomplete evaporation, salt crystals of both hydrated and dehydrated forms were present at the salt/EPS interface and water movement between microorganisms and salts under different RH conditions contributed to the mechanical pressures. Thus MMP enhanced stone water content, providing salts with water of hydration over an extended period.

Conclusions

SEM confirmed the presence of bacteria and biofilm on all substrates, *in situ* and in the laboratory. Bacterial populations and total carbohydrate levels were not favoured on substrates of a particular mineralogy *in situ* or in the laboratory. Although factors other than grain and porosity size control microbial populations *in situ*, the highest final total carbohydrate measurements for the porous stone in laboratory tests indicated a possible preference of microorganisms for EPS production in protected pore niches. In addition, interconnected pores may allow groundwater and bacteria to be transported through the stone matrix although biofilm production within stones may eventually prevent any further penetration. While EPS production is not a selective advantage in the laboratory, total carbohydrate in stone discs treated experimentally with MMP was similar to or higher than *in situ* levels, possibly encouraged by the environmental conditions provided.

No correlation between bacterial populations and total carbohydrate levels was noted *in situ*, but in the laboratory, some bacterial populations were correlated positively, especially under water stress conditions, with or without salts. *In situ*, there were indications that higher populations develop on sheltered locations where the weathering rate of substrates was often slower (higher unconfined strength) and entrapped moisture and nutrients were more readily available. This was confirmed by higher counts of bacteria on mechanically stronger substrates in the laboratory. Moreover, higher selective pressures are more likely to prevail on exposed sampling substrates.

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Paper 5-2: Mechanisms of Microbial Calcium Carbonate Precipitation. Giorgio Mastromei, Chiara Barabesi and Brunella Perito. University of Florence, Italy

The formation of minerals by microorganisms is a very diffuse phenomenon and it is responsible to a great extent for the deposition of minerals throughout the history of Earth (1).

Calcium carbonate precipitation by different bacteria is a relevant example of the important geologic role played by bacteria, suggested since the beginning of the last century. In 1903, Nadson showed that calcium carbonate deposits in lake Veisowe in Karkou, Russia, could have originated from bacterial activity. In 1914, Drew called attention to the role of denitrifying bacteria in calcium carbonate deposition in Great Bahama sediments, the most significant calcium carbonate deposits that exist all over the world (2).

This microbial capability is not restricted to any specific group of bacteria (3). It has been described in different environments such as soils, freshwater and saline habitats and related to the formation of marine calcareous skeletons, carbonate sediments, and soil carbonate deposits (4).

Different mechanisms were proposed to explain the involvement of bacteria in carbonate production. The studies made in this field have pointed out the complexity of the phenomenon that can be influenced by environmental physico-chemical conditions and it is correlated both to the metabolic activity and the cell wall structure of microorganisms (5).

The molecular mechanisms by which bacteria foster calcium carbonate precipitation are not known. In particular, nothing is known about a possible genetic control of the process. A better understanding at molecular and genetic level could improve our knowledge of the process and be useful for biomineralization applications.

Our aim is to develop a biological system, bacteria-mediated, able to induce $CaCO_3$ minerals formation in absence of living cells. We are following two kinds of approaches:

1. Identification of bacterial genes and products (molecules) involved in the biomineralization process.
2. Identification of bacterial cell structure(s) inducing $CaCO_3$ precipitation.

For approach 1, we are using mutants of *Bacillus subtilis* 168, a calcinogenic laboratory strain; these mutants do not form calcite crystals on solid B4 medium (6). At the present time, our attention is focused on a cluster of genes that appear to be involved in the synthesis of intermediates in fatty acid metabolism and, directly or indirectly, related to precipitation.

For approach 2, we are testing dead cells and cellular fractions from calcinogenic strains as potential crystallisation nuclei. We have spent time to work out "a suitable *in vitro* precipitation test" which should be simple, cheap and performable under environmental conditions, also in view of the applicative perspectives.

To evaluate *in vitro* precipitation, we have used the assays described in literature either to test bacterial membrane fractions (7), or to test the calcinogenic activity of molecules (8). Crystal formation was detected by observations at the stereomicroscope for several days. Two kinds of samples were tested: molecules known to induce crystal

formation (8), as controls, and bacterial cells (living and dead) of two calcinogenic strains: *B. subtilis* 168 and a *B. cereus* strain very active in $CaCO_3$ precipitation.

In our hands, the first assay detected calcinogenic activity only with living bacterial cells. Crystal were analysed by FT-IR spectrophotometry and X-ray diffraction and resulted constituted by calcite when produced by *B. subtilis* and by calcite and vaterite when produced by *B. cereus*. The second test, with ammonium carbonate efflux, was positive with molecules able to induce $CaCO_3$ mineralization, as expected from literature (8). Moreover, it was also positive with killed cells (sonicated or autoclaved) of the two *Bacillus* strains. Precipitates were studies by FT-IR spectrophotometry and X-ray diffraction and they were always formed by calcite. Then we tested, as controls, cells of *Escherichia coli*, which does not produce $CaCO_3$ precipitates on B4 medium, and of a *B. subtilis* mutant. Dead cells of *E. coli* did not produce precipitates, while those of the *B. subtilis* mutant had a slower precipitation kinetic. This test resulted useful to distinguish between calcinogenic and non-calcinogenic strains. However, it generates some problems because of ammonia vapours production. To make the test more "suitable", we modified it by directly providing both calcium and carbonate sources in aqueous solution.

The modified assay was applied to all the samples (molecules and dead cells) tested by the assay with ammonium carbonate and the same results were obtained. Different concentrations were also tested to identify the minimal amount of sample needed to induce *in vitro* precipitation. Precipitates were studied by FT-IR spectrophotometry and X-ray diffraction and they were always formed by calcite.

It is important to note that dead cells of calcinogenic strains induced $CaCO_3$ precipitation. It is probably the first time that such a result has been obtained, since we have not found this kind of information in the related literature. Moreover, dead cells of a non calcinogenic strain did not give $CaCO_3$ precipitation. This suggests that calcinogenic strains might have a cellular structure, resistant to the methods used to kill cells, able to promote $CaCO_3$ precipitation. These results are very encouraging and support the possibility to restore monumental stones without using living bacterial cells.

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Paper 5-3: **Biomediated Calcite Precipitation for the Reinforcement of Monumental Stones.** Piero Tiano, Sussana Bracci, and Silvia Rescic CNR, C.s. "Opere d'Arte", Florence, Italy

Monumental stone decay is a consequence of the weathering action of several physical, chemical and biological factors, which induce a progressive dissolution of the mineral matrix. In the case of calcareous stones the material, due to calcite leaching, increases its porosity and decreases its mechanical characteristics. In this sector the products actually applied for stone conservation, such as ethyl silicate and silicones present some drawbacks and they can not fully satisfy the achievement of their safeguard. In fact, restorers involved in monument conservation have an extreme caution to use synthetic products for consolidate or protect stone surfaces. Colour changes, crust formation, glossy appearance and substrate exfoliation, together with environmental pollution are the most frequent drawbacks. These are essentially related with the photochemical modification of these products, which causes a short lasting action and with the use of large amount of organic solvents. Furthermore, the lacking of standard methods for measuring the treatment effectiveness can lead to an over or under estimation of the amount of products to be applied. This situation has induced, together with the past indiscriminate application of all sort of synthetic materials, a real diffidence, both in the operators and in the controlling institutions, towards the use of synthetic products. Thus the historical buildings repair and maintenance field needs innovative conservation strategies and products, specifically tailored.

An old method, based on the application of lime-water, has been recently used to impart a slight water-repellence and consolidation effect, but it creates new calcite with no bond with the substrate.

A new approach to improve this kind of conservative treatments, based on the use of inorganic compounds, is to grow new calcite crystals, inside stone porosity, with a biomineralization process induced by specific organic macromolecules such as the Organic Matrix Macromolecules (OMM) extracted from marine shells. The OMMs are composed usually by acidic glycoproteins rich in aspartic and glutamic acid (protein moiety) and carboxylate and sulphate groups (polysaccharide moiety). In the presence of calcium the protein moiety tends to partially adopt the β-sheet conformation, thus having a high affinity for ions and actively participates to the control of the crystal formation. This process has been deeply investigated and used to control crystallisation of inorganic materials in vitro. But a real application of a conservative method based on the use of the OMM is hindered by the very low yield that we can obtain. For this reason synthetic analogue polypetides, such as poly-aspartate (polyAsp), and the alternating polypeptide poly Aspartate- Leucine (poly Asp-Leu), have been experimented.

The positive factors are that:

- new calcite crystals behave as composite material affecting the mechanical properties of the crystals itself that fractures with a conchoidal cleavage instead of the classical rhomboedral one; and
- new crystals remains strictly bound with the old calcite mineral structure in which the OMM have been absorbed.

The feasibility study and the results obtained on small limestone samples using the OMMs extracted from the shell of a mollusc (*Mytilus californianus*) have shown a good

efficiency of the bio-induced calcite precipitation process with respect to the decrease of the amount of water absorbed and the increase of the superficial strength of the stone material treated.

Contemporary another biomineralisation treatment has been developed applying living cultures of selected calcinogenic bacteria. This treatment has demonstrated its' efficiency even if the application of heterotrophic viable bacteria, inside a monumental stone, need some improvements and caution.

The aim of our work is to develop and validate, a methodology for monumental stones conservation based on the biomediated calcite precipitation process without the use of viable organisms. The molecular biology and the bacterial genetic engineering are the innovative technologies used to improve the biomineralisation process in order to obtain a high amount of low cost and renewable source of macromolecules.

Paper 5-4: ***Biological Mortars as a Solution for Stone Sculpture Conservation*****.** Geneviève Orial[1], Thomas Vieweger, Groux SA, France, and Jean-François Loubiere[1], [1]Laboratoire de Recherche des Monuments Historiques (LRMH), France

Paper 5-5: Biocides and Treatment of Stone: Limitations and Future Prospects.
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Control of biological growth is one of the operations carried out on cultural heritage during restoration. Chemical substances, biocides, are generally used for this purpose; they are applied before and/or after restoration and conservation treatments. Generally biocides are used to eliminate the macro- or microflora responsible for biodeterioration, although more recently some products have been designed to prevent new microbial colonization of restored surfaces and consequently should be applied at the end of the conservation treatment (Urzì et al., 2000). For the conservation of stone materials, many studies have been carried on commercial biocides to test their effectiveness, low toxicity, chemical stability and harmlessness for treated surfaces in order to identify the most suitable products (Koestler and Salvadori, 1995; Warscheid and Braams, 2000). Unfortunately the results of laboratory and *in situ* tests often provide information on a single case or on a specific problem without taking into account the overall conservation project of a monument.

It is well known that biocide treatment should be decided on only after an accurate diagnosis that detects the stone-colonizing microflora and evaluates its role in the deterioration process. When micro-organisms are not responsible for serious damage or the effectiveness of the treatment is uncertain and/or the environmental or substrate conditions are favorable to new colonization and cannot be modified, no treatment should be carried out. Ariño and Saiz-Jimenez (1996) warn of the risk of repeated applications of biocides. Monuments in polluted areas can shelter the development of species that adapt to the urban environment and represent an actual refuge for certain species when the natural habitat is threatened. Moreover, new associations seem to have established themselves on artificial stone substrata in some archaeological areas in the Mediterranean Basin. The risk of an irreversible loss of biological diversity should also be taken into consideration.

Efficacy tests can be performed in the laboratory and/or *in situ*. Selected procedures can strongly influence the results, and a diverse pattern of behavior in biocides has frequently been found between laboratory and outdoor conditions, depending on the type of micro-organisms as well as on practical conditions. Laboratory tests may therefore have a limited prediction value with regard to the microbicidal activity of biocides on stone surfaces. Isolated micro-organisms used in efficacy tests are much more sensitive than those present in biofilms, which are far less susceptible to biocides, so the spectrum of activity of chemicals could be significantly affected by practical conditions. Finally the presence of endolithic micro-organisms and their interaction with biocides has only rarely been considered.

Among other factors that may affect the effectiveness of biocides, we must mention:

- the type of substrate (e.g. the porosity of the stone affects the quantity of the product absorbed and the presence of certain clay minerals, such as illite and smectite, can increase efficacy, absorbing more biocide) (Young et al., 1995);
- the presence of organic materials (dust, pollens, etc.) or pollutants on the surface;

- the solvent used (e.g. water hardness can reduce the activity of quaternary ammonium compounds, while alcoholic solutions can increase their penetration power and sometimes the stability);
- the temperature of the treatment as well as weather conditions (wind, rain) and light intensity.

Some of these conditions can be simulated in the laboratory but it is impossible to recreate a complex ecosystem such as colonized outdoor stone.

In situ applications are very useful as biocide micro-organisms and biocide stone interrelations in the short and long-term can be studied contemporarily. Efficacy tests are in any case carried out in the laboratory, except for some measurements of phototrophic micro-organisms which can be performed in situ (e.g. fluorometric analysis). Chromatic alteration is the only variation of stone parameters that can be measured in situ, as a large quantity of sample, which rarely can be taken, would be required to make other measurements.

In conservation practice, microbiological analysis is only occasionally carried out for various reasons, often due to lack of funds, despite the fact that biocide application is a common procedure. Furthermore, only a few laboratory experiments have resulted in practical suggestions and so an evaluation of the most suitable biocide to use is not carried out and the choice of products and methods is often left to the practical experience of the restorers. A better knowledge of the products employed should however be acquired by restorers; too frequently the mode of action of the chemicals is unknown and consequently the timing of the application is wrong, or recommended concentrations are doubled or trebled in an attempt to improve effectiveness with negative consequences on the final result. Higher concentrations are sometimes far less effective than diluted solutions (Pinck et al., 2002). Treatments with concentrated solutions of a product based on benzalkonium chloride (BAC) caused yellowing of Carrara marble and turned the color of porous limestone containing iron traces pink or red, whereas no change of color was detected using the same product at lower (and recommended) concentrations.

A careful appraisal of biocide interactions with stone materials in the short and/or long-term should be made. Many factors affect the interaction of biocide with stone; the most significant are the chemical composition of the product, the conditions of use (concentration, method of application, duration on substrate, etc.), the mineralogical and petrographic characteristics of the stone as well as the environmental conditions surrounding monuments (Nugari, 1999). Sometimes chromatic alterations can be induced by different commercial formulates with the same active ingredient, because of diverse coformulants or solvents. Different types of stone showed a specific interaction with biocides suggesting the possibility of selecting the product that causes the minimum interference. A biocide (TBTN+BAC) caused a darkening and yellowing of travertine also at low concentrations, while several other products did not interfere with this lithotype. Marble and limestone are particularly sensitive to acid action, therefore biocides with a pH of around 7 or those with added neutralizing substances should be used to prevent damage. Corrosion of calcite and oxidation of minerals producing rusty stains through use of H_2O_2 is well known. Despite this, several products, whose interaction with stone has been clearly demonstrated, still appear in many lists of recommended biocides.

During restoration many operations are carried out for different reasons and with different products. Biocide treatment is only one of them. Combining biocides with cleaning agents, consolidants and/or water repellents or alternatively applying them one after the others should be done with extreme caution in case of possible negative interactions between the different products with the consequent effects on the effectiveness of each individual operation. In order to define the compatibility and the most appropriate procedure for applying different products, exhaustive investigations should be carried out.

Recently the application of protective polymers and biocides at the end of restoration projects to waterproof the stone and inhibit re-colonization for long periods has become common practice. A study on mortars treated either with silicone or with separate applications of silicone and biocide, in two successive steps, has demonstrated biocidal inhibition of microbial growth for a period of 5-8 years, except under continuous conditions of rising damp when phototrophic micro-organisms developed on the silicone alone (Ariño et al., 2002). However, the application of biocides before spreading water repellents seems to modify the characteristics of the latter, thereby diminishing its water-repellent properties. On the contrary, treating the stone initially with protective polymers and then with biocides does not significantly alter the water-repellent characteristics. Moreover, the sequence of applications and the biocide-protective interrelations vary according to the products selected (Malagodi et al., 2000).

The control of stone biodeterioration is a complex question and the use of biocides is currently the most common method. Further studies are therefore necessary to provide efficient and harmless products. Research should focus on establishing harmonized test protocols, choosing specific target organisms from among those affecting monuments, and on developing methods for evaluating biocidal efficacy and biocide-stone interaction both in the laboratory and *in situ*.

Furthermore, better results from biocide treatment could be achieved by improving the methods of application. Besides brushing and paper poultice application, biocides could be added to certain gels (frequently used as bases for solvents or other cleaning agents in the cleaning of paintings) ensuring better biocide-biofilm contact, and thus reducing their spreading within the stone and the consequent negative effects.

In order to guarantee long-term protection of restored monuments against biodeterioration, studies must also be promoted to test biocides for their preservative properties. A few products have shown long-lasting efficacy and it is necessary to verify the duration of residual action and the possible inference on stone over time and under diverse environmental conditions.

Recently the development of new materials for restoration (polymeric metallo-organic matrixes and mortars) displaying antimicrobial activity has been studied. The biocidal activity is due to Cu^{2+} release (Quaresima et al., 1997; Ferone et al., 2000) or to the presence of pentachlorophenol (Martinez-Ramirez et al., 1998). These studies are preliminary reports and require more attentive evaluation (e.g. the use of PCP is banned in many countries for toxicological reasons), but represent an interesting new field of research.

In practice, prevention methods to inhibit or slow down biological colonization should be adopted much more by modifying the environmental conditions and physico-chemical parameters of the stone in order to avoid or reduce repetition of biocide

treatment. Periodic control and maintenance work (i.e. to remove the initial stage of 'dirt' deposition) should be carried out. This is often the principal, and sometimes the only way of preventing biological colonization. Unfortunately, many non-toxic (e.g. modified atmospheres) or physical methods (UV and γ-rays) applied to control biodeterioration indoors, are unsuitable for outdoor application (Bartolini et al. 1999).

A more responsible attitude by researchers, to contribute more emphatically in this field, is required. Research on disinfection treatments should be carried out to provide as much information as possible to set up a "code of practice" for carrying out restoration and/or maintenance work with the best results.

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Paper 5-6: The Use of Metallic Oxides in Control of Biological Growth on Outdoor Monuments. David P. Wessel. Architectural Resources Group, San Francisco, California

The detrimental effect of microorganisms (fungi, lichens, algae, moss, bacteria) on historic building facades and methods to control biodeterioration on outdoor masonry has been the focus of many studies. The processes of biodeterioration on stone has been documented as both chemical and mechanical. For example, lichens can produce organic acids that may damage stone masonry, particularly calcareous rock. An example of mechanical biodeterioration is the process of hyphae penetrating cracks and fissures in stone and thereby causing mechanical damage.

The technique commonly identified in the literature for control of microorganisms is by the use of commercially available chemical biocides typically in liquid form. Wakefield has identified the six most frequently used active ingredients found in masonry biocides (alkyl amine salts, quaternary ammonium salts, inorganic borate/zinc salts, sodium salt, and sodium hypochlorite). Chemical biocide treatment requires periodic reapplication to sustain effectiveness. Reapplication is frequently not possible because of funding constraints. Further, there are possible side effects of chemical treatments to control microorganisms, such as the introduction of soluble salts into the masonry to be treated.

Passing reference has been made in the literature to a biocide alternative for control of microorganisms on buildings, specifically, the strategic positioning of metal strips on building facades (Ashurst and Dimes). Rainwater, washing over the strips, releases oxides that appear to inhibit biological growth. Building surfaces below the metal strips are kept free of microorganisms.

Field conditions documented in this study suggest that rain water runoff passing over either zinc or copper is effective in inhibiting or preventing biological growth on a variety of substrates including wood, architectural concrete and stone masonry. This technique is most effective on new or freshly cleaned architectural materials. Substrates with established colonies of microorganisms are not readily affected by rainwater laden with either copper or zinc oxides. However, one antidotal instance was recorded in which a wood shingle roof was eventually cleaned of dense colonies of mosses after approximately eight years. Field conditions also indicate that exposure of surface as well as slope are factors in the effectiveness of metal oxides in solution in keeping architectural materials clean of microorganisms.

Little research appears to have been done on this method of microorganism control. Given the challenges associated with application and reapplication of chemical biocide, such as maintenance costs and possible health hazards, investigation of this topic is warranted.

This paper will examine the use of two types of metals for control of microorganisms on buildings: copper and zinc. A field test, in place for five years, using zinc strips will be reviewed. Field conditions in which copper strips on wood and masonry that appear to inhibit microorganisms will be examined. Current concern regarding possible adverse environmental effects of the use of copper and zinc oxides as roof runoff will be presented.

Paper 5-7: Conservator as Product Developer. Norman Weiss, Columbia University, New York

Session 6: Analytical Methods

Paper 6-1: Art, Genes and Microbes. Sabine Rölleke, Genalysis GmbH, Germany

The presentation will provide some background information on microorganisms as possible deteriorants of cultural heritage. We will enter into the world of microbial ecology and suggest some answers to the question “Why are bacteria and fungi important to object of art and why can`t we live without them?.”

Furthermore, we will give some good practice expamples in preservation work, as well as some lessons to be learned from microbial eradication strategies and repercussions on cultural heritage conservation.

Some two years ago, we haven proven the impact of microorganisms in cultural heritage deterioration and suggested an analytical method by which all microorganisms present on an object of art can be identified. However, the manual approach of such procedures and the lack of internal standards did not allow this method to be applied in a broader context. Now, Genalysis Ltd. is able to offer a standardized and automized genetic procedure that allows for reliable analysis of all bacteria and fungi present in that sample within 36 hours. In turn, the microbial communities living on and from art can be fully reflected. For the first time in restoration history, this approach allows for a distinct restorators` intervention with a view to ensuring sustainable cultural heritage protection.

Paper 6-2: How Can Cyanobacterial Biofilms Alter Stone in Hypogean Environments? Patricia Albertano, University of Rome "Tor Vergata", Rome, Italy

Methods to control, prevent and monitor phototrophic biofilms that cause damage to rock surfaces are presently very scarce, although the colonisation of stone monuments and archaeological remains by this type of microbial communities is widespread all around the world and particularly abundant in humid areas. Exposed lithic faces are the natural substratum to which microorganisms adhere and grow according to the bioreceptivity of substrata and the environmental selection. Microbial diversity and biodeteriorative activity are dependent on the carbon and energy sources of microorganisms, whose metabolic activity produces irreversible changes and biomineralization of substrata. In this type of terrestrial environment, most of the organisms settle at the surface and form more or less thick biofilms that originate from the development of airborne cells and spores. However, microbial colonisation may produce different patterns and appear as a patchy distribution of cells that accumulate in fissures, cracks or in sub-surface and deep layers depending on the porosity and state of conservation of the material as well as on the ecological requirement of individual species.

Cyanobacteria-dominated biofilms in hypogea

In underground archaeological sites, colonizing microorganisms are usually distributed on the mineral surface layer, while few develop beneath it or actively bore the calcareous substrata. These sites are usually characterised by high relative humidity, constant temperature throughout the year and extremely low photon flux densities provided by sunlight penetrating through holes in the ceilings or artificial light sources. However, the irradiance available for the photosynthesis is usually provided by the lighting systems that are used to allow the illumination of paintings, frescoes, stuccoes and marbles of historic and artistic value during visitors hours. Close to the entrances and throughout the photic zone, phototrophic microorganisms form more or less thick biofilms differently pigmented, that cause aesthetic, physical and chemical damages to the lithic faces. Terrestrial cyanobacteria occur as dominant phototrophic microorganisms, since few species of eukaryotes are able to withstand the low irradiance available for photosynthesis, and only terrestrial species of sciaphilous coccal and filamentous green algae, diatoms, and mosses have been reported. The species composition of these epilithic microbial communities is, therefore, mostly determined by the light regime (Albertano 1993).

However, bacteria, and to a lesser extent fungi, are always present in the same microhabitats colonised by cyanobacteria and microalgae, and populations of heterotrophic bacteria, mostly actinobacteria, have been found in close association with phototrophs (Albertano and Urzì, 1999). Generally, a synergic biodeteriorative effect on stone surfaces is possibly achieved by the concomitant growth of phototrophic and heterotrophic populations as has been shown for other microbial communities in confined environments. Bacteria and fungi, in fact, are able to use the organic matter produced by phototrophs, to release acidic organic compounds and solubilise the minerals of the substratum.

Current methodological approaches

Most of the approaches to the study of microbial deterioration have been dealing with the quantification and identification of the different types of microorganisms within the stone community. Chroococcal and filamentous cyanobacteria have been identified as the most important photosynthetic organisms in Roman hypogea (Albertano and Urzì, 1999). Up to date, the list of cyanobacterial taxa found in Catacombs and Necropolis includes about 20 taxa. Among these, the calcifying species *Scytonema julianum* and *Loriella osteophila*, might be considered as the most deteriogenic because of the capability to mobilise calcium ions from calcareous substrata (Arino et al., 1997; Hernandez-Marinè et al., 1999).

The application of light and electron microscopy methods to the study of microbial communities forming biofilms offered valuable possibilities in the visualisation of cyanobacterial biofilms, providing the means to characterise structural interactions between species and in resolving ultrastructural details at a micrometer and nanometer scale. A variety of microscopic techniques has been used for the examination of microorganisms in their natural environment, to assess the taxonomic affiliation and nutritional status (Albertano 1997; Albertano et al., 1991b, c, 1994, 1995). Light microscopy, epifluorescence, confocal laser microscopy, scanning and transmission electron microscopy allow to understand the organism relationships within the polymicrobial population and the interactions between mineral substratum and microorganisms (Urzì and Albertano, 2001). Noteworthy, most of the microorganisms that develop in this environment are unknown at the genus or species level and some of them have been reported to possess evolutionary interesting adaptive features (Albertano et al., 2000a).

As natural caves, man-made hypogea can be considered environments with extreme light conditions, and similar to other extreme habitats can be thought to harbour low-diversity communities (Albertano, 1993). However, studies on the 16S rRNA of other cyanobacterial mats, showed the presence of many genotypically different cyanobacterial populations with phenotypes morphologically and cytologically similar or essentially identical. In fact, although a large diversity of morphological complexity of many groups of cyanobacteria allows their visual identification, it cannot be assumed that morphologically similar cyanobacteria, e.g. the widespread Oscillatorialean *Leptolyngbya* species, do have the same genotype. It will be, therefore, essential to match genotypes with phenotypes of terrestrial cyanobacteria inhabiting hypogea, and molecular techniques are now available and are being used for phototrophic microbial communities (Ward and Castenholz, 2000).

The role of biopolymers

Despite of biodiversity, another aspect is crucial in the assessment of the biodeteriorative action of microbes on monuments and has to be considered. It involves the understanding of biofilm function and of the metabolic machinery that leads to the transformation of the lithic substrata and to a consequent change of the monument ecosystem. New approaches are, therefore, focusing on these aspects in the attempt to provide the information needed for the establishment of control and prevention strategies against biodeterioration processes.

The microbial strategies for the adhesion to the stone seemed to be generally based on the production and secretion at the cell surface of mucilaginous compounds. Exopolymeric substances secreted by the microorganisms (glycocalyx, sheath or envelope) act in sticking the cells to the substratum, and their adhesive properties contribute to the formation and cohesion of biofilms. A polysaccharide matrix containing cell debris and significant amounts of inorganic material adsorbed from the substrate makes up most of the cyanobacterial films to which airborne particles, bacteria, and spores adhere increasing masking effects, transformation and corrosion of the surfaces. In addition, the anionic nature of exopolymers maintains highly hydrated the fibrous matrix, strongly adsorbs cations and dissolved organic molecules from the minerals, and stabilises dust particles. Calcium ions can be easily subtracted and precipitated on polysaccharide sheaths of cyanobacteria in form of calcium carbonate and to a greater extent macronutrients as nitrogen and phosphorus are mobilised from the substrata and metabolised or stored into the cells. Moreover, the photosynthetic activity that sustains the production of new biomass into the ecosystem, supports the development of numerous populations of heterotrophic bacteria and fungi that graze on cyanobacterial and algal exopolysaccharides, metabolites and cell debris, therefore increasing the deterioration of stone by a synergistic action. Accordingly, studies on the phototrophic microbial communities present in Roman Catacombs have been undertaken in order to characterise the nature of exopolysaccharides produced by cyanobacteria (Albertano et al., 2000b, Albertano and Bellezza, 2001), as well as to assess their ability to make use of irradiances available in those sites and to acclimate to different light conditions (Albertano et al., 1991a; Bruno and Albertano, 1999).

Non-destructive assessment of cyanobacterial activity

The increasing cultural, artistic and religious relevance, gained by Roman hypogea in the last years, has recently boosted the interest towards new *in-situ* non-invasive approaches in order to monitor and asses damage and biodecay processes. From this point of view, microsensors could be used to study changes in composition of chemical species mobilised upon stone surfaces during microbial metabolism, in order to evaluate the biodeteriorating activity of microorganisms on the colonised surfaces. The advantage of the use of microsensors relies in the facility of the assemblage and in a good signal stability, sensitivity and reproducibility (Albertano and Compagnone, 1999).

The growth of cyanobacterial films, as a result of their photosynthetic and respiratory activity, can induce more or less pronounced variation of the chemical parameters that characterise the microhabitats, and possibly cause deteriogenic effects on the colonised substrata. Amperometric oxygen microsensors were applied to measure photosynthesis and respiration in phototrophic biofilms that develop in Roman catacombs in order to provide new insights on the microbial community function and biogeochemical fluxes (Compagnone et al., 1999).

S*cytonema julianum* (Fig. 1), a filamentous heterocystous cyanobacterium widespread in Roman necropolis and catacombs, can be a very dangerous species because of its ability to precipitate carbonate crystals on the polysaccharide sheath and, therefore, to mobilise calcium from calcareous surfaces. The simultaneous recording of curves of photosynthesis at increasing iradiance (P/I) and the application of potentiometric microelectrodes for the measurements of pH has shown that the

photosynthetic activity of this species can shift pH values to a sufficient extent to induce precipitation of calcium carbonate (Albertano et al., 2000c, d). In addition, the more recent development of K^+ microelectrodes showed that a decrease of potassium concentration occurred during light exposure in strains of *S. julianum* possibly due to an active uptake sustained by the photosynthetic activity of these cyanobacteria, while no appreciable variation of soluble calcium due to the metabolic activity was observed in natural and 'artificial' biofilms of the same species.

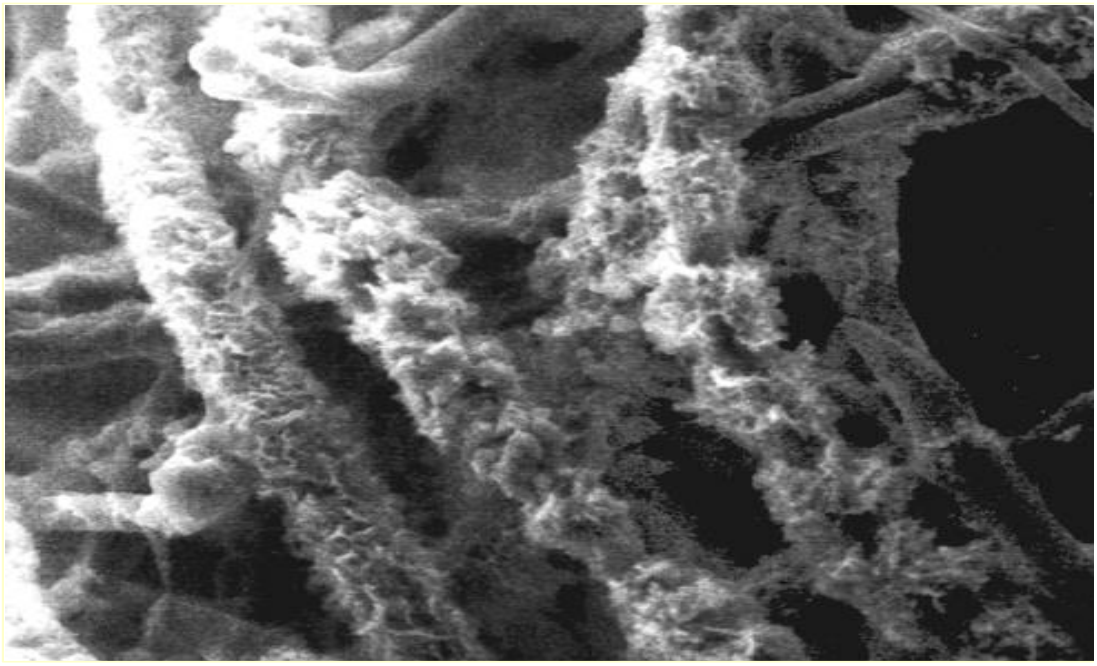

Figure 1. Environmental scanning electron microscope image of hydrated filaments of the calcifying species *Scytonema julianum* in biofilms from Christian catacombs in Rome (Italy).

What conservation strategy can be safety applied in Roman hypogea?

Current research is trying to develop and integrate physical and biotechnological methods intended to limit microbial growth on valuable archaeological surfaces, and to apply analytical methods to monitor the presence and the extent of the damage that is consequent to the development of phototrophic and heterotrophic microorganisms. Pilot studies will investigate the possibility of using new lighting systems providing wavelengths poorly used by cyanobacterial photosynthesis. This could drastically decrease the growth of cyanobacteria and, therefore, the quantity of organic matter available to the associated heterotrophic populations.

In addition to the physical approach, newly identified biomolecules related to iron metabolism and cell-to-cell signalling pathways could be checked for their ability to interfere with bacterial and, especially, cyanobacterial metabolism by removing factors indispensable to microbial development. Finally, the application of non-destructive microsensor methodologies to the analysis of biodeteriorative processes, and the understanding of the interactions occurring *'in situ'* among microorganisms and the

surrounding physico-chemical environment are presently recommended as the best approach for the assessment of conservation strategies in these archaeological sites.

Acknowledgements

This work was supported by the EU Programme Energy, Environment and Sustainable Development, in the frame of CATS Project, contract EVK4-CT-2000-00028 and by the Research National Council of Italy, C.N.R. – Progetto Finalizzato Beni Culturali grant n° 01.00472.PF36.

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Paper 6-3: Non-destructive Versus Destructive Sampling Methods: Limits and Advantages for Studying Microbial Communities Colonizing Monument Surfaces.
Clara Urzì, Filomena De Leo, Paola Donato, Violetta La Cono, Univ. of Messina, Italy

Introduction

In terms of conservation of works of art, an important step is the acquisition of information concerning the state of deterioration of the artifact, object of study. When organisms are involved, it is often necessary to sample significant amounts from the valuable object in order to clarify, if and which type of (micro-)organism is involved and the relationship that it takes with the material itself.

Current approaches deal with the quantification and identification of different types of microorganisms within the stone community, in order to:

1. understand the steps of the colonization and the relationship among the different populations on the surfaces;
2. identify the most dangerous microorganisms;
3. make previsions on the eventual future risk of biodeterioration of the substrate and to address the intervention.

The level and the variety of the possible analysis, are affected by the sampling modality and of the amount of sample taken from the surfaces. Working with valuable objects of cultural relevance, it is almost impossible to take relevant amount and thus non-destructive sampling methods are obviously preferred to destructive ones.

A method to be widely accepted should give valuable information on the microflora connected with a specific alteration, on the relationship between the surface and the colonizing microflora, and eventually inform on the taxonomy of the species involved.

In recent years, several non-destructive methods were proposed for the direct or indirect evidence of microbial colonization (e.g. agar fingerprinting, adhesive tape stripes; sterile needle sampling, etc.) and advantages and disadvantages were listed by Urzì and De Leo (2001).

However, destructive samples are unavoidable in order to get information on the pattern, depth and extension of biodeterioration.

We report some results we have obtained from different deteriorated surfaces, using different sampling methods accordingly with the sampled surface and give a brief description of methods and their applications with limits and advantages.

Experimental

Samples were taken in the frame of European Project EVK4-CT-2000-00028 “CATS” from different hypogean environments (in the Christian Catacombs of St. Callistus and Domitilla, in Rome) and in Spain (at Zuheros caves) in correspondence of surfaces affected by heavy microbial colonization (Figs. 1 a and b).

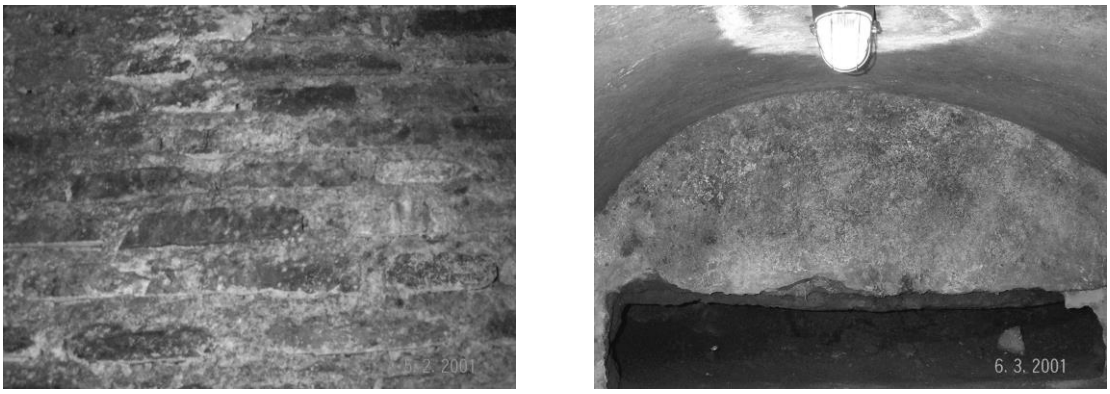

Fig. 1. Two different type of biofilm in hypogean environments. (a) white patina on a brick wall; (b) green patina on mortar in correspondence of a source of artificial light.

Adhesive tape stripes and biofilm removal through scalpel and or tweezers were the chosen methods. In both cases the surfaces were very little affected by the sampling procedures.

Adhesive tape stripes.

This method is a non-invasive sampling technique that offers the possibility to sample microorganisms present on the surfaces and to detect them directly under the microscope.

It was used to get information on the morphology of microorganisms and on their relationships with the colonized material surfaces directly under light and epifluorescence microscope. In addition, we carried out cultural analysis inoculating small pieces of adhesive tape on suitable cultural media (Urzì and De Leo, 2001) and we also applied molecular methods for single cells detection (FISH, Fluorescent In Situ Hybridization) (Urzì and Albertano, 2001).

This technique was tested by us, for the first time, directly on the sample taken with adhesive tape and needed only few adjustments respect the classical protocol for pure culture (Stahl, Amann, 1991). The targeted microorganisms were eubacteria and especially Gram positive bacteria with high content of G+C.

Scalpel, tweezers, needle, etc

Small amounts were taken with the opportune tools accordingly with the sampled material and its importance and status of conservation. When biomass present on the surface was abundant, scalpel or tweezers were safely used without affecting the integrity of surface. In this case the sample taken was mainly concerned on the microbial community colonizing that specific area and, only in part, on the interactions between the material and the microorganisms.

Information concerning the deterioration was achieved through microscopic examination, cultural analysis and the application of different molecular techniques for the fingerprint of microbial population (DGGE, TGGE, RISA, SSCP, etc.).

In our laboratory we tested the SSCP technique (Single-Strand-Conformational-Polymorphism) for the analysis of microbial communities colonizing Catacombs and hypogean environments and the protocol utilized is described below.

SSCP technique used was that one described by Schwieger and Tebbe (1998). Total genomic DNA from biofilm samples were isolated by using a modified protocol of Zhou et al.(1996) and amplified by universal primers. PCR products were purified and the digestion of the one strand was carried out used lambda exonuclease (Pharmacia Amersham Biotech, Italia). The samples were electrophorezed in a 0.6 MDE gel (FMC Bioproducts, Rockland, Maine), and gels were stained with silver stain system by a protocols recommended by the manufacturer (Promega).

Results and Discussion

The sampling technique of adhesive tape gave valuable information on the microorganisms involved in the biofilm. Microscopical examination of samples were easy to perform and different microscopic techniques were carried out (Light microscopy, epifluorescence with or without fluorochrome.

Cultural analysis from adhesive tape gave qualitative results and the isolation was successful only for fungi and algae, while bacteria were more difficult to isolate.

The FISH technique applied directly on adhesive tape was successful and allowed a better evidentiation of bacterial community in the biofilm (Fig. 2a). However, the application of the technique was limited by:

the contemporary presence of phototrophic and heterotrophic organisms, due to the chlorophyll autofluorescence disturbing the evidentiation of fluorochrome bound to the molecular probe (Fig. 2 b); and

the choice of the right probe to apply for that specific sample; in our case, of the biodeteriogen responsible of the damage.

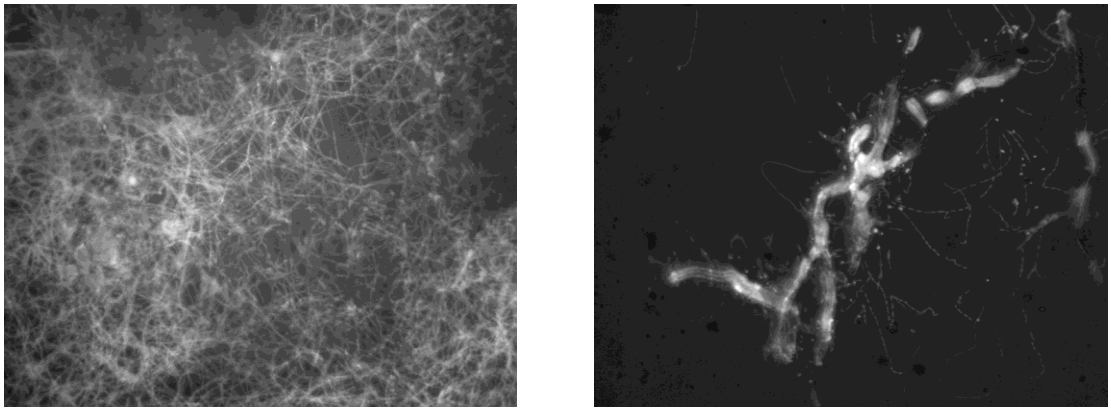

Fig. 2. Evidence of microbial community from two different samples taken with adhesive tape stripes from the Roman Catacombs of Domitilla (left) and St. Callistus (right). (a) Amasses of *Streptomyces* hyphae evidenced with an universal probe EUB 338 labeled with FITC; (b) autofluorescent filamentous algae and bacterial filaments evidenced with EUB 338 labeled with FITC.

Biomass analysis rendered possible the evidence of culturable microflora and it

was also possible to carry out the extraction of total DNA for further molecular analysis. Microbial population coming out from the growth media were useful to gain information on the most representative strains and on their taxonomy; it was possible, thus, to design and choose the appropriate molecular probe to be used with FISH technique.

Studies of the microbial community by SSCP were very useful in order to evidence differences and similarities existing among the populations taken from the different biofilms (Fig. 3). In fact, each band obtained with SSCP technique corresponds to different genera/species. Identification of microorganisms involved was possible through the DNA extraction from the gel and its sequencing.

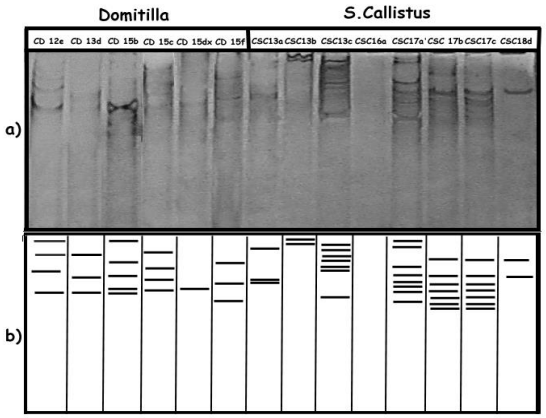

Fig. 3. SSCP electrophoretic patterns obtained from the biofilm samples taken from the Roman Catacombs.

From our results, it is evident that the combination of different methods, is needed for the detection of microflora colonizing marble probes to reduce the damage during the sampling procedure at the minimum level and to get useful information concerning the microorganisms responsible of biodeterioration.

A summary of sampling methods and type of possible analysis is shown in Table 1.

Table 1 – Non-destructive and destructive sampling methods.

Methods	When to use	Type of analysis
Adhesive tape stripes (negligible or no amount of sample is required)	Any suitable surface before and/or after conservative treatments	• Microscopic observation with or without staining • Cultural analysis • Molecular methods for single cells detection (FISH)
Sampling with scalpel, tweezers, etc (amount 50 mg – 1 g or more)	Detached material, hidden part, quarry material, etc.	• Microscopy with or without staining • Cultural analysis • Molecular methods for community fingerprinting, single cells identification, and taxonomy

Acknowledgments

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Session 7: Treatment and Prevention

Paper 7-1 Visual Effects of Selected Biocides on Water-based Paints. Jun Suzuki and Robert J. Koestler*. Museum of Fine Arts, Boston; *The Metropolitan Museum of Art, New York

Introduction

Virtually all art materials are susceptible to microbial attack. When it occurs an immediate response is needed to minimize the damage to the material. This response may include application of a biocidal treatment. In order to make an informed selection of what biocide treatment to use, it is important to have some idea of the side effects of any treatment. A technique to assess for visual change in paint samples was presented in Koestler et al., (1993). This technique has been used to assess the changes to four water-based paints: watercolor, acrylic color, egg tempera and Japanese paint after treatment with four different biocides: two were gases--argon gas and nitrogen gas; the others were liquids--an architectural biocide, D2) and an experimental pine resin extract.

Materials and Methods

Watercolor and acrylic color were proprietary brands (for watercolor, Winsor and Newton, England and for acrylic color Liquitex, USA). Egg tempera was made with dry pigments then ground into the medium of 1:1 egg yolk and water (all pigments Holbein, Japan). The dry pigments for Japanese paint were ground into the solution of Japanese animal skin glue, *Sanzenbon-nikawa* (all pigments were Japanese products). Each paint had twelve colors (Table 1).

Supports

Watercolor and acrylic color were applied on watercolor paper (Fabrian, cellulose 100%, Cartiere Miliani Fabriano, Italy). Egg tempera was applied on a poplar plywood panel, 4 mm thickness. The panel was sized with 7% rabbit skin glue solution and prepared with gesso ground (for lower 6 layers, gesso grosso; for upper 4 layers, gesso sottile with 7% rabbit skin glue solution). Japanese paint was applied on Japanese paper (*Kumohada-mashi*, a hemp and *Kozo* fiber 100%, sized with animal skin glue, Uematsu Co., Japan).

Paint application and dryness

Each paint was applied in 2.5 cm wide strips on each support. And each paint was diluted with water in the usual painting method, and applied about 4 to 6 layers with a soft-flat brush to avoid unevenness as much as possible. Triplicate strips were prepared as control and each experimental sample. Control and experimental samples were stored under identical light conditions before and treatment. The samples were dried for about 10 months under similar conditions (they were hung on a wall, in dim light).

Biocides and application

Four biocides were selected: two of them are fumigants--argon gas (Ar) and nitrogen gas (N_2); and two liquid fungicides--D2 (an architectural antimicrobial, Prosoco, Inc., Lawrence, KS), and an experimental pine emulsion (Tampere Univ. of Technology, Finland).

Samples treated with Ar or N_2 gas were enclosed in Marvel Seal 360 (a heat-sealable aluminized mylar) and brought down to an oxygen level under 100 ppm. Packets of oxygen scavenger (Freshpax D-2000cc, Multisorb Technologies, Buffalo, NY) were place inside each bag to maintain the low oxygen level. The humidity level was about 55%. Triplicate samples were kept in this environment for 1 month.

Triplicate sample strips of each paint sample were sprayed with the D2. The pine emulsion was tested on canvas and found to severely stain the canvas brown, so further testing with this product was not conducted.

Evaluation method

The evaluations of the change after treatment were carried out by visual observation by two persons, and by measurement with a Minolta Spectrophotometer CM-2002. A statistical binary procedure was used to assess the visual data (as per Koestler et al., 1993). Changes were assessed in the following five categories: visual color change; change in gloss; appearance of cracks, bubbles, blisters; blanching effect; and topography changes.

The color values of CIE 1976 L*a*b* of control and experimental samples were measured before and after treatment.

Table 1 Selected colors of each paint

Watercolor	Acrylic Color	Egg Tempera	Japanese Paint	
			English Name	Japanese Name
Chinese White	Zinc White	Zinc White	Chalk	*Gofun*
Titanium White	Titanium White	Titanium White	White Lead	*Enpaku*
Alizarin Crimson	Alizarin Crimson	Rose Madder		*Iwashiro* *
Cadmium Red	Cadmium Red	Cadmium Red		*Iwamomo* **
Terre Verte	Chromium Oxide Green	Terre Verte	Light Red	*Taisha*
Winsor Emerald	Emerald Green	Oriental Green	Vermilion	*Shu*
Prussian Blue	Prussian Blue	Prussian Blue	Red Lead	*Entan*
Cobalt Blue	Cobalt Blue	Cobalt Blue / Deep	Verdigris	*Rokusho*
Cadmium Yellow	Cadmium Yellow	Cadmium Yellow	Synthetic Verdigris	*Shin Iwarokusho*
Raw Sienna	Raw Sienna	Raw Sienna	Azurite	*Iwagunjo*
Burnt Umber	Burnt Umber	Burnt Umber	Gold	*Ao-Kindei*
Ivory Black	Ivory Black	Ivory Black	Silver	*Jun-Gindei*

Results

Some of the results of the visual and spectrophotometric readings will be presented at the meeting, and the complete set will be published in the full paper.

Acknowledgements

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Paper 7-2: Detection of Life in Art and Introduction of Anoxia Eradication of Insects and Fungi. Franc Pohleven, Crtomir Tavzes, Jure Pohleven, and Robert J. Koestler*. University of Ljubljana, Slovenia, *The Metropolitan Museum of Art, New York

Introduction

Objects of cultural and historical heritage are irreplaceable and should therefore be protected from damage caused by insects and wood-decay fungi. Wooden objects may be attacked by various wood-boring insects, e.g., the furniture beetle or the powderpost beetles, which can over long periods of time cause severe damage. The furniture beetle, in particular, likes to attack wood that has been infested by fungi. Wood-attacking fungi can decay wooden objects very rapidly and in a short period completely destroy it.

The anoxic treatment method has been developed to eliminate insect infestations with no side-effects to the object (Gilberg, 1991; Koestler, 1992, 2001). This approach has proven very successful, especially when coupled with measurement of insect repiration (Koestler et al., 2000, Stusek et al., 2000). Eliminating a fungal infestation may be more difficult. Dry wood is nearly completely protected against infestation (germination) by fungal spores, but if the object is already infested with fungal mycelia, drying will not always eradicate the infestation. For example, the dry rot fungus, *S. Lacrymans,* can not be eliminated by drying and a more active procedure should be employed (Pohleven, 2000).

Since the anoxic treatment has proved so successful for insect control, we have decided to try to modify it for fungal control, too. There have been a few studies on the effect of different oxygen concentrations on growth and survival of fungi and the extent of decay that they cause. Jensen (1967) reported that fungal biomass production decreased rapidly at an oxygen concentration of 15% and ceased in the absence of oxygen. Otherwise, if the oxygen concentration was kept at a certain level and the cultivation differed in the carbon dioxide concentration, the amount of dry weight produced decreased with an increase in carbon dioxide concentration. The ability of several fungal species to degrade wooden blocks was severely retarded when oxygen content was as low as 1% or CO_2 levels were raised to 10% (Highley *et al*., 1983). On the other hand, delignification was not affected by an atmospheric CO_2 content of 14% and was only slightly retarded at 7% of O_2 in the bulk gas phase; concentration inside the wood particles could be lower (Reid, 1985). The ability of fungi to survive in an environment with a low oxygen concentration is best described by Scheffer (1986). There it was shown that the fungi that survived the longest were more often heart-rot than the sap- and products-decay fungi. Some of the fungi were even able to survive in sealed vessels for more than two years. However, most of the fungal species studied died within three months and cultures of two of the species died within a week.

Nevertheless, none of the above-cited authors used pure argon or nitrogen gas to deplete the atmosphere in experimental vessels of oxygen and carbon dioxide. The aim of this research was to determine whether treatment with argon or nitrogen, separately, could reduce the exposure time of selected fungal species to anoxic conditions needed for their eradication. Another aim of this research was to ascertain whether the choice of substrate (PDA nutrient medium or wood blocks) influenced asphyxiation of selected

fungi with either argon or nitrogen gas. Optimisation of the asphyxiation method and establishment of extremely low oxygen concentrations could shorten the time needed for eradication of fungal attack (Tavzes *et al*., 2001). If the process is feasible, the ultimate goal is to apply it to fungal-infested art objects.

Materials and Methods

The effects of low-oxygen conditions, achieved with either argon or nitrogen gas, on the viability of wood decay fungi *Coniophora puteana, Antrodia vaillantii* and *Trametes versicolor,* cultivated on PDA medium and infested wood samples, were examined. *Serpula lacrymans* was anoxically treated on infested wood samples only. The fungal cultures were exposed to low oxygen concentration (below 10 ppm) for one to five weeks in hermetically sealed vessels or gas-impermeable plastic enclousures. Anoxic treatment did not affect *T. versicolor* cultures in the time span of the experiment. Therefore treatment of only *C. puteana* and *A. vaillantii* mycelial cultures was extended to 10 and 16 weeks. After treatment, respiration and regeneration of mycelium were tested by measurements of CO_2 production and the ability of hyphae to resume their growth on fresh PDA medium. The effect of anoxic conditions on the mycelia of treated fungal species was expressed as an increased time needed for regeneration or as a complete absence of growth of inocula taken from the exposed cultures or wooden blocks reintroduced on new nutrient medium.

Results

Cultures that were slowed by the low oxygen concentration consequently produced less CO_2. For *C. puteana* cultures, the effects of anoxic treatment became evident in the second week of the treatment. The number of affected cultures rose steadily with the prolongation of anoxic treatment. By the sixteenth week of the anoxic treatment, 80% of the inocula of *C. puteana* did not regenerate (see Figure). *A. vaillantii* inocula regeneration was not affected until after the fourth week of treatment, and similarly for infected wood samples, after five weeks. The influence of anoxic treatment on the cultures of this species was more pronounced on the tenth and especially after the sixteenth week, when 77% of inocula did not regenerate (see Figure). In general, fungal species were differently sensitive to asphyxiation. *T. versicolor* cultures were not affected by anoxic conditions, caused by either argon or nitrogen gas, and *A. vaillantii* mycelial cultures proved to be less sensitive than those of *S. lacrymans* and *C. puteana*. In the tests with fungal-infested wood blocks, argon proved to be more effective than nitrogen gas at reducing or eliminating fungal activity.

Figure

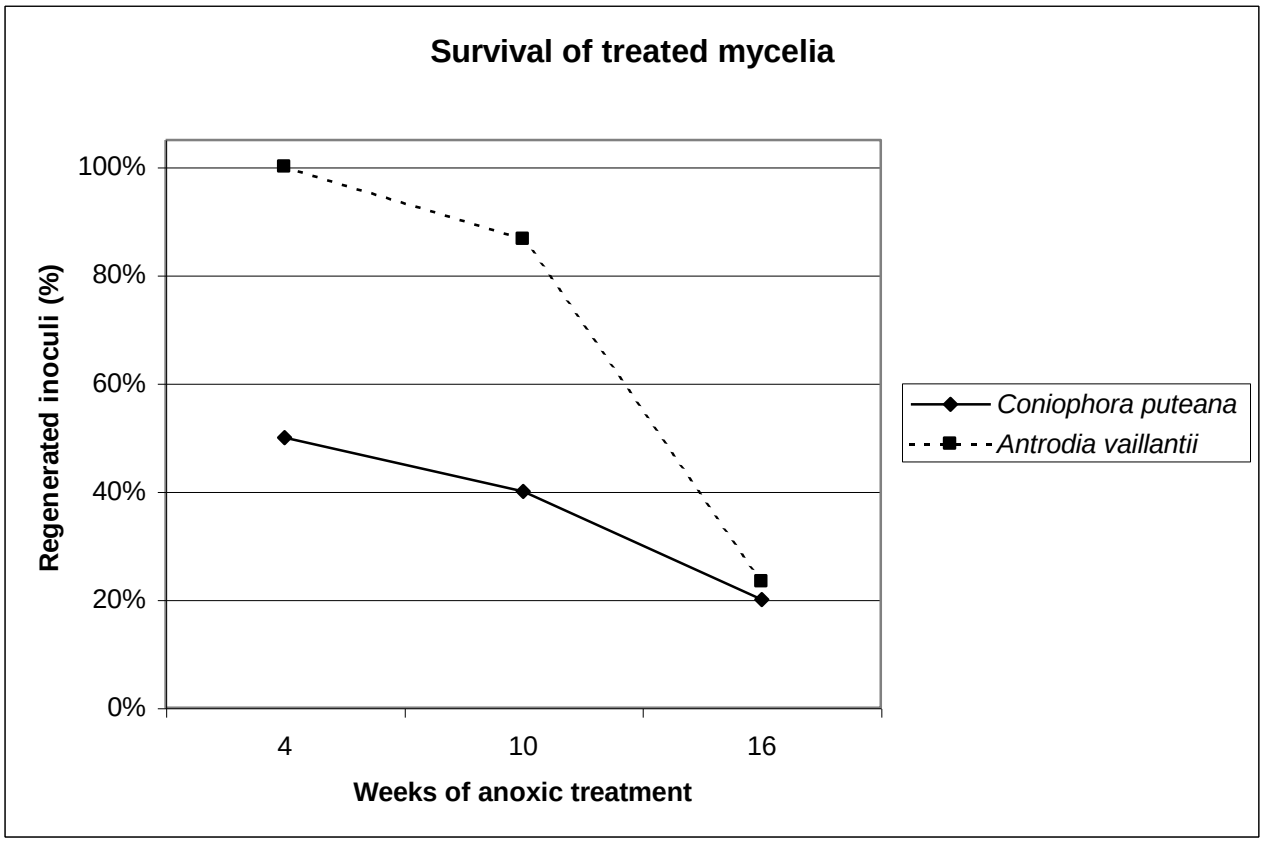

Figure 1: The effect of anoxic treatment length of *C. puteana* and *A. vaillantii* cultures on survival of inoculi.

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Paper 7-3: Results of a Novel Germicidal Lamp System for Reduction of Airborne Microbial Spores in Museum Collections. Harold W. Rossmoore[1], Katalin Rossmoore[1], Mohammad Sondossi[2], & Robert J. Koestler[3]. [1]Biosan, Inc., Detroit, Michigan; [2]Weber State University, Utah; [3]The Metropolitan Museum of Art, New York

The airborne transmission of microorganisms is a well-known vehicle in the spread of infectious disease. This is equally true for potential biodeterioration of natural and manufactured materials. Prevention of infection is certainly preferable to treatment. Among susceptible materials no substrates are more in need of prevention of biodeterioration than cultural properties, especially post-creation protection. Methods and treatments must be limited to those that do not react with the properties being protected. The use of UV irradiation of indoor air to reduce viability of airborne microbial populations falls in that category i.e. only the air is treated. To treat a large volume of air in a reasonable period of time i.e. to prevent surface establishment of viable organisms requires an improvement in existing technology. The results presented here are based on the following, 1) greater UV intensity (output per arc length), 2) a chamber of 10 micron mirror-finish aluminum increasing the reflectance of UV over other surfaces and 3) increase in air flow through the irradiation chamber. A pilot system has been evaluated in the laboratory to validate the microbicidal effect against organisms with known high resistance to UV at 254nm. These include spores from *Bacillus cereus*, *thuringiensis subtilis* and *stearothermophilus*. An exposure time of 2 seconds using a 30 watt UV lamp reduced the viable population >95% (initial population 10^7 – 10^8 spores/ml). Conidia of a well-known resistant fungal contaminant *Aspergillus niger* were similarly irradiated using 30 watt and 70 watt UV lamps. For the 30 watt, average viability reduction was 77%. For the 70 watt, average viability reduction was 98% (inoculum 10^5/ml for both 30 and 70 watt). An increase in the inoculum to 10^7/ml reduced viability loss to 94%. These laboratory data are for a single pass of 1.4 ft^3/min. through the test chamber. Field units currently under test involve continual recycling of room air through the UV chamber. Bear in mind the test contamination levels used here were at least three orders of magnitude higher than any expected pretreatment room levels. With an air flow rate of 550 ft^3/min. a room 40 x 60 x 15 would be treated in one hour. Results from these trials are expected for this presentation.

Paper 7-4: How to Prevent Microbial Damage on Glass and Glazed Art Objects

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Introduction (historical)

Glass and glazed (ceramic, clay, brick) objects of art belong to the oldest representatives of the physical cultural heritage. Only natural glass (obsidian), some rocks and especially rock carvings and mural paintings (rock art) reached comparable ages. However, the oldest clearly dated man-made glass object is part of a glass eye from an Egyptian statue and some glass pearls made only about 3500 years ago. About 1300 B.C. the Egyptians developed the technique to make glass bottles. The heated and liquid glass was molded around a stick. Initially glass was a luxury item. 700 B. C. is the date of the first technical description of how to make glass (Assubanipal): Take 60 part sand, 180 part ashes from sea weeds, 5 parts chalk – and you get glass. It is uncertain how glass was discovered. Pliny the Elder suggested that sailors landing on a sandy beach (quartz), unloaded some of their cargo from Wadi Natrun (natron, alkali) to support their cooking pots. They started a fire (heat) and were astonished to get glass. Pliny's is a good story, but most likely glass came about as a side product of metal making. The idea of ashes of seaweed also may explain the alkali content very convincingly. About 300 B.C. people near Syria and Phoenicia developed the technique of blowing glass. A glass object could be blown when a large mass of molted glass was put at the end of a hollow pipe and blown into a hollow mold and shaped artfully. This was a revolution in the making of glass, making the process faster and the product cheap. Since that time glass objects of all kinds including windows and stained windows were produced. The oldest European stained glass windows, however reach back only to the 12th Centurry. The oldest of all are the five figures in the clerestory of the Augsburg Münster, Germany, which are dated from 1050 to 1150. Many other early examples are found in France and England including the wonderful North Rose window of Notre Dame, Paris dating from 1250. Since about then worries of how to care for and protect these precious objects from natural and vandalistic decay and perishing.

Glass is regarded as a liquid to crystal. The transition is a thermodynamic one. The crystal is energetically more favorable than the liquid below the melting point. The glass transition is purely kinetic. The disordered glassy state does not have enough kinetic energy to overcome the potential energy barriers required for movement of the molecules past one another. The molecules of the glass take on a fixed but disordered arrangement. Glasses and super-cooled liquids are both meta-stable phases rather than true thermodynamic phases like crystalline solids. A glass could theoretically undergo a spontaneous transition to a crystalline solid at any time. Sometimes old glass devitrifies in this way especially if it has impurities. There are three main types of molecular arrangement (1) crystalline solids: molecules are ordered in a regular lattice; (2) fluids: molecules are disordered and are not rigidly bound; (3) glasses: molecules are disordered but are rigidly bound. Today, however, scientists made quasi-crystals halfway between crystal and glass. These do not fit into the above scheme. Polymerised materials such as any organic tissue or rubber show a clear glass transition at low temperatures but are normally considered to be solid in both the glass and rubber (polymer) conditions. There have been many claims (especially by tourist guides) that glass deforms because it flowed

slowly down the window panels over the centuries. This has become a persistent myth, but close inspection shows that characteristic signs of flow, such as flowing around, and out of the frame, are not present. Examples of rippling in windows of churches can be accounted for, because the glass was imperfectly flattened by rolling before the float glass process came into use. Robert Brill (1962) of the Corning Glass Museum has been studying antique glass for over 30 years. In his opinion the notion is untrue. He says, examples of sagging and ripples in old windows are also most likely physical characteristics resulting from the manufacturing process. Just for the locality of the second Symposium on "Microbes and Art" it may be stated here that American glass making was the first industry in Jamestown, Virginia in 1608. The production was not successful and closed before the settlement itself disappeared. The first successful glass making enterprise in North America was founded 1739 in Wistarberg, New Jersey.

"Blow hot, cut cold" was the subject of a very successful art exhibition in 2001, when the sister of one of the authors of this article, world-known glass engraver and glass-recycling artist Karin Hubert and her son Korbinian Stoeckle (Director of the museum glass blowing unit of the Industriemuseum Bochum in Ovenstedt) put their skills and art together in a world-wide covered art show. For these artistic and many other cultural concerns glass window panels (stained and engraved), precious blown, engraved, carved and cut glass objects world-wide cause increasing concern about durability and change for the above mentioned reasons of the instability, fragility and vulnerability of glass. What is to be found then about microbial attack and biodeterioration? What about preventing environmental and microbial damage? These questions are the topic of our communication to this Symposium.

Which Microbes Attack Glass by what Processes?

Present day knowledge of microbial attack on glass is manifold and very well developed. Krumbein et al. (1991) have summarized the state of the art of biological research giving credit to Mellor (1922) as the first author clearly pointing at the main causes of microbial attack and damage as well as to ways of preventing the damage. However, the main processes of glass destruction by microbes were studied and described gradually in later time. Key publications stem from Oberlies and Pohlmann (1958) and Bacon and Raggon (1959). Krumbein (1969) was the first to describe bio-pitting, bio-chipping and bio-cracking of glass (chert). He had the chance to learn directly from Dr. Oberlies, a former scientist of the Max-Planck-Institute of Silicate Research (Würzburg), now Fraunhofer-Institute of Glass Research. However, Oberlies, Mellor and Krumbein should give credit to Bachmann (1904) an early worker on biodeterioration of silicate and carbonate materials by lichens. In a joint M. Sc. Thesis project between the Material Science Institute of the University of Erlangen and the Geomiccrobiology Unit of Oldenburg University (Birrenbach, 1997, Weissmann and Drewello, 1996, Krumbein et al., 1996) new techniques and new approaches enabled modern science to prove, what Mellor (1922) had conceived. The microbial attack of glass is mainly a physical/mechanical process and only in a sub-ordinate way a chemical one. What really happens is not yet entirely clear. However, microbes create considerable damage to surfaces and deeper parts of glass objects of the physical cultural heritage by several different physical processes. These are (1) differential heat transfer; (2) differential turgor pressure; (3) individual hardening and stabilisation of microbial cell surfaces comparable

to heat processed steel materials; (4) polymer embedding into the cell walls; (5) differential attachment mechanisms; (6) active movements; (7) cell arrangement in substrate adapted microcolonies; (8) physical use of water potential and water physical characteristics; (9) active transport of water and other compounds through microcolonies and hyphal extensions; (10) chemical conditioning through excreted compounds and minerals. Several of the physical processes and especially their thermo-dynamical and kinetic outcome and time related intensity are still to be analyzed in deep. It became evident, however, that mechanical attack is more important and detrimental, than chemical processes and that biological processes general rule the decay status and speed under present day environmental conditions.

Prevention and Protection

One of the major issues in modern conservation and restoration problems is microbiology. One should not wonder about this. The spoiling of food and any other so called "good" is mainly a microbial process, as all global physical and chemical processes (biogeochemical) are ruled by microbial activity. Thus thermodynamics teach us, that live processes and living matter are the strongest and most important factor in changing conditions and materials (including physical cultural heritage) on Earth. In order to understand the importance of micro-organisms one could also think of all available techniques of preparing the human body for "eternity" i.e. mummification. The art of creating mummies is basically the art of preventing microbial attack of organic material and mineral material produced by organisms. This, in modern times has been extended to the potential production, maintenance and destruction of any mineral material or near-mineral material such as glass. Constant poison injection into a certain living organisms will make it less vulnerable to microbial attack. This is the secret of biocides. Absolute cleanliness prevents a body and the parts of it (like teeth) not always but sometimes from rapid decay. Thus we just have to apply the rules of cleanliness to the conservation and restoration sciences of objects of the physical heritage. In this way we arrive exactly at the conclusions taken by Mellor (1922), when she published her thesis on biological decay of old church window panels defended at the Sorbonne. These conclusions are comparable in their repeated value to the US constitution or the declaration of the Rights of Mankind. Our contribution consequently ends with the repetition of the summary of the Mellor doctorate thesis written clear-sightedly in 1921. Admittedly we have slipped in some modern scientific jargon into her elegant summary of work and thought:

(1) The immediate cause of the destruction of objects of art is a mechanical action exerted by biofilms establishing themselves on chemically pre-conditioned surfaces and substrates.

(2) The chemical corrosion (pre-conditioning) of the substrates exposed to atmospheric conditions (pollution) is always accelerated by sub-aerial microbial biofilms.

(3) Inhibitory substances such as heavy metals or organic antagonists may cause exceptional resistances of the substrates in question.

(4) Annual or at least regular careful and cautious cleaning of the substrate surfaces in question helps to avoid physical/mechanical attack and destruction of the substrates. The establishment of a mature sub-aerial aggressive biofilm needs a considerable

period of time, which is definitely longer than in the case of sub-aquatic detrimental biofilms.

Thus the protection of church glass panels and other atmosphere exposed glass objects is easier and more promising, than the protection of water immersed materials of any kind.

Acknowledgements

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Session 8: Wood and Archaeological Materials

Paper 8-1: Deterioration in Historic and Archaeological Woods from Terrestrial Sites. Robert A. Blanchette. University of Minnesota, St. Paul, Minnesota

Wood exposed to environmental influences will deteriorate over time from microbial decay and non-biological degradation processes. This paper provides an assessment of the types of deterioration that have been found in historic and archaeological woods from different terrestrial environments and gives insights to better understand decay processes that affect wood. Historic and archaeological woods that survive adverse environmental influences and the many forms of biological and chemical deterioration are extremely valuable resources since they can provide important information about past cultures. These woods can also be used to obtain accurate dating of materials in dendrochronology studies (Schweingruber, 1993), determine where objects have originated by tree species identification (Tennessen et al. 2002) and provide information on past environments by examining chemical and biological signatures that have been left in the wood (Blanchette, 2000; Blanchette et al. 1994; Filley et al. 2001).

Wood decays rapidly in most temperate and tropical environments and may totally decompose leaving little to no evidence of the original substrate. In some environments, however, wood is protected from biological and chemical attack and may remain relatively unaltered. But even in the most extreme environments that restrict microbial growth and limit non-biological deterioration processes, wood is not completely free from attack. Over long periods, extremophiles (microbes able to grow in very adverse environments) degrade the wood and chemicals, such as salts, cause a slow deterioration of wood surfaces. Most, if not all, historic and archaeological woods are affected by some degree of degradation. To obtain information on the major types of deterioration that occur in wood, several different historic and archaeological sites were selected for study. These sites represented very diverse environments and locations from the Antarctic and Arctic regions to the semi-arid areas of Turkey, Egypt and the southwestern United States.

Samples reported in this investigation are from archaeological woods from buried tombs and historic woods from world heritage sites, including:

i. The buried wooden tomb found in Tumulus MM at Gordion Turkey, thought to be the tomb of the legendary King Midas (this work is from collaborative research with Elizabeth Simpson, The Baird Graduate Center for Studies in the Decorative Arts, New York; G. Kenneth Sams, Department of Classics, University of North Carolina, Chapel Hill and Timothy Filley, Department of Earth and Atmospheric Sciences, Purdue University);

ii. Wood from ancient tombs in Egypt (in collaboration with Robert Koestler, Metropolitan Museum of Art, New York and Pamela Hatchfield, Museum of Fine Arts, Boston);

iii. Wood from buried tombs in Siberia (in collaboration with Elizabeth Simpson, Leonid Marsadolov, State Hermitage Museum, St. Petersburg, Russia and Joel Jurgens, University of Minnesota);

iv. Wood from the historic huts of the Ross Sea region of Antarctica built by Robert F. Scott and Ernest Shackleton (1902 – 1911) during their expeditions to the South

Pole. (In collaboration with the Antarctic Heritage Trust, Christchurch, New Zealand; Roberta Farrell, University of Waikato, New Zealand; and Benjamin Held, Department of Plant Pathology, University of Minnesota);

v. Wood from Fort Conger built by Adophuls Greely (1881) and huts built by Robert Peary (1900) in northern Ellesmere Island of the Canadian high Arctic during their explorations of the North Pole regions. (In collaboration with the Quttinirpaaq National Park, Parks Canada; Nunavut Research Institute; Benjamin Held, Joel Jurgens and Michael Sombrio, Department of Plant Pathology, University of Minnesota; and

vi. Wood from Chaco Culture National Historic Park and Aztec Ruins, National Monument, New Mexico (in collaboration with the US National Park Service and The Getty Conservation Institute, Los Angeles).

Moisture is essential for microbial decay to take place and there are few natural environments where moisture is completely excluded. Even in buried tombs from arid regions some moisture is usually present allowing selective microbes to grow and progressively decay the wood. In addition to moisture, many other factors influence microorganism growth, such as oxygen, nutrients, temperature and pH. Favorable conditions allow rapid colonization by fungi and fast decomposition, but unfavorable conditions usually allow slower growing organisms that tolerate the extreme environmental conditions to dominate. A major type of decay commonly found in the woods examined in this study was caused by different species of soft-rot fungi. These fungi, apparently well adapted to extreme environments, can cause extensive decay over time. Soft-rot fungi are classified as Ascomycota and Deuteromycota and can be distinguished from other decay fungi by their decay patterns produced in wood. Typically, soft-rot fungi produce cavities that spiral within the secondary wall of wood cells following the microfibrillar orientation of cellulose (Type I attack). In transverse sections of wood, holes of varying sizes can be observed in the secondary wall of wood cells, whereas in longitudinal view they are elongate cavities often with pointed ends. In some woods, a different form of attack occurs (Type II) and the entire secondary wall is gradually eroded leaving a relatively intact middle lamella. Soft-rot fungi are often associated with waterlogged woods and the term soft-rot is used to describe the decay because the affected wood surface appears soft in wet environments. In the investigations reported at this meeting, soft rot was commonly found at the opposite extreme in relatively dry sites.

Investigations of wood from the King Midas tomb showed a soft-rot fungus was the primary organism to decay the furniture, coffin, and wood of the tomb structure. ^{15}N isotope analyses demonstrated that the fungus utilized nitrogen from the King's body and subsequently translocated this nitrogen as it grew throughout the tomb (Filley et al. 2001). The environment of the tomb with limited moisture availability, high pH (from the limestone rubble place over the tomb), and the nutrient-rich body of the king, were likely the major influences for only one type of degradation and possibly one specific fungus to colonize and cause decay in all woods of the tomb over an exceedingly long time.

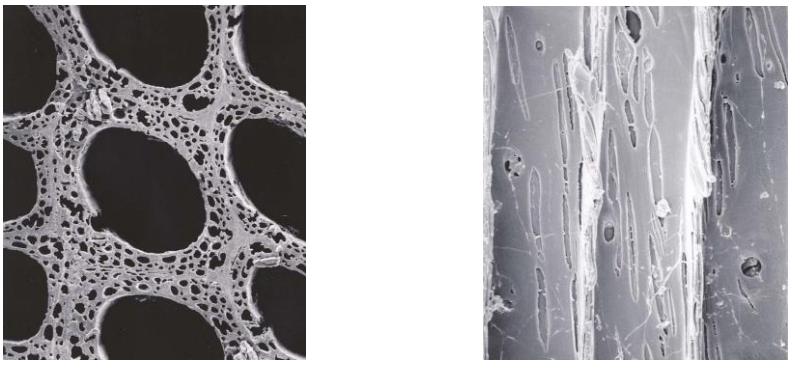
Soft-rot fungi were also the major decay causing organisms in woods from the historic huts of both the North and South Polar Regions. In these areas, temperature and

salts have a strong influence on microbial growth. The fungi responsible must tolerate very cold conditions (there are only a few weeks during the summer months when temperatures are above freezing) and high salt concentrations. These fungi are also well adapted to colonize wood and remain dormant for most of the year only to reactivate when conditions are conducive the following summer. Although the rate of decay in these harsh polar environments is slow, the accumulative effects over decades of fungal growth and subsequent soft-rot attack results in very substantial amounts of decay occurring.

Another type of decay that was found in some of the samples of ancient Egyptian wood, the Siberian tomb woods and the historic wood from New Mexico is a brown-rot that is caused by wood destroying basidiomycetes. This type of decay is characterized by an attack on the cellulose and hemicellulose components of wood. In very early stages of decay, cellulose deploymerization occurs accompanied by a drastic loss of wood strength. Moderate levels of moisture, association with wood destroying insects and low concentration of salts (lower pH) are some of the factors that appear responsible for favoring this type of degradation in woods from these sites. In advanced stages of decay it is difficult to visually differentiate brown rots from soft-rots and microscopic observations must be made to determine the specific degradation patterns.

Another very important type of degradation found in woods from several different sites examined for this study is a chemical deterioration of wood caused by high salt concentrations that accumulate on wood surfaces. The most serious problems occur when wood absorbs water with dissolved salts and later evaporates the moisture resulting in high concentrations of salts to accumulate at the evaporative surface. This non-biological attack causes a corrosion of wood components by deteriorating the lignin within the cell wall (Blanchette et al. 2002). A defibration of the wood results and cells readily separate from one another. This deterioration results in large masses of defibrated cells to be removed from the wood. This situation is most severe in environments were rainfall is lacking and leaching of accumulated salts from the wood surface does not occur. It was found in the North and South Polar regions and Chaco Canyon and Aztec Ruins sites. The decay can be very extensive if woods are exposed for long periods to these conditions. Similar adverse chemical reactions have been observed in surfaces of wood from several ancient Egyptian woods examined. In these situations, unique environments exist when wood contacts high alkaline soils or mud brick containing high salts. At sites with limited moisture the effects are restricted to only the surface wood cells but these cells can be greatly altered and extremely fragile.

Soft rot in wallboard from the interior chamber of the King Midas Tomb



Scanning electron micrographs of soft-rotted wood from the King Midas tomb showing numerous holes within the cell wall in transverse section (right) and cylindrical cavities in radial section (left).

Exterior wood from Scott's hut at Cape Evans, Antarctica showing defibration caused by salts.

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Paper 8-2: Degradation Patterns in Waterlogged Wood and the Two-Step PEG Treatment for Archaeological Finds: The Case of the Bremen Cog. Per Hoffmann, Deutsches Schiffahrtsmuseum, Germany

Archaeological wood is a term used by conservators for wood excavated by archaeologists. It is normally wet, waterlogged, or wet and frozen. Waterlogged soils, sediments or deposits can provide anoxic or low oxygenated environments, where the degradation of the organic material is restricted to the action of slow-working specialist microbes: soft rot fungi and/or bacteria. The wood substance metabolized by the microbes is substituted by water, the wood thus keeps its shape. To prevent the archaeological wood from shrinking, warping, and disintegration it has to be stabilized before it may dry. The microbial attack on the wood can produce different degradation patterns, different distributions of more or less degraded cells. In large wooden objects the type of degradation patterns governs the choice of the appropriate stabilization method. This is exemplified in a series of ship conservation projects, culminating in the conservation of the Bremen Cog from 1380, a huge 24 m cargo ship stabilized in the largest conservation tank ever operated.

Paper 8-3: The Conservation of the Wooden Objects from Gordion, Turkey: Methods for the Treatment of Dry Archaeological Wood. Elizabeth Simpson. The Bard Graduate Center for Studies in the Decorative Arts, New York

A large collection of rare and unusual wooden objects was excavated at the site of Gordion, capital of the kingdom of Phrygia, between the years 1950 and 1973. Excavations were carried out by the University of Pennsylvania Museum of Archaeology and Anthropology under the direction of Rodney S. Young. At least 37 pieces of ornate wooden furniture and 56 wooden objects were recovered from three royal tombs—Tumulus P, Tumulus MM, and Tumulus W, excavated in 1956, 1957, and 1959 respectively. In addition, many carbonized wood fragments were found in the "destruction level" of the City Mound.

New C-14 dates for organic samples from the City Mound establish a date for the destruction level in the late 9th century B.C.; Tumulus W and Tumulus P are approximately contemporary. Tumulus MM is now dated to the second half of the 8th century B.C., the period of the Phrygian King Midas or his immediate predecessor (Simpson, 2001).

Many of the wooden objects were well preserved inside the wooden tomb chambers, which had been covered over with huge mounds of earth (tumuli). The high clay content of the earth had kept the moisture levels inside the tombs fairly constant and low enough to inhibit major deterioration. The wooden objects were studied briefly and underwent conservation treatment at the time of excavation. The pieces were then placed in storage at Gordion and in the Museum of Anatolian Civilizations, Ankara. The excavation and finds from the three tombs were published posthumously by Young (Young, 1981).

During the preparation of Young's volume for publication, numerous errors were found in the field drawings of the wooden furniture, and a new study of the pieces was begun by Elizabeth Simpson in 1978. Investigation of the artifacts in Turkey indicated that the wood was extremely fragile and in need of further conservation. A project to retreat the wood was begun in 1981 under the auspices of the University of Pennsylvania Museum, directed by Simpson with the participation of Robert Payton, then conservator for the British Institute of Archaeology in Ankara. Last summer (2001), the conservation project was completed after 21 years of field work in Turkey, the last 11 years under the supervision of the project's senior conservator Krysia Spirydowicz, professor of objects conservation at Queen's University, Kingston, Ontario.

The first piece to be retreated was the fancy inlaid table from Tumulus MM (Simpson, 1983; Payton, 1984). Although most of the wood was in relatively good condition, several pieces were warped or damaged, apparently by water that had seeped into the tomb, in part due to the drilling down from the surface of the mound in 1955 and 1956 in order to locate the tomb chamber. After the objects were excavated, an effort was made to conserve the wood. The pieces were immersed in a solution of alcohol and water in an attempt to dry the wood. They were then treated in a solution of wax and gasoline, in the hope that the wax would enter the pores of the wood. It did not, instead remaining as a thick deposit on the surface of the pieces, attracting dust and dirt in the years after its excavation.

The new treatment involved cleaning the wood of the waxy deposit with cotton swabs soaked in the solvent toluene. The wood was then consolidated under vacuum in a solution of polyvinyl butyral (Butvar B-98), dissolved in a mixture of toluene and ethanol. After consolidation, the wood was removed from the solution, wrapped in polyethylene, and allowed to dry slowly over several months. The wood was then unwrapped, and a stronger solution of Butvar B-98 was applied to reinforce cracked or damaged areas. Finally, the surface was cleaned of excess consolidant. The treatment was a success, lightening the color of the wood and strengthening it considerably; the pieces could finally be handled safely and the reconstruction drawing and photography of the table completed (Simpson and Payton, 1986). This method, refined over the next several years by Payton, Spirydowicz, and other members of the conservation team, has been used for most of the wooden objects from Gordion (Spirydowicz, 1996; Simpson and Spirydowicz, 1999). The procedures and concentration of the consolidant were optimized according to the results of investigations carried out in collaboration with Robert Blanchette, Arno Schniewind, and other scientists (Spirydowicz et al., 2001). The process has proved highly effective for the consolidation of dry archaeological wood.

At the time of excavation, certain of the wooden objects were treated with a solution of the consolidant Alvar dissolved in acetone, which helped preserve the surface of the most degraded pieces but did not serve to strengthen the wood. During retreatment, the Alvar was removed with acetone, aided by the application of gel poultices consisting of a solution of Klucel HF in ethanol or an ethanol-acetone mixture (Spirydowicz et al., 2001). The wood was then consolidated with Butvar B-98.

Wood fragments from Tumulus W, carved in openwork and covered with bronze studs, were apparently the remains of an elaborate serving stand. The wood had degraded, and the surviving studs were held in place only by means of the Alvar coating applied at the time of excavation. The stand was removed from the Gordion depot, where it had been stored since 1959, and taken to Ankara for retreatment, a process that would take eight years of painstaking work to complete. The wood was first cleaned of the Alvar coating, and the studs were cleaned and treated with a solution of Benzotriazole for bronze disease, which they had developed during their years in storage at Gordion. Tests conducted to determine the effect of Butvar B-98 on the bronze studs indicated that the acidic nature of the consolidant actively promoted corrosion of the bronze. A method was developed which allowed the wood to be soaked in the consolidant without immersing the studs in the solution. The studs were retreated with Benzotriazole following the wood's consolidation.

The king's coffin from Tumulus MM was left unconsolidated because of the extremely degraded condition of the wood. Studies carried out on the coffin were extremely productive in terms of the anatomy and identification of the wood (Blanchette and Simpson, 1992). The reconstruction of the coffin on paper (Simpson, 1990) led to a reconstruction of the king's funeral and even the items on the menu at the funerary banquet (Simpson, 2001).

Special studies have revealed the species of woods used, the techniques of construction, and previously unrecognized aspects of the design and function of these important objects. Four major pieces of furniture have been reconstructed on Plexiglas mounts for display in the Museum of Anatolian Civilizations, installed in custom display cases made in Turkey. Steel storage cabinets were fabricated in the United States, made

in modules, shipped to Ankara, and assembled in the Gordion storage room. The wooden objects have been placed in the drawers of the storage cabinets, set into ethafoam bedding, cut to conform to the shapes of the pieces. The effective collaboration of the conservators, scientists, archaeologists, and other specialists participating in the project has produced much new information about the craft and technology of ancient woodworking, contributed to the study of wood science, advanced the field of wood conservation, and, thankfully, insured the preservation of the artifacts.

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Paper 8-4: Deterioration and Conservation Issues Associated with Antarctica's Historic Huts. Brian W. Held[a], Robert A. Blanchette[a], Robert L. Farrell[b], Shona Duncan[b]. [a]Univ. of Minnesota, Minnesota; [b]Univ. of Waikato, Hamilton, New Zealand

During Antarctica's heroic era, explorers used Ross Island as a starting point for expeditions to the South Pole and other regions. Two huts built by Robert F. Scott in 1902 and 1911, and one built by Sir Ernest Shackleton in 1908 (Fig. 1) were used to escape the extreme Antarctic environment during their explorations. Large quantities of supplies brought to sustain the expeditions for several years were stored in and around the huts. Once the expeditions were completed, the huts and unused supplies were abandoned. Antarctica's dry, cold environment has provided some protection to the huts and artifacts, but significant deterioration has occurred over the past 9 to 10 decades and serious conservation issues must be addressed if the huts are to be successfully preserved. Biological degradation is common in the huts. A soft-rot type of decay was found to be attacking wood in contact with the ground at several locations (Fig. 2). The fungal species responsible for this decay are being characterized and studied. Actively growing fungi have been observed and isolated from clothing, leather, wood, foodstuffs and other artifacts within the huts (Fig. 3). Data loggers placed at various locations throughout the three huts have revealed that during the austral summer, temperatures rise above freezing and relative humidity within the structures is often over 80% providing conditions conducive to mold growth. The impact of visitors on relative humidity inside the huts is also being studied. Non-biological deterioration was also evident in the huts resulting in a defibration of wood surfaces. This unusual corrosive type of chemical degradation is caused by salts that affect the lignified regions of the woody cell wall resulting in cell separation. Snowmelt carrying dissolved salts is absorbed by the wood and evaporated, causing an accumulation of salts at the evaporative surface. Elemental analyses have shown that very high concentrations of salts are present in affected woods. Wind abrasion has also caused significant erosion to woods exposed to prevailing winds (Fig. 4). Wood treatments are being studied on test racks to determine their effectiveness as possible protectants for the exterior of the huts. These test racks have been erected at three different locations near each of the huts in highly exposed areas to evaluate the effectiveness (Fig. 5). Results from this work are providing important insights into how wood deteriorates in the polar environment and is creating a foundation of information for conservation plans to preserve these historic sites for future generations.

Fig. 1. Shackleton's hut at Cape Royds built in 1908.

Fig. 3. Active mold growing on a historic crate inside Cape Evans hut.

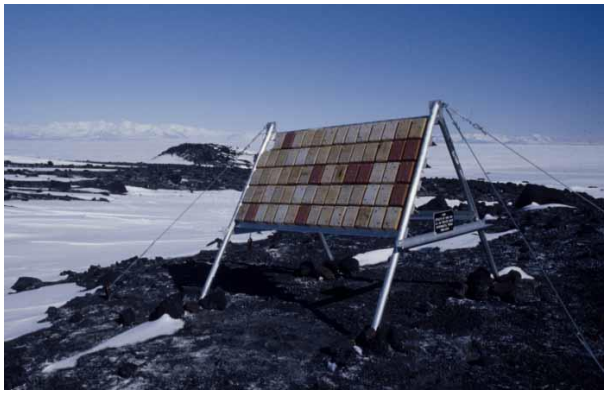

Fig. 5. One of three test racks to evaluate various wood treatments for potential use in protecting exterior woods of the historic huts.

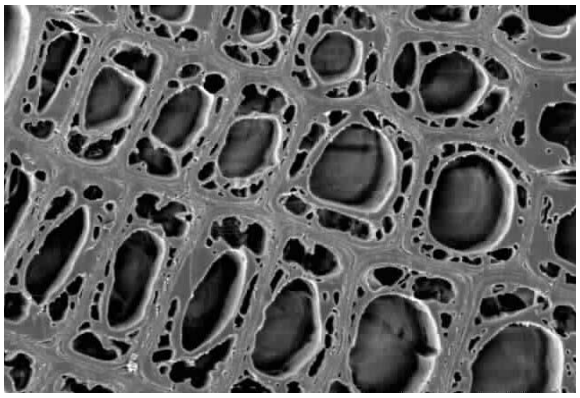

Fig. 2. Scanning electron micrograph showing a transverse section of spruce (*Picea sp*.) with soft rot cavities from Cape Royds hut.

Fig. 4. Corner post of Discovery Hut exhibiting extensive wind erosion. Earlywood cells are being eroded at a faster rate than latewood cells.

Paper 8-5: Evaluating the Wooden Remnants of the Tektas Burnu Shipwreck. Joel A. Jurgens[a], Robert A. Blanchette[a] and Deborah N. Carlson[b]. [a]University of Minnesota, St. Paul, Minnesota, [b]Institute of Nautical Archaeology, College Station, Texas

A small vessel thought to be a Greek merchant ship sank to the floor of the Aegean Sea near Tektas Burnu, Turkey in 440-425 BC. Scattered among the many remaining artifacts were a few small remnants of the ships hull and other wooden objects. These remains were investigated to determine the type of wood used in the construction of the ship and to obtain information on the degradation processes that have occurred in the wood over the past several millennia. Seven small segments of wood were all that remained of the ship, and these woods have been identified as *Pinus* (3 samples), *Quercus* (2 samples), *Ulmus* (1 sample) and one very small sample remains unidentified. Micromorphological studies indicated the woods were severely degraded and lacked structural integrity. In all samples examined, the secondary wall layers of the wood cells were extensively degraded with minute holes and tunnels present among disrupted cell wall material. The middle lamella between the cells remained and was not eroded. The deterioration in the secondary wall layers has characteristics indicating that bacterial degradation was the major type of microbial decay. The advanced stage of decay and changes that have occurred from environmental influences and secondary scavenging bacteria makes it difficult to determine what type of bacterial attack had taken place. Erosion and tunneling bacteria as well as secondary scavenging bacteria most likely were involved with the deterioration observed. Decay by erosion bacteria is characterized by a depletion of the cellulose and hemicelluloses in the secondary wall, leaving only the lignin-rich framework of the middle lamella. Tunneling bacteria attack the secondary wall producing small tunnels and may penetrate and degrade the middle lamella to a limited extent. In sediment covered, waterlogged woods where oxygen is limited both erosion and tunneling forms of bacterial degradation may be found. Some of the ship wood samples were infiltrated with copper and other metal corrosion products that may have helped to inhibit microbial degradation and aid in their preservation. Identification of the wood species found in the shipwreck has provided information on the types of woods used for building this ship and the characterization of decay has documented the current condition of the woods and elucidated the type of microbial degradation occurring in this waterlogged environment.

Figure 1. Diver and submersible excavating the Tektas Burnu shipwreck.

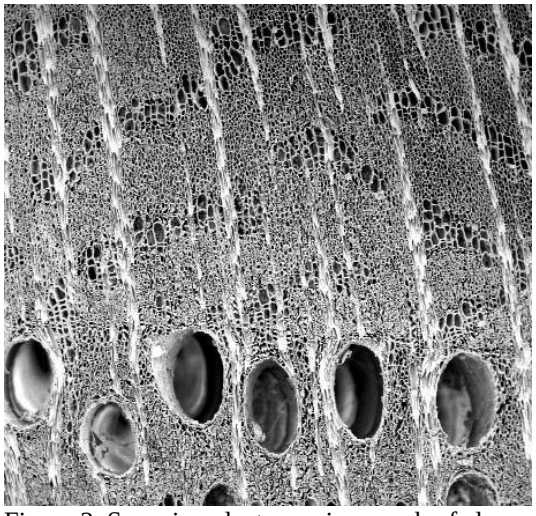

Figure 2. Scanning electron micrograph of elm (*Ulmus sp*.) recovered from the shipwreck.

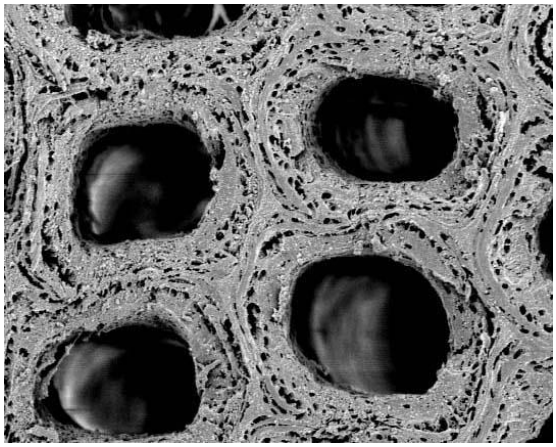

Figure 3. Transverse section of pine (*Pinus sp*.) showing small holes indicative of bacterial degradation.

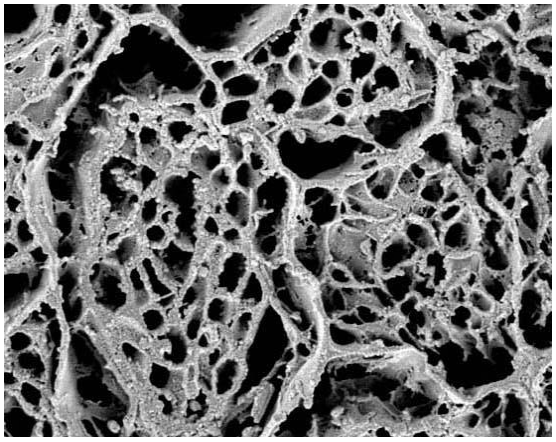

Figure 4. Late stages of bacterial decay in oak (*Quercus sp*.) with extensively degraded secondary walls and relatively intact middle lamella.

Poster Session:

P-1: Biodeterioration of coatings for the protection of outdoor bronze monuments. C. J. McNamara, M. C. Helms, M. Breuker*, and R. Mitchell**.** Harvard University, Cambridge, Massachusetts, *National Parks Service, Lowell, Massachusetts.

Introduction

Outdoor monuments are very susceptible to corrosion, and organic coatings are widely used for protection of the metals (Leidheiser, 1986). A wide range of polymers are used for this purpose, including lacquers, waxes, and resins. Natural and synthetic waxes, which are utilized on bronze monuments, produce a barrier that excludes moisture and oxygen from the metal surface (Moffett, 1996), and are often used in conjunction with acrylic coatings as a further protective layer (van Zelst and Lachevre, 1983).

Most coatings are highly susceptible to microbial attack (Campbell, 1995). Microorganisms capable of forming biofilms and causing deterioration include bacteria, fungi, and algae. Biofilms degrade protective coatings through acid and enzyme production (Mitton et al., 1993; Gu et al., 1998). This activity frequently leads to failure in the form of blisters or cracks, delamination, and changes in porosity, ultimately resulting in the deterioration of the underlying metal (Mitchell and Gu, 1996).

In the current study, the susceptibility to biodeterioration of Incralac (StanChem Inc., East Berlin, CT), a coating commonly to protect outdoor monuments, was investigated. Microorganisms capable of degrading the coating were isolated using enrichment cultures and breakdown of the coating was analyzed using electrochemical impedance spectroscopy (EIS).

Methods

Microorganisms were collected by swab sampling in April 2001 from the George Washington Monument, New York City. Samples were inoculated into sterile minimal salt medium (l^{-1}: 0.22 g $(NH_4)_2SO_4$, 1.20 g KH_2PO_4, 0.23 g $MgSO_4 \cdot 7H_2O$, 0.25 g $CaCl_2$, 0.024 g yeast extract) containing cured pieces of Incralac. These enrichment cultures selected for microorganisms capable of using the coating as the sole source of carbon and energy. After 10 days, media from the enrichment cultures was plated on nutrient agar and isolates were collected.

To insure that isolates were capable of growth on Incralac and not simply dormant organisms revived on nutrient agar, the isolates were re-inoculated into flasks of minimal salt medium with cured pieces of Incralac. For those isolates capable of growth (determined visually by turbidity of cultures), the Gram Reaction and morphology of isolates was determined.

Biodeterioration of Incralac was analyzed using EIS. In order to obtain a thin coating, Incralac was mixed to a final concentration of 10% with toluene and then applied with a brush to one side of a 316 stainless steel coupon (5.0 cm x 5.0 cm). The Incralac was cured overnight at room temperature. A 5.0 cm long acrylic tube (3.2 cm I.D., 3.8 cm O.D.) was then adhered to the coated coupon using Amercoat (Ameron International, Alpharetta, GA) 90HS resin and cure in a ratio of 4:1. The Amercoat adhesive was dried overnight at room temperature and cured at 37ºC for 72-96 hrs. Two cells were

constructed and were surface sterilized using UV irradiation in a laminar flow hood. Cells were filled with minimal salt medium. One cell was inoculated with an isolate obtained through the methods described above, and the second was maintained as a sterile control.

The EIS analytic system consisted of a Schlumberger 1250 frequency response analyzer with a Schlumberger 1286 electrochemical interface (now manufactured by Solartron Analytical, Houston, TX). Z-Plot (Scribner Associates Inc., Southern Pines, NC) was used to control the instruments and analyze the data. EIS cells were held at their open circuit potential and a sinusoidal perturbation of 20 mV was applied. The impedance response was measured over a range of frequencies from 65 KHz to 1 mHz. A trielectrode system was used in this study; a saturated calomel reference electrode, platinum mesh counter electrode, and the stainless steel coupon as the working electrode. All measurements were made in a laminar flow hood to prevent contamination of the cells.

Results and Conclusions

Five isolates capable of growth using Incralac as a sole carbon and energy source were isolated. Three of the isolates were identified as yeasts and the remaining two isolates were unidentified bacteria. One of the yeast strains, designated GWM1, appeared to grow substantially faster than the other isolates and was used in the EIS experiments.

EIS is one of the least destructive and most informative techniques for the electrochemical analysis of polymeric coatings on metal surfaces (Mansfeld and Tsai, 1991). This technique has been used to microbial deterioration of other polymers, such as polyimides (Gu et al., 1996), polyurethanes (Mitchell et al., 1996), and fiber-reinforced composites (Gu et al., 1997).

The impedance response of Incralac at the initiation of the experiment is shown in Figure 1A. After 15 days, impedance values for the inoculated cell had decreased, particularly in the low frequency range, while the sterile cell had not changed (Fig. 1B). It is likely that deterioration of the Incralac by GWM1 resulted in increased penetration of water and ions into the polymer matrix, thereby decreasing the impedance response of the inoculated cell.

Incralac was developed by the International Copper Research Association (INCRA) and is composed of the acrylic resin Paraloid B-44 and benzotriazole dissolved in toluene. Biodeterioration of acrylic polymers is fairly common (e.g., Hayashi et al., 1994, Kawai et al., 1995). Our results indicate that biodeterioration of Incralac has the potential to occur rapidly, leading to corrosion damage to the underlying monument.

In addition to Incralac, we are currently analyzing the biodeterioration of beeswax and carnuba wax, which are frequently used in conjunction with Incralac (van Zelst and Lachevre, 1983). Microbial utilization of large polymers requires that the polymer first be broken into smaller units (i.e., oligomers, dimers or monomers), which often occurs enzymatically. Therefore, we have also begun monitoring exo-esterase activity in conjunction with EIS.

Figures

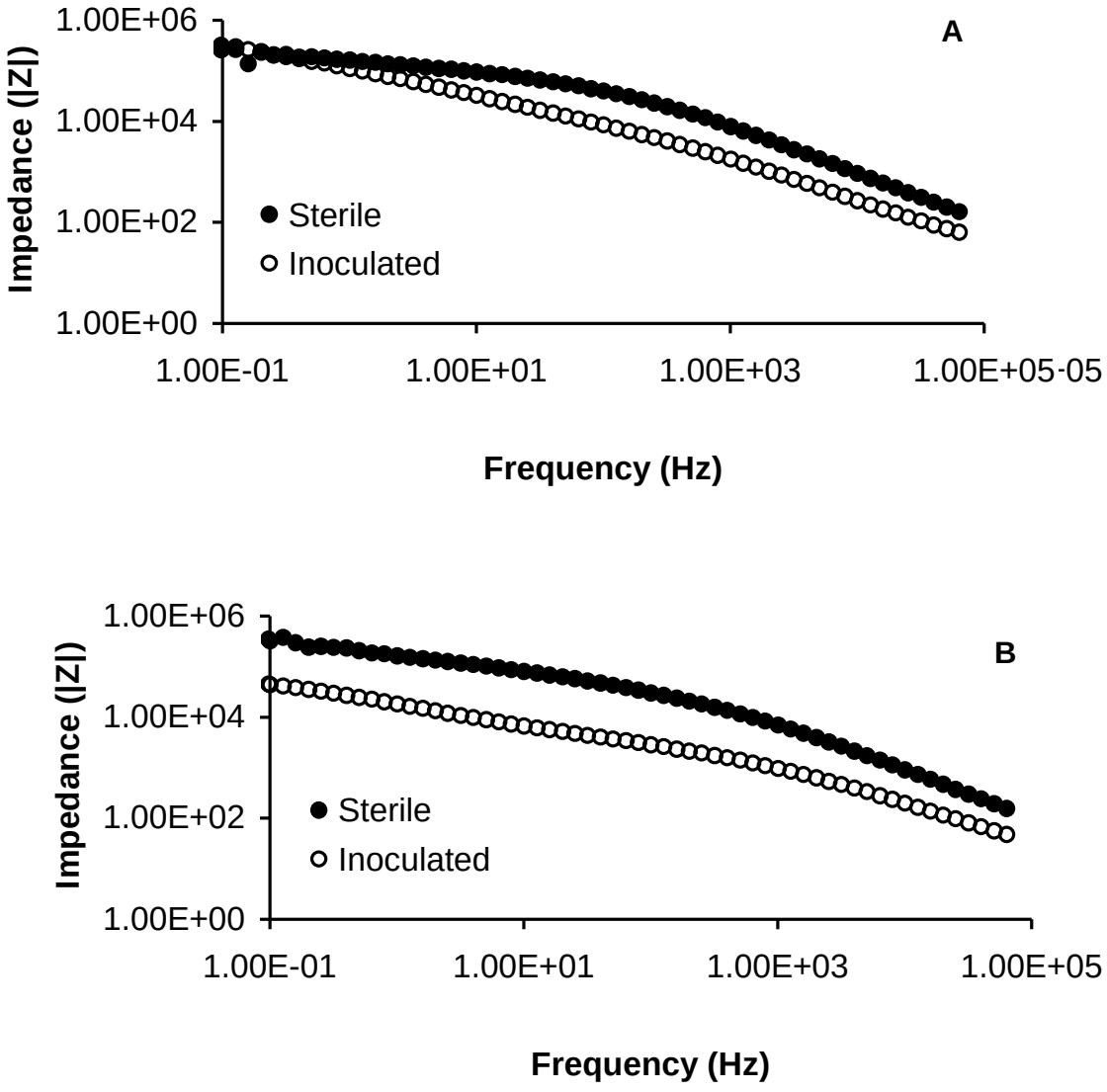

Fig. 1. Bode magnitude plots of Incralac (A) at initiation of the experiment and (B) after 15 days.

Acknowledgements

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P-2: Wood Biodeterioration in Historical Buildings: Biological Sanity and Structural Analyses. Sérgio Brazolin, Maria Beatriz B. Monteiro, and Takashi Yojo. Gonzalo A. C. Lopez Instituto de Pesquisas Tecnológicas do Estado de São Paulo S.A. Brazil

This work presents the methodology developed by the of Forest Products Division of IPT for the evaluation of wood structures in historical buildings, deteriorated by biological agents – decaying fungi, termites and wood bore beetles.

The structural analysis takes into consideration the biology of the organisms and the biological sanity of each wood component to evaluate the safety condition of the whole structure.

The curative and preventive measures are based on the different biodeterioration degrees and service life expectations of the components in the structure, the wood species used and the preservative methods and products existing in Brazil to control these organisms.

A case study is presented where the biodeterioration was caused by wood decaying fungi and subterranean termites. Environmentally safe products and processes were proposed to control these organisms.

P-3: CATS: Interactions and Diversity of Microorganisms Isolated from the Roman Catacombs of St. Callistus and Domitilla, Rome, Italy

Urzì, C., De Leo, F., Donato, P., La Cono, V. Univ. of Messina, Italy

P-4: Hunley Submarine, Charleston Harbor, and 1864 textiles. Paul Mardikian and Mary Ballard, SCRME, Washington, D.C.

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