

# Application of Biology to Cultural Heritage

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This Special Issue of the *Applied Sciences*, entitled “Application of Biology to Cultural Heritage” aimed to cover all the latest outstanding progress of biological and biochemical methods developed and applied to cultural heritage. As you can see cultural heritage biodiversity and biodeterioration has received much research attention in recent years. This Special Issue intended to provide a comprehensive examination of the science of biology in various fields and areas, and its practical application for the preservation of cultural heritage. Full research articles and reviews on all aspects of biological causes, modes of action, biocidal treatment, protection, and prevention of cultural heritage, are here presented as well as long term studies on the biodeterioration of cultural heritage sites and monuments. Analyses and testing of macro- and microorganisms affecting the preservation of cultural heritage were also addressed.

The knowledge that has arisen from the published papers about the studies on new techniques and new products applied to the cultural heritage area may now be translated into new conservation and restoration treatments in similar objects, sites and supports. This was our main goal and was achieved in a mission that we carried out with great pleasure!

This issue addresses researchers from both academia and industry, working in microbiology and biotechnology. We hope that you enjoy and find it useful in your current and future work, research projects and careers.

In this Special Issue eleven excellent papers (including review and full research articles) were published and all went through a hard and demanding reviewing work to assure maximum quality. In the following paragraphs, a summary of these 11 papers, with their most relevant contributions, is presented.

In the paper by Klügl and Di Pietro [1] an atlas of micromorphological degradation of archaeological birch bark, dating from the Neolithic to the Middle Age, was developed in order to describe the decay of archaeological birch bark. The characteristics of contemporary non-degraded mature birch phellem was first investigated and compared with the characteristics of contemporary decayed in-nature phellem. The morphology of 13 samples from ice-logged, waterlogged and in-cave recovered archaeological birch barks was then investigated, using light and electron transmission microscopy and, the authors made, for the first time, a comprehensive description of microscopic degradation features of birch bark and correlated those with macroscopic visible features. These results can be very useful for conservators working with archaeological birch bark.

The next three studies are related to stone biodeterioration and possible treatments.

Bartoli et al. [2] present a very interesting paper on the efficiency of biocides on stone cultural heritage. The authors describe and investigate the biocide effects of two different



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biocides encapsulated in two different silica nanosystems. The *in vitro* experiment was performed using *Chlorococcum* sp., a green algae commonly found in biodeteriorated stone. The two biocides selected were: Zosteric sodium salt (ZS), a green biocide, and the commercial biocide, 2-mercaptobenzothiazole (MBT), widely used in the treatment of cultural heritage. The analyzed systems were the following: silica nanocapsules (NC) and silica nanoparticles (MNP) not loaded with biocides, two nanosystems loaded with ZS and MBT, and free biocides. The results confirmed the interest of the silica nanosystems coupled with biocides as a potential protective coating for stone monuments. Nanoparticles, synthesized for the formulation of a multifunctional coating, proved better than nanocontainers as being promising tools in the conservation of stone cultural heritage.

The paper by Santo et al. [3] describes a multidisciplinary study on the state of conservation of white marbles from the Florence Cathedral and the microbial community involved in their deterioration. This remarkable study is focused on the widespread dark discoloration of marble analyzed in two differently exposed sites of the Cathedral: NW and SE. The authors used chemical and petrographic analyses, *in situ* and *ex situ* microscopy, and cultivation and identification of microorganisms. According to the obtained data the darkening is mainly due to the growth of black fungi and dark cyanobacteria at both the study sites. The biodiversity of the lithobiotic community seems to be similar in the investigated areas, especially that of the photoautotrophic components. On the contrary, the pattern of microbial colonization on/inside marble seems to be different at the NW and SE sites depending on the distinct climatic conditions of the two study areas, in particular on solar radiation exposure, which influences marble's bioreceptivity. This work presents the first report on the lithobiotic community inhabiting the Florence Cathedral marbles. These results are very important for future interventions to control microbial growth.

In the Roman wall of Lugo (NW Spain), declared a World Heritage site by UNESCO in 2000, three separate treatments were applied, in the past 20 years. In this interesting work, Prieto et al. [4] used a combined laboratory and field research to examine the possible alterations caused by herbicide treatments applied to the construction materials of the Roman Wall (schist and some granite, bound with mortar). Their results showed that herbicidal treatments should focus on environmentally friendly technologies with low risk to human health and should not damage the construction materials. In this respect, *Origanum vulgare* L. and *Thymus vulgaris* L. essential oils seem to be the appropriate, as they did not provoke any mineralogical alterations or changes in the salt content and original colour.

Nanhai No. 1, a Chinese Song Dynasty shipwreck, was discovered in 1987 near the Chuanshan archipelago in the South China Sea, Guangdong Province. In 2007, Nanhai No. 1 was salvaged and displayed in "the Crystal Palace" of the Guangdong Maritime Silk Road Museum. Due to the high humidity and high temperature in the storage environment of Nanhai No. 1, and the fact that wooden cultural relics are natural organic matter, this provides suitable conditions for the growth and reproduction of microorganisms. Microorganisms are very harmful to wooden relics. Some bacteria and fungi can destroy wood by degrading cellulose, hemicellulose and lignin. In this fascinating paper by Huang et al. [5], the authors report the inhibitory effect of cinnamaldehyde on the main destructive microorganisms of Nanhai No. 1 Shipwreck. The results showed the inhibitory effects of cinnamaldehyde, a natural biocide that proved to be effective as a control agent for many of the main destructive microorganisms identified in the Nanhai No. 1 Shipwreck. This is an important step towards the development of safer products – both for the environment and the safety of the professionals responsible for the application of such products.

In this newsworthy work from Fuentes, Carballeira and Prieto [6] the granite biodiversity is analysed. The microorganisms' diversity present in historical rural granite buildings was identified for the first time. This granite has been used throughout history in Galicia (NW Spain), forming the basis of much of the region's architecture. For the identification of the granite biofilms the authors used a combination of culture-dependent and next generation sequencing (NGS) techniques. For this work three granite-built churches located

in rural areas of Galicia, in the northwestern part of the Iberian Peninsula, were selected. Since the orientation of buildings is one of the factors that most strongly influences the development and composition of subaerial biofilms, as it determines the amount of light and the availability of water on the substrate, the role of exposure was also considered by comparing the biodiversity on north and west walls of the three churches. The authors also compared their results with previous work, made on biodiversity on historical granite buildings in urban settings. The results proved that orientation is an important factor regarding both the diversity and abundance of microorganisms on the walls, with environmental factors associated with the north orientation factors favouring a higher diversity.

This excellent paper, from Dyda et al. [7], also addresses the stone biodiversity topic. In this case the biodiversity of different biocenoses on the historical sandstone of the Northern Pergola in the Museum of King John III's Palace at Wilanow (Poland) are analysed. The authors aimed at describing seasonal changes of microbial community composition in situ in different biocenoses: winter and summer, of the same historical sandstone. Another objective of this work was to describe the seasonal changes of microbial community composition in situ in relation to biodeterioration patterns or with anthropogenic factors, e.g., air pollution. The microbial biodiversity was analyzed by the application of Illumina-based next-generation sequencing methods. The metabarcoding analysis on fungi, bacteria and lichenized fungi are presented and compared between winter and summer. The authors found that the diversity of organisms in the biofilm ensures its stability throughout the year despite the differences recorded between winter and summer. On the other hand, analyses of bacterial and fungal communities' biodiversity in biocenoses from historic sandstone Pergolas revealed that the observed changes were mostly associated with the biodeterioration patterns.

This Special Issue also presents four very excellent reviews.

In this very interesting work by Macedo, Vilarigues and Coutinho [8], the authors review the 21st century literature (2000 to 2021) regarding the biodiversity and biodeterioration of glass-based historical building materials, particularly stained glass and glazed tiles. These authors study the provenance of the stained glass and glazed tiles with biological colonization, presented in the 21st century literature. They also compile a list of fungi, bacteria and phototrophic microorganisms reported on glass-based historical building materials and make a critical overview regarding the organism's biodiversity and the colonized materials. They also compare the biodeterioration patterns from case studies with laboratory-based colonization experiments, showing that many deterioration patterns and corrosion products are similar. Finally, the authors make suggestions regarding further studies that are urgent to do in order to fully understand the biodiversity and biodeterioration of stained glass and glazed tiles.

In addition, in this special issue, there is an excellent review by Ortega-Morales and Gaylarde [9] dealing with bioconservation of historic stone buildings. In this review, the authors seek to provide updated information on the innovative bioconservation treatments that have been or are being developed. According to the authors there is an urgent need to replace traditional conservation treatments by more environmentally acceptable, biologically-based, measures, including bioconsolidation and biocleaning. Bioconsolidation can use whole bacterial cells or cell biomolecules while biocleaning can employ microorganisms or their extracted enzymes to remove inorganic and organic surface deposits such as sulphate crusts, animal glues, biofilms and felt tip marker graffiti. The great advantage of bioremediation based on microbial cells or their products, compared with traditional chemical, physical and mechanical methods, is that biological treatments are not destructive of the underlying substrate, simply removing unwanted overlying materials, in the case of biocleaning, or producing new stone, in the case of biocalcification.

The interesting review paper by Trovão and Portugal [10] attempts to provide an overview of biodeteriorative profiles obtained by amended plate essays, while also providing a summary of currently known fungal species putative biodeteriorative abilities. Both the essays available and the results concerning distinct fungal species biodeteriorative

profiles obtained by amended plate essays, remain scattered and in need of a deep summarization. In this way, the authors also provide a series of checklists that can be helpful to microbiologists, restorers and conservation workers when attempting to safeguard cultural heritage materials worldwide from biodeterioration. This review highlighted that, so far, isolates from more than 200 fungal species have been showed to exhibit biodeteriorative abilities when studied by specific plate essays. This review can be very interesting also for the broad target public from researchers to end users.

Finally, an excellent review by Passaretti et al. [11] on metal heritage cleaning by biologically derived gel formulations, is presented. The authors review biologically derived gel formulations already proposed for artistic and historical metal as reliable tools for cleaning. An overview on the common practices for cleaning metallic surfaces in cultural heritage is presented. The potentialities of gel-alternatives and in particular those with a biological origin are discussed. In this review water-gels (i.e., hydrogels) and solvent-gels (i.e., organogels) together with particular attention to bio-solvents, are discussed in detail. Nevertheless, most of metals conservators rarely use this bio-gels. This could be due to the relatively high price, the unawareness of either risk related to traditional methods or existence of reliable alternatives, and the effort required to change long-established habits in favour of unprecedented methodologies.

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