

REVIEW

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# Rainbow code of biodeterioration to cultural heritage objects



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## Abstract

The vast majority of cultural heritage objects consist of materials that can be subjected to biodeterioration. Currently, there is significant number of publications showing which materials are capable of destroying certain organisms, and what conservation and restoration procedures are required. However, there is neither a clear classification of these diverse events nor their visual representation. In our review, for the first time, an attempt is made to compare the type of biodeterioration, based on the destruction of a particular material, with a specific color of the rainbow. In this regard, a cultural heritage objects made of a single material are designated as one color icon; and those made of composite materials are designated as pictogram consisting of several icons of corresponding colors. For example, a stone sculpture, in accordance with the rainbow code, is assigned a gray color, which was introduced to visualize stone materials. The drum corresponds to a pictogram consisting of violet (corresponds to leather) and brown (corresponds to wood). A work of easel painting on canvas corresponds to a pictogram consisting of a red color icon (corresponds to canvas) and a gold color icon (corresponds to painting materials). We used cold color shades to denote basic inorganic materials, and cold color shades to denote organic materials. The proposed rainbow code for biodeterioration is an open platform that can be expanded by adding new colors for new materials introduced, and allows to translate potentially any cultural heritage object into a pictogram with colors that correspond to the materials used in its manufacture. Such a graphical interpretation can help both systematize the storage conditions of museum exhibits and facilitate understanding of the processes of biodeterioration of composite materials.

**Keywords** Rainbow code of biodeterioration, Objects of cultural heritage, Bacteria, Fungi, Monumental art, Easel art, Restoration

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## Graphical Abstract



## Introduction

Microorganisms, along with abiotic environmental factors, are the most important factors in the destruction of cultural heritage. This is due to the fact that the various materials that make up the artwork art can be exposed to specific microbial destructors, causing structural and aesthetic damage due to their specific metabolic activities [1]. Biodeterioration can occur with almost any art object, from paper manuscripts [2, 3], tempera or oil art materials [4–6], textiles [7] to stone statues, rock or wall paintings, or objects from archaeological excavations [8–11]. If we go deeper into the origins and quote Hueck: “Between organisms and materials, i.e. the substance or matter of which anything is made or to be made, exist many relations”, we understand that these relations can cause “undesirable change in the properties of a material caused by vital activities of organisms” which is the definition of the word biodeterioration [12]. The material can be subject to damage due to various processes. This may be mechanical damage from rodents or insects, or action of microorganisms using the material as food or destroying it by excretion products, etc. This work proposes a color structuring of biodeterioration processes of different materials by microorganisms to help restoration specialists. The practice of conservation and restoration involves both ethical and professional aspects and is based on the manifesto of the Venice

Charter of Restorers, adopted in 1964. The manifesto is based on three ideas: restoration of the historical object in its original form; maintaining the object as intact as possible; identification and coordination of the historical and artistic value of the object [13, 14]. That is, when determining the goals of the restoration impact, it is necessary to find a balance between the historical and artistic components and carry out restoration work on the object with minimal restoration intervention [15]. If biodestruction is detected in a cultural heritage site, a comprehensive scientific approach is required, including three levels of microbiological research [16]: (i) culture-independent or culture-dependent metaomics analyses of whole microbial community (metagenomics [17–19] and metatranscriptomics, metabolomics, metaproteomics (MALDI-ToF, ESI-Q-ToF, XRC); (ii) culture-dependent analysis of isolates (taxonomic classification based on barcodes amplicons, micro and macromorphology, etc.), functional tests (for content of primary and secondary metabolites, enzymes, lipids, polysaccharides, etc.) [20, 21], tests for metabolic activity under various conditions (temperature, pH etc.); (iii) physico-chemical analysis of degraded/renovated material (AFM, GC-MS, SEM, TEM, ATR-FTIR, XRD, XRF, etc.) [2, 22–26]. An integrated approach to the analysis of the research object makes it possible to compile its individual passport, identifying the historical causes of biodeterioration. In this

review made an attempt to classify the different types of biodeterioration occurring to objects of cultural heritage based on the types of materials underlying them. For example, artistic inlaid parquet consists of wood (often of various species). A work of easel painting on canvas consists of fabric (canvas) and applied painting materials. For a more visual perception, we introduced a rainbow code to various types of biodestruction, correlating the material with one or another color of the rainbow. We also divided classified materials into inorganic and organic, assigning inorganic materials with cold color shades and organic materials with warm color shades. The introduction of the rainbow code of biodeterioration opens up the possibility of representing potentially any object of cultural heritage in the form of a formalized pictogram consisting of colors corresponding to the materials used in its creation. The rainbow code of biodeterioration is an open platform that allows to add new colors to introduce new types of materials. It can potentially be used both for cataloging museum exhibits in order to regulate storage conditions, and for understanding the processes of biodeterioration of composite materials.

### Introduction of rainbow code of biodeterioration to cultural heritage objects

Various microorganisms are capable of effectively degrading certain materials, which allows them to inhabit a wide variety of ecological niches [27]. In many cases, the effective destruction of cultural heritage objects occurs due to a consortium of microorganisms specializing in this, which may include representatives of bacteria and fungi, mosses, lichens and algae [24, 28, 29]. Although the same microorganisms are capable of biodegradation of various materials, destruction is usually caused by the work of several types of microorganisms, including those that form biofilms [16]. The first colonizers are bacteria, usually with low nutritional requirements, but later, due to the accumulation of organic components of artistic and restoration materials, fungi become the main colonizers, causing phenomena of physical and chemical destruction [30].

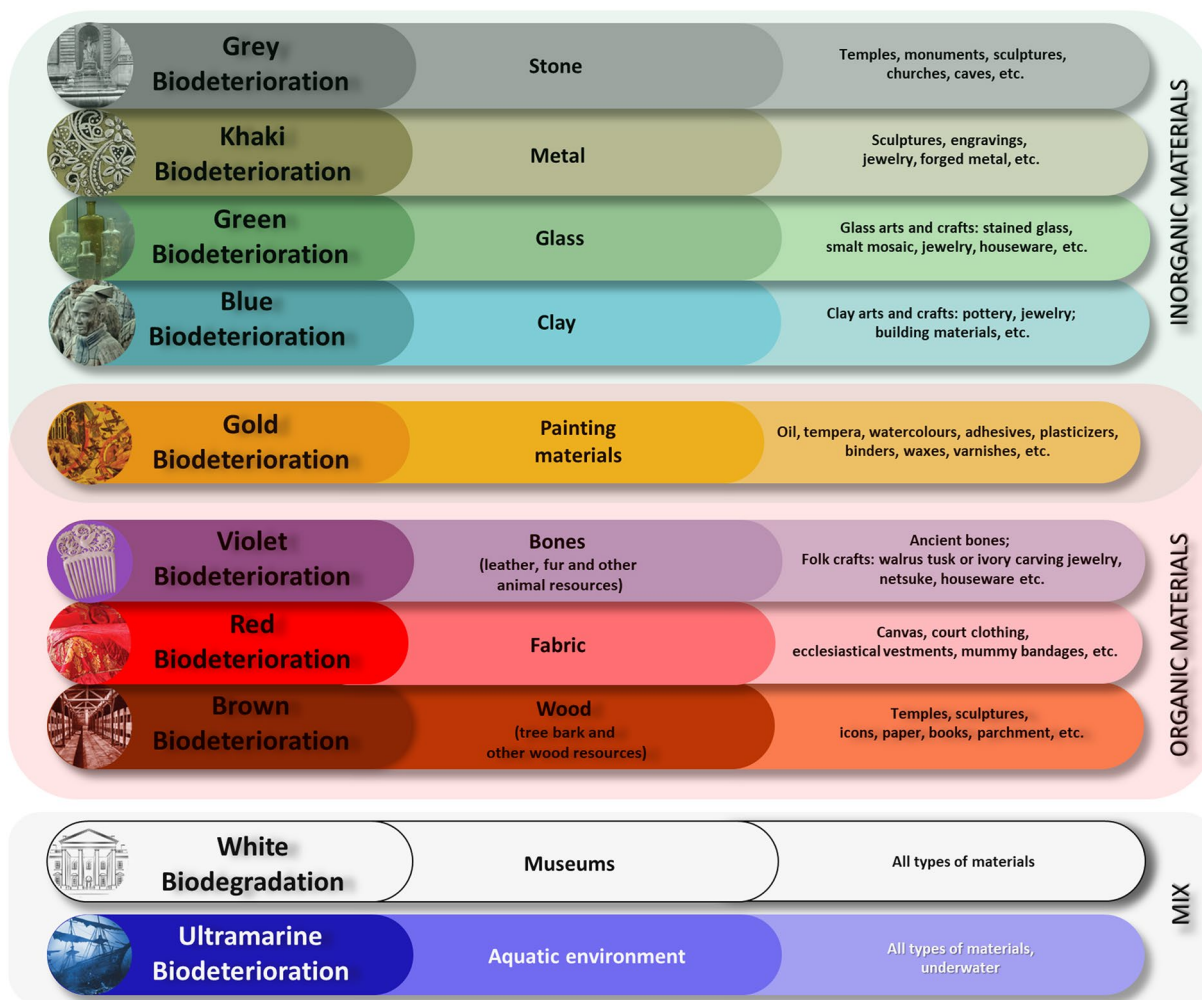
In some cases, bacterial and fungal communities are extremely dangerous for works of art, since they are adapted to biodeterioration due to extracellular enzymes of fungi, such as esterases, lipases and proteases. Lipases and esterases catalyze the hydrolytic cleavage of esters [31]. Important enzymes that support the deterioration process include endo-*N*-acetyl-*P*-*D*-glucoamidases (ENGases), which hydrolyze the glycosidic bonds of *N*-acetyl-*P*-*D*-glucosamine and convert oligosaccharides into monosaccharides. There are three types of ENGases: ENGase I—cleaves murein, the main framework substance of the bacterial cell wall, ENGase II—cleaves

chitin, the main component of the fungal cell wall, and ENGase III—cleaves *N*-glycans [32]. ENGase I and ENGase II do not directly destroy the pigment paint layer, however, they use bacterial and fungal cells as a substrate for the further growth and development of the fungi themselves.

It has been shown that several bacteria belonging to genera *Staphylococcus*, *Bacillus*, *Acinetobacter*, *Agrococcus*, *Janibacter*, *Rhodococcus*, and *Stenotrophomonas* exhibited endocellulolytic activity, implying their potential involvement in degradation of canvas, probably in synergy with fungi [33]. The ability to form biofilms has been demonstrated for the most important representatives of the genera *Candida*, *Aspergillus*, *Cryptococcus*, *Trichosporon*, *Coccidioides*, *Pneumocystis* [34, 35]. Biofilms have been found in cultural heritage sites, such as churches built in grottoes in the Campania region (Italy) [24], on the walls with polychromy degradation of mural paintings of the Senhora de Saude fortification (Spain) [36]. As the example of infection of some historic and artistic objects in the city of Lisbon it was shown that textiles, sculptures and paintings are infected mainly by *Aspergillus*, *Cladosporium* and *Penicillium* as well as by bacterial strains [37]. And it was noticed that the great bacterial contamination is related to greater fungal contamination. Some fungal strains [20, 38] as well as bacterial strains [10] are able for pigmenting the art works producing a rainbow of colors.

The examples listed above are just the tip of the iceberg of current knowledge about biodeterioration of cultural heritage sites, and this knowledge is growing rapidly in connection with emerging new technologies [39]. In this regard, we have made an attempt to systematize knowledge about biodeterioration of cultural heritage objects, introducing the so-called “rainbow code of biodeterioration” (Fig. 1).

We made this attempt because a similar attempt to systematize knowledge in the field of biotechnology, undertaken in 2004, was crowned with significant success [40]. The so-called rainbow code of biotechnology is used in numerous classifiers to distinguish between industries in this field of activity. The classification we introduced can also have a practical application, since it visualizes the composition of materials of various objects, which can facilitate both their cataloging and storage conditions. In our classification, we assigned cold color shades to inorganic materials such as stone, iron, glass, clay, and we assigned warm color shades to organic materials based on plant and animal origin (Fig. 1). This allows for more efficient visual “sorting” of cultural heritage objects, which is important since there are a number of fundamental differences between inorganic and organic materials, such as possible elemental composition,



**Fig. 1** Rainbow code of biodeterioration to cultural heritage objects. Inorganic materials are assigned with cold color shades; organic materials are assigned with warm color shades. Painting materials (golden color) are on the border since they can contain both inorganic and organic materials. A museum can have exhibits made from any type of material; so it is designated by the color white, according to the white light, which can be created by mixing all the lights of the rainbow. Objects of cultural heritage located under water are subject to special effects, so another additional color is allocated for them, ultramarine, which denotes not the type of material, but a specific storage environment, as in the case of a museum

hygroscopicity, flammability and others. In the current classification, we have separately placed the so-called painting materials (and assigned the gold color to these materials) because they are extremely important for the creation of numerous cultural heritage objects. However, this group of materials is extremely heterogeneous in its composition, depending on the era and direction of painting, it can include both organic materials (binders, plasticizers, varnishes, organic pigments) and inorganic materials (for example, inorganic pigments). We did not split this type of material into organic painting materials and inorganic painting materials, but placed it at the intersection between organic and inorganic materials (Fig. 1). We understand that this breaks the symmetry of

the rainbow code, but we are forced to make this important exception for painting materials (placing them in the border region), because there are numerous examples of complex paints that have an organic base and inorganic pigment. For example, the red paint Cinnabar, often used in ancient icon painting, has an organic base, an emulsion based on egg yolk, and an inorganic pigment, a mercury salt. In addition, we assigned a separate color to museum storage, where exhibits made of any type of material could potentially be found. Therefore, in the most general terms, the color for the museum will be white, since it is formed by adding all the colors of the rainbow. The justification for introducing a special color for the museum, as well as for cultural heritage objects located under

water (ultramarine color), as well as the detailed application of the rainbow code to simple and composite materials, is discussed in the next section.

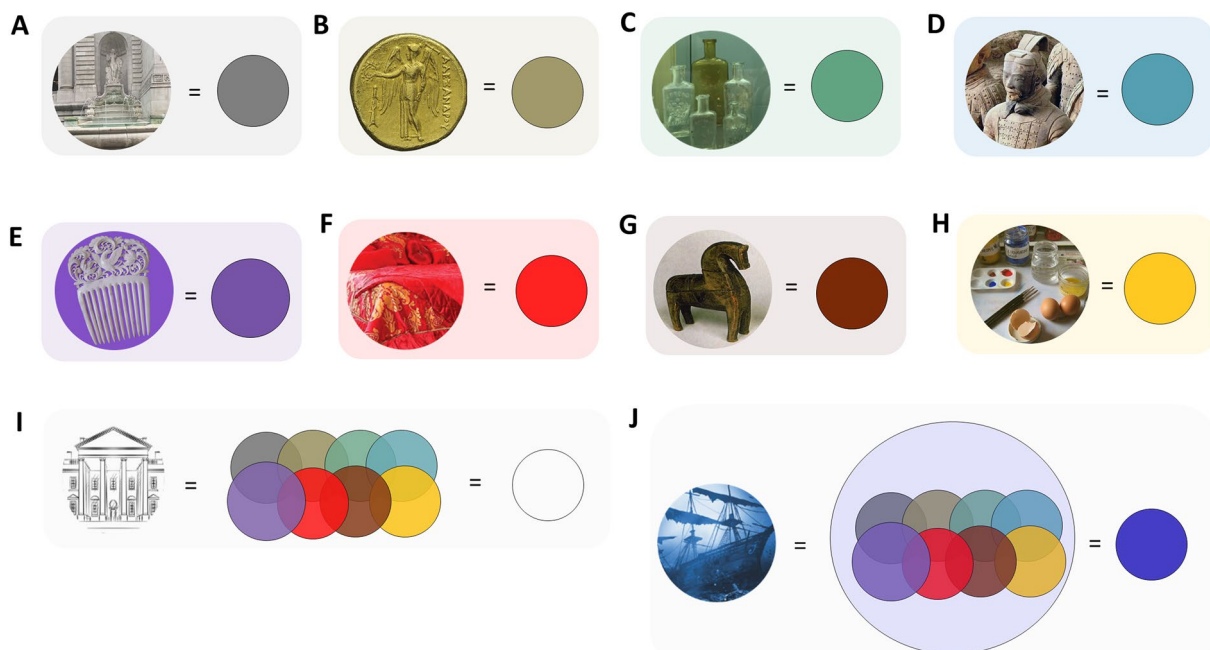
### Application of the rainbow code of biodeterioration to create pictograms for various cultural heritage objects made from single or composite materials

In order to understand how the proposed rainbow code of biodetermination works in practice, at the first stage it is necessary to consider objects for the creation of which a single type of material was used in accordance with their previously introduced classification (Figs. 1, 2).

Thus, in this example, the statue is made of stone and is therefore susceptible to biodeterioration of the gray color that corresponds to this type of material (Fig. 2A). Therefore, next to the stone statue there is a pictogram consisting of a single circle filled in gray. This is the rainbow code for possible biodeterioration for this object. The coin is made of metal, so next to it there is a pictogram represented by a single circle, shaded in khaki color, which, in accordance with Fig. 1, is attributed to metals (Fig. 2B). A pictogram with a green circle is placed next to the glassware (Fig. 2C), a pictogram with a blue circle is placed next to the terracotta warrior (Fig. 2D), a

pictogram with a violet circle is placed next to the bone comb (Fig. 2E), and a pictogram with a red circle is placed next to the brocade (Fig. 2F), next to the figurine of a wooden horse there is a pictogram with a brown circle (Fig. 2G), next to the tempera paints there is a pictogram with a gold circle (Fig. 2H). Next to the image of the museum there is a pictogram with all the above circles (Fig. 2I). Moreover, circles painted in colors of cool shades, indicating inorganic materials, are placed in the top row, and circles painted in colors of warm shades, to indicate organic and painting materials, are placed in the bottom row. The equals sign between the pictograms shows that the superposition of all these individual colors can be replaced by a single icon in white (according to the white light, which can be created by mixing all the lights of the rainbow). Behind the icon symbolizing sunken cultural heritage sites (a sunken ship), there is a pictogram with all the above circles, which are placed in one large circle, shaded in ultramarine color (Fig. 2J). The equals sign between the pictograms shows that the superposition of all these small circles and a single large circle according to the classification being entered can be replaced by a single icon in ultramarine (Fig. 2J).

Cultural heritage objects in many cases may consist of several types of materials. And each type of material



**Fig. 2** Examples of pictograms based on the Rainbow Code of Biodeterioration for cultural heritage objects consisting of a single color. **A**—stone; **B**—metal; **C**—glass; **D**—clay; **E**—Bones and other materials of animal origin; **F**—Fabric; **G**—Wood and other materials of plant origin; **H**—Painting materials; **I**—Museum collection; **J**—Underwater cultural heritage objects. Since the museum can contain exhibits made of any type of material, which corresponds to the entire color range, to simplify the designation, a special pictogram of white color (according to the white light, obtained by mixing all the lights of the rainbow) is introduced. Also, since cultural heritage objects made of any materials can be located underwater, and the aquatic environment differs significantly in its characteristics, an additional ultramarine-colored pictogram is introduced

has its own characteristic destructor organisms. Since we have assigned different colors to individual materials, a composite material can be schematically represented as pictogram consisting of two or more circles of different colors (Fig. 3).

Figure 3 provides a series of examples of assigning pictograms for various cultural heritage objects made from two or more materials. Examples apply both to objects consisting only of inorganic compounds and to objects consisting only of organic compounds or combining materials of organic and inorganic nature. To simplify perception if the object consists of both inorganic and organic (or paintings) materials, the following

design of pictogram is used: circles corresponding to inorganic materials are located at the top, and the circles corresponding to organic (or paintings) materials are located at the bottom part of the pictogram.

### Different types of biodeterioration according to the rainbow code

In this section, we will take a closer look at how the introduced rainbow code can be used to characterize the type of biodeterioration. In doing so, we will focus on cultural heritage sites associated with monumental and easel art.



**Fig. 3** Examples of pictograms based on the Rainbow Code of Biodeterioration for cultural heritage objects consisting of composite materials. **A**—stone and metal (precious stones and gold); **B**—metal and glass; **C**—stone and metal; **D**—leather and wood; **E**—fabric and painting materials; **F**—wood and painting materials (varnish); **G**—stone and painting materials (tempera paints); **H**—metal and painting materials (enamel); **I**—glass and painting materials (smalt); **J**—clay and painting materials; **K**—stone, metal and wood; **L**—stone, leather and wood; **M**—stone, metal and fur; **N**—metal, glass, wood and painting materials; **O**—stone, metal, fabric and painting materials; **P**—metal, animal derived material (pearl), wood, fabric (pavoloka), painting materials (tempera). If the object consists of both inorganic and organic/paintings materials, then the circles corresponding to inorganic materials (colored in cool shades of colors) are located at the top of the pictogram, and the circles corresponding to organic/paintings materials (colored in warm shades of colors) are located at the bottom of the pictogram

### Monumental art

Monumental art is an architectural and sculptural compositions, fresco paintings and mosaic panels, reliefs, stained glass windows in a synthesis with the local landscape form an ensemble. The themes and stylistic orientation of the monuments are somehow connected with the general social climate and the atmosphere prevailing in public life. Human beings can record significant information for understanding the ancients when contemplating architecture and art monuments such as level of economy, politics, society, culture, art, religion, technology and scientific advances of a region. For example wall painting can trace human civilization back to prehistoric times. An archive of information is in the pigments used for the painting layers and painted objects. If we are talking about stone monuments as cultural objects, then we must take into account that these are minerals with a rich chemical composition—copper, nickel, calcium, sodium, magnesium, iron, aluminum, etc., which are populated with different preferences by microorganisms and are subject to weathering at different rates. Also a climatic region with its weather plays a huge role in terms of the colonization of specific microflora. Such as the primary colonizers of stone surfaces are photosynthetic microorganisms, algae and cyanobacteria are able to survive climate extremes [41]. Northern Europe and comparable regions in U.S. are characterized by regular rainfalls and moderate temperatures, while Mediterranean regions as well as South America have comparable high temperatures and less rainfalls, and in tropical climate is a strikingly different correlation between temperature and humidity. Thus, most of the microorganisms-destroyers manifest their aggressive destroying action within monuments in warm and humid climates [41]. It has been noted that algae and cyanobacteria can produce thick green biofilms in areas of high humidity, but if the monument or even part of it is located in more dry region/area, the color of biofilms may vary. An example would be the filamentous alga *Trentepohlia*, which has a pink (non-green) pigment, and affects the surface of the monument of the Mayan buildings at Edzna, Campeche, Mexico, where UV levels are high and temperatures can be up to 36 °C. At the same time, it was shown by the example of various ancient monuments in Turkey that microorganisms could grow on stone surfaces under air pollution and continental-cold climatic conditions of – 10 to – 25 °C [42]. All these factors lead to an awareness of the adaptability of certain consortia of microorganisms to a certain object in a certain ecological niche.

As we know in accordance with International Charter for the conservation and restoration of monuments and sites “the restoration must be preceded and followed by an archaeological and historical study of the monument”




[13], one way or another the recovery of archeological objects implies considerable changes in the environmental conditions of the material. For instance, cultural heritage sites excavated from the soil or stored in dark and oxygen-free conditions and exposed to a corrosive atmosphere may experience accelerated biodegradation due to the aggressive effect of different elements, one of each is oxygen [43]. On the other hand, if the object should remain in its native environmental conditions, under soil, or volcanic ash, etc., it could be more degraded by anaerobic microorganisms. In this way, the intensity of the microbial attack is determined by its environmental conditions [44]. So, in order to formulate effective strategies for conservation and restoration to prevent biodeterioration it requires an efficient analytical approach which, in addition to physical–chemical and biochemical analysis, includes microbiological analysis: destructive microorganisms should be correctly isolated and genotyped to define the mechanisms by which they colonize and affect the monuments [45].

### Grey biodeterioration

#### *Temples, stone monuments*





According to the location in the stone microorganisms can be distinguished to their location. If located on the surface of the rock they so-called epilithic, and endolithic if they live inside the rock within cracks and pores in granites [46] (Table 1). The pioneers that first colonize the stone surface are chemioautotrophic sulphur oxidizing and ammonia oxidizing bacteria and phototrophic microorganisms such as microalgae, cyanobacteria, and lichens [47, 48]. Among chemolithotrophic bacteria are nitrifying and sulphur-oxidizing bacteria. The first one oxidize nitrogen or nitrogen dioxide for energy and transform them into nitric and nitrous acid. Sulphur-oxidizing bacteria such as *Thiobacillus* have the ability to convert inorganic sulfur to sulfate in the form of sulfuric acid [49]. Many reports have shown that nitrifying and sulfur-oxidizing bacteria are present on stone monuments [49, 50]. Thus, sulfur-oxidizing bacteria and the hydrocarbon-degrading microflora penetrated into the historic limestone materials caused by atmospheric pollutants was found in Massachusetts, USA [51]. As for phototrophic microorganisms, if the environmental conditions become favorable (moisture, light, temperature and nutrition) these microorganisms, usually occupying a wide water biotopes, can easily settle on rocks, stone monuments, historic buildings, etc. They produce stains and form colored biofilms and incrustations, which alter the original aspect, causing unaesthetic perception, producing structural damages [47, 52]. In Macedo et al. [53] was shown the occupation by cyanobacteria (most widespread *Gloeocapsa*, *Phormidium*, *Chroococcus*) and green

**Table 1** The most abundant microorganisms degrading cultural heritage objects

The object	Microorganisms	Refs.
<b>MONUMENTAL ART</b>		
Grey Biodeterioration (Stone)	Cyanolichens: ( <i>Zwackhiomyces</i> , <i>Peltula</i> , <i>Psorotichia</i> , <i>Lichinella</i> ) Green algae: ( <i>Coccomyxa</i> , <i>Chlorella</i> , <i>Chlorococcum</i> , <i>Klebsormidium</i> , <i>Trebouxia</i> , <i>Trentepohlia</i> , <i>Stichococcus</i> )	[53, 56–59]
	Bacteria phyla: Acidobacteriota ( <i>Acidobacteria</i> ), Actinomycetota ( <i>Nocardia</i> , <i>Micrococcus</i> , <i>Microbacterium</i> , <i>Modestobacter</i> , <i>Pseudonocardia</i> , <i>Rhodococcus</i> , <i>Rubrobacter</i> , <i>Streptomyces</i> ), Ascomycota ( <i>Aureobasidium</i> ), Bacillota or Firmicutes ( <i>Listeria</i> , <i>Bacillus</i> , <i>Staphylococcus</i> ), Bacteroidota ( <i>Flavisolibacter</i> , <i>Hymenobacter</i> , <i>Pseudozobellia</i> , <i>Rhodocytophaga</i> , <i>Spirosoma</i> ), Cyanobacteria ( <i>Acaryochloris</i> , <i>Chroococcidiopsis</i> , <i>Chroococcus</i> , <i>Gloeocapsa</i> , <i>Leptolyngbya</i> , <i>Phormidium</i> , <i>Scytonema</i> ), Deinococcota ( <i>Truepera</i> ), Deinococcota ( <i>Truepera</i> ), Nitrospirota ( <i>Leptospirillum</i> ), Planctomycetota ( <i>Planctomyces</i> ), Pseudomonadota ( <i>Acidithiobacillus</i> , <i>Chryseomonas</i> , <i>Lutibacterium</i> , <i>Lysobacter</i> , <i>Methylobacterium</i> , <i>Ochrobactrum</i> , <i>Roseomonas</i> , <i>Pseudomonas</i> , <i>Sphingomonas</i> , <i>Stenotrophomonas</i> )	[10, 11, 45–47, 49, 53, 54, 60–64]
	Fungal phyla: Ascomycota ( <i>Acremonium</i> , <i>Aspergillus</i> , <i>Alternaria</i> , <i>Chaetomella</i> , <i>Cladosporium</i> , <i>Curvularia</i> , <i>Geomyces</i> , <i>Geosmithia</i> , <i>Emericella</i> , <i>Epicoccum</i> , <i>Engyodontium</i> , <i>Exophiala</i> , <i>Fusarium</i> , <i>Herpotrichiellaceae</i> , <i>Isaria</i> , <i>Kraurogymnocarpa</i> , <i>Lasiodiplodia</i> , <i>Ochroconis</i> , <i>Penicillium</i> , <i>Tolypocladium</i> ), Basidiomycota ( <i>Volvariella</i> , <i>Rhodotorula</i> ), Ascomycota ( <i>Alternaria</i> , <i>Aspergillus</i> , <i>Engyodontium</i> , <i>Cladosporium</i> , <i>Devriesia</i> , <i>Epidermophyton</i> , <i>Fusarium</i> , <i>Lepraria</i> , <i>Curvularia</i> , <i>Penicillium</i> , <i>Rhinochlamydia</i> , <i>Trichophyton</i> ), Chytridiomycota, Mucoromycota ( <i>Mucor</i> , <i>Rhizopus</i> )	[38, 42, 45, 46, 49, 55, 65]
Khaki Biodeterioration (Metal)	Green algae: ( <i>Apatococcus</i> )	[66]
	Bacteria phyla: Pseudomonadota ( <i>Actinobacteria</i> , <i>Shewanella</i> , <i>Sphingomonas</i> , <i>Stenotrophomonas</i> , <i>Thiobacillus</i> , <i>Vibrio</i> ) Bacteroidota ( <i>Chryseobacteria</i> ), Proteobacteria ( <i>Desulfomicrobium</i> ), Thermodesulfobacteriota ( <i>Desulfosarcina</i> , <i>Desulfovibrio</i> ), Euryarchaeota ( <i>Methanococcus</i> )	[16]
	Fungal phyla: Ascomycota ( <i>Alternaria</i> , <i>Arthrinium</i> , <i>Aspergillus</i> , <i>Candida</i> , <i>Cladosporium</i> , <i>Clonostachys</i> , <i>Chrysosporium</i> , <i>Debaryomyces</i> , <i>Exophiala</i> , <i>Fusarium</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Pichia</i> ), Basidiomycota ( <i>Antrrodia</i> , <i>Cryptococcus</i> , <i>Poria</i> , <i>Rhodotorula</i> )	[16, 37]
Green Biodeterioration (Glass)	Green algae: ( <i>Apatococcus</i> , <i>Chlorella</i> , <i>Klebsormidium</i> , <i>Oocystis</i> , <i>Trentepohlia</i> ), Diatoms	[67]
	Bacteria phyla: Actinobacteria, Bacteroidetes, Cyanobacteria ( <i>Gloeocapsa</i> , <i>Chroococcidiopsis</i> , <i>Iphinoe</i> , <i>Nostoc</i> , <i>Oscillatoria</i> , <i>Scytonema</i> <i>Tolipothrix</i> ), Chloroflexi, Firmicutes, Nitrospirae, Proteobacteria	[67, 68]
	Fungal phyla: Ascomycota ( <i>Acremonium</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Aureobasidium</i> , <i>Capnobotryella</i> , <i>Chaetomium</i> , <i>Cladosporium</i> , <i>Coniosporium</i> , <i>Didymella</i> , <i>Engyodontium</i> , <i>Fusarium</i> , <i>Geomyces</i> , <i>Hortaea</i> , <i>Kirschsteiniotelia</i> , <i>Leptosphaeria</i> , <i>Myrothecium</i> , <i>Penicillium</i> , <i>Penidiella</i> , <i>Phoma</i> , <i>Stanjemonium</i> , <i>Trichoderma</i> , <i>Trimmatostroma</i> , <i>Verticillium</i> ), Basidiomycota ( <i>Rhodotorula</i> , <i>Sistotrema</i> , <i>Ustilago</i> )	[67–69]



**Table 1** (continued)

The object	Microorganisms	Refs.
<b>MONUMENTAL ART</b>		
Blue Biodeterioration (Clay)	Cyanolichens: ( <i>Zwackhiomyces</i> , <i>Peltula</i> , <i>Psorotichia</i> , <i>Lichinella</i> )	[59, 70]
	Bacteria phyla: Actinomycetota ( <i>Arthrobacter</i> ), Bacillota or Firmicutes ( <i>Bacillus</i> ), Pseudomonadota ( <i>Pseudomonas</i> )	[59, 70]
	Fungal phyla: Ascomycota ( <i>Aspergillus</i> , <i>Cladosporium</i> , <i>Curvularia</i> , <i>Penicillium</i> )	[59, 70]
Violet Biodeterioration (Bones, leather, fur and other animal resources)	Bacteria phyla: Actinobacteria, Bacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Proteobacteria, Plantomycetes	[71]
		
	Fungal phyla: Ascomycota ( <i>Aspergillus</i> , <i>Cladosporium</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Streptomyces</i> , <i>Stemphylium</i> , <i>Trichoderma</i> , <i>Trichophyton</i> )	[72–74]
Red Biodeterioration (Fabric)	Bacteria phyla: Actinomycetota ( <i>Brevibacterium</i> , <i>Corynebacterium</i> , <i>Rhodococcus</i> , <i>Streptomyces</i> , <i>Arthrobacter</i> , <i>Cellulomonas</i> , <i>Microbispora</i> , <i>Nocardia</i> , <i>Streptomyces</i> ), Bacillota or Firmicutes ( <i>Bacillus</i> , <i>Clostridium</i> ), Bacteroidota ( <i>Cytophaga</i> , <i>Sporocytophaga</i> ), Pseudomonadota ( <i>Alcaligenes</i> , <i>Pseudomonas</i> , <i>Cellvibrio</i> , <i>Pseudomonas</i> , <i>Vibrio</i> )	[46, 75–78]
		
	Fungal phyla: Ascomycota ( <i>Acremonium</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Aureobasidium</i> , <i>Cephalothecium</i> , <i>Chaetomium</i> , <i>Chrysosporium</i> , <i>Cladosporium</i> , <i>Fusarium</i> , <i>Memnoniella</i> , <i>Myrothecium</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Stachybotrys</i> , <i>Trichoderma</i> , <i>Trichothecium</i> , <i>Scopulariopsis</i> , <i>Stachybotrys</i> , <i>Ulocladium</i> , <i>Verticillium</i> ), Zygomycota ( <i>Mucor</i> ), Mucoromycota ( <i>Rhizopus</i> )	[37]
Brown Biodeterioration (Wood, tree bark and other wood resources)	Green algae: ( <i>Apatococcus</i> , <i>Chlorella</i> , <i>Chlorococcum</i> , <i>Trebouxia</i> , <i>Klebsormidium</i> , <i>Stichococcus</i> )	[79]
		
	Bacteria phyla: Actinomycetota ( <i>Actinobacteria</i> , <i>Corynebacteriales</i> , <i>Cutibacterium</i> , <i>Cryobacterium</i> , <i>Intrasporangium</i> , <i>Mycobacterium</i> , <i>Nocardia</i> , <i>Micrococcales</i> , <i>Streptomyces</i> , <i>Promicromonospora</i> ), Bacillota or Firmicutes ( <i>Bacillus</i> , <i>Streptococcus</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> ), Bacteroidota ( <i>Flavobacteria</i> , <i>Cytophagia</i> ), Cyanobacteria ( <i>Anabaena</i> , <i>Chroococcus</i> , <i>Lyngbya</i> , <i>Microcoleus</i> , <i>Nostoc</i> , <i>Oscillatoria</i> , <i>Plectonema</i> , <i>Scytonema</i> ), Planctomycetota ( <i>Isosphaera</i> ), Pseudomonadota ( <i>Acinetobacter</i> , <i>Alcanivorax</i> , <i>Alphaproteobacteria</i> , <i>Buchnera</i> , <i>Burkholderia</i> , <i>Caulobacter</i> , <i>Devosia</i> , <i>Chelativorans</i> , <i>Cupriavidus</i> , <i>Enterobacteriales</i> , <i>Escherichia</i> , <i>Halomonas</i> , <i>Marinobacter</i> , <i>Moraxella</i> , <i>Psychrobacter</i> , <i>Pseudomonas</i> , <i>Ralstonia</i> , <i>Salmonella</i> , <i>Sphingomonas</i> , <i>Stenotrophomonas</i> , <i>Xenorhabdus</i> )	[21, 64, 78, 80]
	Fungal phyla: Ascomycota ( <i>Alternaria</i> , <i>Aspergillus</i> , <i>Aureobasidium</i> , <i>Cladosporium</i> , <i>Chaetomium</i> , <i>Doratomyces</i> , <i>Eurotiomycetes</i> , <i>Emericella</i> , <i>Geosmithia</i> , <i>Nigrospora</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Saccharomyces</i> , <i>Scopulariopsis</i> , <i>Sordariomycetes</i> , <i>Stemphylium</i> , <i>Thielavia</i> , <i>Trichophyton</i> , <i>Ulocladium</i> ), Basidiomycota ( <i>Rhodotorula</i> ), Mucoromycota ( <i>Mucor</i> , <i>Rhizopus</i> , <i>Syncephalastrum</i> )	[21, 37, 64, 78, 80]

**Table 1** (continued)**EASEL ART**Gold Biodeterioration  
(Painting materials)

Bacteria phyla: Actinomycetota (*Arthrobacter*, *Brachybacterium*, *Brevibacterium*, *Microbacterium*), Actinobacteria (*Agrococcus*, *Brachybacterium*, *Janibacter*, *Microbacterium*, *Rhodococcus*), Bacillota or Firmicutes (*Bacillus*, *Clostridium*, *Stenotrophomonas*, *Virgibacillus*, *Paucisalibacillus*, *Paenisporosarcina*, *Staphylococcus*, *Sporosarcina*, *Veillonella*), Bacteroidota (*Myxococcoides*, *Clostridium*, *Prevotella*, *Sporocytophaga*), Proteobacteria (*Aeromonas*, *Achromobacter*, *Acinetobacter*, *Pelomonas*, *Pantoea*, *Pseudomonas*, *Pseudoalteromonas*, *Stenotrophomonas*), Pseudomonadota (*Achromobacter*, *Acinetobacter*, *Alphaproteobacteria*, *Cellvibrio*, *Oxalobacteraceae*, *Pantoea*, *Pelomonas*, *Pseudoalteromonas*, *Rhodanobacter*) [20, 31, 43, 69–71, 81]

Fungal phyla: Ascomycota (*Alternaria*, *Aspergillus*, *Aureobasidium*, *Candida*, *Chaetomium*, *Cladosporium*, *Emericella*, *Eurotium*, *Fusarium*, *Myrothecium*, *Memnoniella*, *Neurospora*, *Pichia*, *Penicillium*, *Simplicillium*, *Scopulariopsis*, *Stachybotrys*, *Stemphylium*, *Trichoderma*, *Ulocladium*, *Penicillium*), Basidiomycota (*Bjerkandera*, *Filobasidium*, *Porostereum*), Mucoromycota (*Mucor*) [5, 20, 21, 33, 46, 78, 82–88]

**AQUATIC ENVIRONMENT**Ultramarine Biodeterioration  
(Aquatic environment)

Bacteria phyla: Actinomycetota (*Arthrobacter*), Bacillota (*Clostridium*, *Bacillus*), Bacteroidota (*Cytophaga*, *Flavobacterium*), Pseudomonadota (*Cellvibrio*, *Pseudomonas*, *Spirillum*) [89]

Fungal phyla: Ascomycota (*Cladosporium*, *Acremonium*, *Fusarium*, *Chaetomium*) [89, 90]

algae (most widespread *Chlorella*, *Stichococcus*, *Chlorococcum*) different lithotypes present in the monuments in Mediterranean Basin such as limestone, marble, granite, sandstone, travertine, dolomite. The secondary colonization by heterotrophic bacteria and fungi is followed by the metabolic activity of autotrophs. For example, consortium of heterotrophic bacteria, actinomycetes and fungi was identified in the Certosia of Pavia, Italy [54]. Microorganisms have adapted to survive in their ecological niche in a variety of ways. One of them is the release of protective pigments [41]. These are endopigments such as photosynthetic chromophore pigments: chlorophyll and phycobilins produced inside the red algae, cyanobacteria and other algae, and exopigments emitted outside the cell as fungal pigments (black, violet, blue, green, and purple). The black pigment known as melanin protects fungi against environmental threats and cellular lysis [46]. Mycosporines and carotenoids ( $\beta$ -carotene, s-carotene, phytoene, torulene, and torularhodin) may

protect fungi against oxidative stress and exposure to visible light or UV irradiation [55].

Fortunately, modern molecular biology methods make it possible to diagnose the whole consortia of microorganisms inhabiting a cultural object. Thus, metagenomic sequencing of the bacterial 16S rRNA gene and fungal ITS sequences was used to analyze the diversity and variability of the microbial communities colonizing the ancient stone brick monuments around West Lake, Hangzhou, China [49]. It was shown that Cyanobacteria are the primary component of the bacterial communities of all bacterial phyla isolated within investigated objects. Cyanobacteria are probably the most resistant of the microbial communities on the surface of stone monuments with their ability to resist high UV radiation and dehydration. The phyla Proteobacteria, Actinobacteria and Acidobacteria are the next largest members of the bacterial communities [49]. This bacterial phyla are frequently present on ancient mural paintings [11, 91]. Proteobacteria and Firmicutes are associated with earthy and

arid environments, while Actinobacteria are considered the dominant microbial taxon in subterranean environments, such as mural paintings in caves and on tombs [10]. Ascomycota was the predominant fungal taxon as it was established in erfs. [49, 92]. It has long been known that fungi isolated from soils and weathering rock can solubilize a range of synthetic and natural silicates by producing various organic acids [93].

Wall Painting or murals are often an integral part of the interior decoration of temples, and they are generally classified as *secco* or *fresco*, which represent different Eastern and Western production techniques. The name *fresco*, or “fresh” in Italian, stems from the practice of painting with a mix of water and pigment onto freshly laid wall plaster [94]. This is the main technique used for traditional murals originating from the Aegean of ancient Greece and Rome. *Secco* painting is a fundamentally different technique. The canvas for *secco* is a dried plaster wall, and the paint contains the color pigment and a binder like *tempura* egg yolk, oil, or glue [94]. This kind of technique had been originated in Asia and parts of Africa, and has been used widely in the Mediterranean regions. First of all, it is logical to begin with history of *fresco* painting in Egypt and finish by considering some contemporary *fresco* styles. The earliest *fresco* examples found in the Hierakonpolis tomb in Egypt date back to between 3500 and 3200 B.C. Other early *frescos* in Israel, Egypt, and Crete date back to 2000 B.C. These *frescos* typically adorn the walls of tombs and palaces and depict various parts of ancient life, depiction of many gods and goddesses, battles and farming scenes [95]. The Investiture of Zimri-Lim is one fantastic *fresco* example from eighteenth century B.C. Mesopotamia. These earlier tomb *frescos*, particularly the ones in Egypt, use the *secco* technique [94]. Classical antique *frescos* can be found in the ruins of Herculaneum and Pompeii [23] demonstrate the Roman style of *fresco* painting. The late Roman Empire *frescos* can be found in the catacombs beneath Rome. Other Roman *frescos* depicting Byzantine icons exist in Antioch, Crete, Cappadocia, and Cyprus. Beside *frescos* in Ancient Rome and Greece this period also saw *fresco* paintings in Sri Lanka and India [52, 65]. There are many different locations in India with preserved *frescos* dated between 200 and 400 B.C. Many of these ancient heritage monuments are located far from human habitations inside forests. The cave monument of Ajanta is 30 caves of decorated mural paintings representing the past lives and rebirths of the Buddha, pictorial tales and rock-cut sculptures of Buddhist deities carved in the second century B.C. [96]. Next on the timeline is the Italian Renaissance which saw an explosion of experimentation with *fresco* techniques. Well known Michelangelo and Raphael and other Italian Renaissance

artists experimented with depth and perspective by carving into the wet *intonaco* plaster before painting. Well known Michelangelo’s The Sistine Chapel ceiling in Vatican is about 35 m long and 14 m wide, with the ceiling rising to about 20 m above the main floor. Michelangelo painted the altar part, on which the famous *fresco* “The Last Judgment” is located, and the Plafond. The painting on the ceiling of the Sistine Chapel is made in the technique of *buon fresco*. That is, paint was applied to the fresh coating. In addition to this method, Michelangelo also painted on dry plaster using the *secco* technique, which was used only to retouch any details. Also the famous Renaissance period *fresco* is The Last Supper by Leonardo da Vinci. This is his only surviving *fresco* and his largest painting. Like an investigator he used a modified *secco fresco* technique. In nineteenth and twentieth centuries *fresco* painting techniques saw a revival. Public mural paintings became incredibly popular, and the movement was spearheaded by those associated with the Arts and Crafts Movement and Pre-Raphaelite painters [94]. All of these nuances in history techniques must be known to understand the mechanisms of restoration and conservation of certain cultural heritage objects.

So if we turn to the ancient Egyptian manner of execution, for example murals in tombs in Lower Egypt [97], it can be noted that mixed pigments were used, such as hematite, limonite, azurite blue, lamp black and green malachite or synthesis pigments such as Egyptian blue with different binding media such as arabic gum, animal glue, egg yolk and bees wax. The study [97] shows that *tempera* painting is affected mainly by bacteria of the genus *Streptomyces*, which are capable of producing certain enzymes, in particular collagenase for animal glue breakdown; phospholipases for phospholipids hydrolysis to ammonia when recycling egg yolk; various esterases for recycling beeswax, and also hydrolyze the polysaccharides that make up gum arabic. The work [98] shows the symbiotic existence of streptomycetes and molds; they produce enzymes that hydrolyze both bacterial and fungal cell walls and polysaccharides of the bacteria themselves. A study [61] in the Royal Palace of Casas Pintadas in the city-museum of Évora (Portugal) identified a symbiotic microbiological community of Renaissance wall paintings in areas of loss and destruction of the paint layer. Thus, bacterial strains (cocci, bacilli), actinomycetes, yeast-like fungi, and classic manifestations of mold of the genera *Aspergillus*, *Cladosporium* and *Penicillium* were discovered. It was shown that these microorganisms are able to exist on a nutrient medium of a coated calcite layer and pigments of earthy origin: ochre with clay minerals (aluminosilicates) enriched with iron oxides and hydroxides—goethite (FeO(OH)) and hematite (Fe<sub>2</sub>O<sub>3</sub>),

manganese oxide ( $\text{MnO}_2$ ), burnt bone with the presence of calcium (Ca) and phosphorus (P) [61].

Many biological damages of cultural heritage objects begin after they reactivation after long-term isolated storage. So, as a result of a volcanic eruption in the first century AD in Pompeii (Italy), numerous objects with unique wall paintings were buried under a layer of volcanic material. As a result of numerous excavations (1822, 1832, 1846 and beyond), a residence the House of Ariadne was discovered, consisting of number of 40 decorated rooms located around two peristyles.

During a thorough cleaning in 2005, it turned out that the walls were under a layer of patina caused by the activity of microorganisms. In investigation [23] to assess the degree of biological damage to the Pompeian mural paintings, complex physical and chemical analyzes were carried out using Raman and X-ray spectrometry methods. To identify microorganisms, micro- and molecular biological tests were carried out, and electron microphotography of samples of pathogenic flora was carried out. As a result, it was shown that the upper part of the premises (excavations in 1988) was practically not damaged by microorganisms, while the lower part (excavations in 2005) was completely covered with filaments of mold fungi, mainly of the genus *Penicillium*. This is due to the presence of nutrients in deposited ash and centuries-old surface contamination, as well as increased roughness from the volcanic eruption [23].

Mural paintings stand in confined and semi-confined environments with extreme humidity and abundant nutrients, which promote the germination of fungal spores and mycelia growth [30], and as it is known the cracking and peeling of the paint layer may occur. To strengthen the paint layer consolidants may be used. As a rule, these are two-component systems consisting of a filler and a binder which may be of natural or synthetic nature. The natural one, for example, transparent dispersion of casein, obtained from lime and casein, may easily be degraded because of its organic origin. The polymer Palaroid B72, actually the ethyl methacrylate–methyl acrylate copolymer, was found to protect and fix the surface of the pictorial layer [30], but it reduces the permeability to vapors, and if the surface was not well purified from fungal hyphae, microorganisms may be sealed inside the system. Violation of the temperature, humidity, light exposure may accelerate oxidation and cause blackening of damaged areas fixed with such consolidant [61]. It was found that popular for pictorial surface fungal phyla such as *Penicillium* [92], *Aspergillus*, *Ulocladium* [30], *Cladosporium*, *Chaetomium* [99], etc. can absorb pigments on dolomitic limestone, can dissolve calcite  $\text{CaCO}_3$  from the substrate by synthesizing organic acids. Acidification of the substrate can cause the degradation

of consolidants. Therefore, in order to protect certain objects of art, in particular murals, it is recommended to use consolidants together with biocides during restoration, as was successfully done in the investigation [30].

Rock art is a rather vague term which denotes prehistoric man-made markings on natural stone. Similar terms include: “rock carvings”, “rock engravings”, “rock inscriptions”, “rock drawings” and “rock paintings”. This type of Stone Age art is traditionally divided into two main categories: (1) Petroglyphs: meaning, rock engravings or carvings; this category also includes works of prehistoric sculpture that are part of the rocks themselves (known as parietal art), such as relief sculpture. (2) Pictographs: meaning, paintings or drawings [100]. There is also a third, smaller category of rock art and this is Megaliths or Petroforms. These are large stones used to build prehistoric structures or monuments. They are widely distributed from Sweden to the Mediterranean. The most famous Megalith in Europe is Stonehenge stone circle. Petroglyphs are generally made by removing the surface of the rock, by carving, scratching, drilling, or sculpting. The markings can be dyed or painted, or enhanced through polishing. Petroglyphs have been discovered all over the populated world, notably in parts of Africa, Scandinavia, Siberia, southwestern North America, Northern and Western Australia, and the Iberian Peninsula [100]. Pictography is the creation of monochrome or polychrome images through the application of pigments, like carbon, manganese and various oxides. The pigments were rubbed across rock walls (yellow, red, and brown ochre), and contours were drawn with a charcoal stick. Also it has been discovered that ancient dishes could be used for mixing pigments with fat and smeared with the hand.

The Mediterranean Basin is characterized by huge number of karstic caves, for example, in Greece (the majority in Crete), in Slovenia, Italy, Spain, France, and many other. Vivid examples of rock art from Upper Paleolithic period are, for instance, Altamira and Lascaux caves, located northern Spain and southwestern France respectively, famous for their magnificent multi-coloured painting and rock engravings. Before the discovery of these caves mankind had no idea about prehistoric art. For instance, since Altamira was discovered at the end of the nineteenth century, it has aroused considerable archaeological interest due to its famous polychromatic paintings located on the walls [10]. The paintings in Altamira are unique for its different colors (up to three colors in a single animal); the animals—twenty-five of which are depicted in life-size proportions with accuracy. The bison are especially well rendered. The cave, before opening it to a wide audience, was mothballed under a layer of karst deposits, the entrance collapsed

and covered the cave, creating a stable climate inside. In the 60–70 s of the last century, a large number of tourists began to visit the cave. The ingress of daylight, moisture and micro-organisms brought by visitors has greatly influenced the change in the eco-climate in the premises. In refs. [8, 10] a wide variety of microbial communities were detected that settled on walls with drawings, the dominants of which are phototrophs, mainly cyanobacteria, Gram-positive and Gram-negative bacteria, filamentous bacteria of the genus *Actinobacteria* [63] that form biofilms, as well as numerous mold fungi of the genera *Cladosporium*, *Trichoderma*, *Pochonia*, *Aspergillus*, *Penicillium*, *Acremonium*, etc. It's interesting, the researchers note [101] that bacteria are responsible for the formation of carbonate deposits such as moonmilk, which is usually composed of calcite, aragonite, or hydro-magnesite, although it can be formed by other carbonate and noncarbonate minerals, in Altamira Cave. Bacterial communities, mainly consisted by heterotrophic micro-organisms, can form white colonies generated a micro-environment with high pH, which induces carbonate precipitation under cave conditions [102]. Nowadays, cave management tends to reduce anthropogenic impacts by controlling visitors and microclimate [63]—only some visitors, randomly selected by a lottery, are allowed to see the royal cave of Altamira every Friday morning. Other visitors can get acquainted with the cultural heritage without visiting the cave by visiting duplicate rooms that reproduce the decoration in miniature.

A similar story befell another famous cave Lascaux (France), discovered in 1940, also with drawings from the Paleolithic times, from the walls of which two new species of mold fungi *Ochroconis lascauxensis* and *Ochroconis anomala* were mainly isolated and characterized [62, 103]. It is noteworthy that the mold had black and purple colonies, which the authors explained by the presence of biogenic manganese in the composition of fungal hyphae. It had been previously established the first evidence for biological oxidation of manganese was found in soils [104]. Manganese(II) oxide has long been known to be mediated by microorganisms, especially bacteria but also fungi [105], especially it common in hypogene caves, that are typically deeper and contain significant chemolithoautotrophic microbial communities [106].

In the Etruscan crypt Tomba del Colle [91] unique wall paintings are degraded by bacteria of the genus *Rhizobiales* (Alphaproteobacteria), capable to live in conditions with reduced oxygen availability. Another Etruscan tomb—Tomba della Scimmia (Tuscany, Italy), discovered in 1846, was damaged by Actinobacteria. The stone surfaces of the Abbey of Chaly, (France) [79] are affected mainly by Alphaproteobacteria and Actinobacteria [11].

### Easel art

Not only monumental objects but also easel art, which involves a non-stationary and non-utilitarian base (wood, canvas, silk, paper, etc.) using various paint and varnish artistic materials [107–109], are affected by undesirable microflora. Wood and canvas, be it icon or just painting, are prepared by masters according to traditional canons. The first layer is an artistic primer, consisting of two components—inorganic (a mixture of gypsum, chalk, white) and organic drying binder (glue, oil, resin, etc.). Several technological sizing layers can be applied on top of the primer, the last of which is imprimatura [110]. Glues of animal (gelatin, albumin, casein, and wax) and vegetal origin (starch, resins, gums, and gluten) have been used as adhesive agents in various historical periods [46, 111]. Gelatin is obtained from collagen which is an existing protein in the skin or cartilage; the sturgeon glue is the collagen from the inside of the swim bladder of the fish; albumin (protein of egg or blood plasma), casein (protein of milk), and wax (secreted by bees composed of a mixture of esters, hydrocarbons, and fatty acids). The starch is a polysaccharide of vegetable origin, which is formed predominantly of amylose and amylopectin. Vegetal resins are a mixture of organic compounds principally terpenes and derivatives. Next, an underpainting is applied to the prepared primer, done either with charcoal or paints in a monochrome style [110]. The purpose of its use is only to provide the artist with comfort and confidence when painting. Pigments, the most important components, are applied next; they are either natural or synthetic origin [46]. Next, varnishes are applied; they provide brilliance, protect the paint layer from destruction by UV radiation, moisture ingress, and therefore from biodegradation [36, 86]. The manifestations of the damage of art work can be physical/mechanical, chemical, biological. The physical one could be the lack of adhesion of the binder, damage caused by the movement to another exposition, etc. Chemical damage can cause a gradual degradation and depolymerization or crosslinking of adhesives, oils, etc., damage caused by light, oxidation of art materials [4, 46]. A biological attack can occur if a temperature/moisture drops, or as a result of craquelures appearance, or dust settling so far as the rich organic composition of the multi-layered art canvas contributes this.

### Gold biodeterioration

#### Canvas painting

Canvases as the basis of paintings were firstly used in Italy by Venetian artists [112]. The canvas began widely used in the countries of Northern Europe in the seventeenth century, in Russia a little later—at the end of the 17th and beginning of the eighteenth centuries. Such an

innovation, invented by masters in that time, favorably distinguished the canvas from the heavy and rough board on which icons were painted, since this material could be easily transported by rolling it into rolls. Until the nineteenth century linen, hemp (fibers from cultivated hemp) served as the main material for making canvas. Later, jute, cotton, and even sometimes synthetic materials began to be used—nylon, lavsan [110].

The biodeteriorable character of the canvas is conditioned by the characteristics of the fabrics that are its support formed by cellulose fibers which is a polysaccharide whose constituent unit is D-glucose linked by glycosidic bonds  $\beta$  (1–4) forming linear chains, which in turn are links in parallel fibers called microfibrils [46]. For cellulose, degradation involved different cellulases refer to a group of enzymes that catalyze the breakdown of cellulose to form oligosaccharides, cellobiose, and glucose. Cellulases are generally divided into four major classes on the basis of their mode of action; exoglucanases, endoglucanases,  $\beta$ -glucosidases, and cellobiohydrolases [113]. These enzymes are produced by fungi and bacteria which may appear on the canvas under adverse storage conditions. Microorganisms act in their own interests—causing the breakdown of cellulose into an utilizable energy source in the form of glucose. Cellulolytic fungi and bacteria differ in structure and mode of action of the cellulases. *Alternaria*, *Aspergillus*, *Fusarium*, *Memnoniella*, *Myrothecium*, *Neurospora*, *Penicillium*, *Scopulariopsis*, *Stachybotrys*, *Stemphylium*, and *Chaetomium* are the main fungi associated to this process and as cellulolytic bacteria: *Cellvibrio*, *Sporocytophaga*, *Myxococcoides*, *Cellufalcicula*, and also *Clostridium* sp. as anaerobic bacteria has been reported [46]. This cellulolytic process is favored in conditions of relative humidity or water condensation where the fiber of the fabric loses consistency and elasticity becoming brittle and falls apart [37, 46]. The most aggressive microorganisms that destroy pictorial paintings are fungi, and they can affect the substrate in two ways—from the surface to its interior and from the back side into the surface. In addition to canvas, paintings are known to contain many organic compounds, including primers, adhesives, pigments, binders, plasticizers, and varnishes. Penetrating inside the artwork fungi digest organic matter, and they require not only cellulases, but also extracellular hydrolytic enzymes, such as lignocellulases, proteases, lipases, pectinases, chitinases, etc. [114]. More over the back side of the canvas paintings used to coat with organic glue pastes, and fungi may also find it as a rich source for nutrition. Fungal hyphae may extend deep into art materials, or fruiting bodies may form on their surface. For example, it can be pigmented fungi, such as black *Aspergillus niger*, or brown *Ulocladium chartarum*, or white *Simplicillium lamellicola*, or blue to

blue-green *Penicillium chrysogenum* with yellow pigment known as colored secondary metabolite sorbicillinoid [21, 115]. In addition, as the fungus ages in the cracks of the paint layer, it can release the so-called colored exudate, as *Aspergillus versicolor* does—releases brownish red, orange cinnamon or scarlet red one [116]. As a result of depigmentation, shedding, loss of the original author's paint layer, the authenticity of the work is lost. It is necessary to carry out an expertise of the art work and its further restoration.

Many researches devoted to the defeat of oil painting on canvas. Thus, the investigation [117] “Coronation of the Virgin Mary”, painted by the Italian artist Carlo Bononi, 1617 in the classical traditions of the Baroque style, showed some areas with biological damage. This large mobile canvas (280 cm in diameter) was mounted on the ceiling of the Basilica of Santa Maria in Vado (Ferrara, Italy). After the earthquake that affected this city in 2012, the painting was dismantled and was placed on a stone floor against the wall, where it continued to accumulate potential damage from biodeterioration (the basilica had high humidity, more than 65%, and a temperature of around 26 °C). During microbiological studies, significant damage by mold and bacterial microflora was discovered on the back side of the painting; these are fungi of the genera *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria* and bacteria *Staphylococcus* sp.

The front side of the canvas was predominantly affected by *Aspergillus* sp., *Penicillium* sp. and bacteria *Bacillus* sp. Fungi of these genera are often found in museum repositories [118–120]

The canvas painting of Serbian modernist artist Petar Lubarda—“The Battle of Kosovo” dating from 1953 was investigated for suspension for bacterial degradation of back side of the art work in Pavić et al. [33]. The artist used “special tempera” for pigment layer. Before starting research, investigators should to immerse into the history of particular art work creation. Relying on literature data of Lubarda's opus, and the results of analysis performed on other artist's paintings authors of [33] have selected six chemically different pigments (Ivory black, Red ochre, Yellow ochre, Zinc oxide, Cobalt deep green, and Cadmium red). First three of them were suitable for bacterial growth. These pigments have been used in the art since the Paleolithic Era as we mentioned before, and some of these pigments had been used in Altamira cave [10], Lascaux cave [62, 103]. Ivory black (or bone black) contains carbon, calcium, and phosphate [121] the principal coloring matter in Yellow and Red ochres, is iron(III)-oxide present in form of minerals goethite and hematite, respectively [122]. These materials are natural pigments and contain clay [122] which is good for microbial growth. Non-spore-forming bacteria *Staphylococcus*,

*Acinetobacter*, *Agrococcus*, *Janibacter*, *Rhodococcus*, and *Stenotrophomonas* genera were isolated from easel painting. It was shown that isolated bacteria possess capacities to deteriorate Ivory Black, and Red and Yellow ochres as a sole resource of nutrients [33]. Most of bacteria tested in this study can discolor pictorial layer containing Zinc white and/or Cobalt green pigments due to bacterial ability to produce different organic acids in media in glucose metabolisms or by acid-metal complex formation. The strongest negative effect on bacteria was observed for Cadmium red. Bacteria that were able to grow in the presence of this pigment changed a colony color into red or yellow. It's interesting that *Stenotrophomonas* sp. isolated in this study changed the colony color into gray. It can be suggested that some bacteria can accumulate heavy metals and thus tolerate their toxicity [33]. The destructive effect of *Stenotrophomonas maltophilia* was discovered in combination with the *Aspergillus versicolor*, destroying the colorful layer of tempera paints on a 16th-century icon. Fungal communities interact with bacterial and form complex biofilms which could be considered as one meta-organism [16].

#### **Paper painting**

The main components of paper are fiber or fibrous material (hemp, cotton, linen, bagasse, rice straw and wood) and functional additives (sizing, optical brighteners, and consolidating agents such as gelatin, cellulose acetate and carboxymethylcellulose). Cellulose fiber is the major component with a lower proportion of lignin, hemicellulose, and other acromolecules, its quality depends on the source of the raw material used, and the procedure applied to obtain the fiber. Its mechanical resistance depends on its degree of polymerization and its inter fiber links [46]. Various pigments can be applied onto the paper, depending on the depending on technical tasks. For example, ink the main component of Chinese painting since ancient times, consist of a liquid that durable, odorless, with variable pH, and is composed of a pigment, a diluent and a binder [46]. Among the oldest ones are ferrous ink, whose components are iron sulfate, galotanic acid and a binder, usually gum arabic. Over time the components of plant and animal origin have been replaced by synthetic compounds [46]. Main paints for art paintings on paper are watercolor, gouache, tempera, acrylic, and they fundamentally differ from each other in manufacturing technology, and specifically in the composition of the binder. The meaning of using watercolor is to create an airy perception of the picture, to create a luminous image through the transparency of colors. So, in watercolors the pigments are very tiny colloidal suspension of chemical compounds in a light watery solution of bindings (gum arabic, dextrin, etc.) and plasticizers

(glycerin, honey, etc.) [84]. Gum is added to watercolor for better capacity to stick to paper and for stable dispersion of pigment particles in water until the film has dried on the surface [84]. Thickeners in watercolors can be vegetable gums, cellulose ethers, amorphous silicon dioxide, modified starches [123]. The composition of gouache paints is similar to watercolors, with the difference that opacity is imparted to them through the addition of white, and they have greater covering properties. Both watercolor and gouache are highly soluble in water. Tempera and acrylic are also non-transparent paints. The binder of tempera paints is an emulsion, that is, a mixture of substances insoluble with each other (latin "*temperare*" means "*to connect*" or "*mix*"). There are casein-oil, egg, wax-oil and polyvinyl acetate tempera, depending on the binder [102, 124] Most tempera paints do not dissolve or wash off after drying. In acrylic paints, the pigment is mixed with a synthetic binder made of polyacrylates. After drying, acrylic paints are no longer dissolved and washed off with water. Also artists are usually use soft, free-flowing materials such as pressed charcoal, sanguine, sepia, sauce, pastel to create sketches, underpaintings. In fact, these are pressed pigments of natural shades, made from natural materials, clay, chalk, iron oxide yellow–brown pigments, etc. Finished graphic work or watercolored paper may be covered by a fixative of polymeric film former in organic solvent to prevent crumbling, smudging, fading, and discolouring and biodegradation as a result. Despite protection with fixative, as a result of a violation of the storage regime of paper works, they may be degraded by undesirable microflora, since, as you can see, the paper itself and various components of paints are rich in organic nutrients.

Contrary to what has been said, sometimes some pigments can provide bioinhibition properties to the carrier. Perhaps this is due to the selective toxicity of the pigments themselves for the growth and development of microorganisms or with the synthetic fillers inside them, which is not a rich nutrient medium. In Soleymani et al. [85] special samples of handmade Japanis paper coated with plant dyes, watercolours, and acrylic paints were tested for *Aspergillus niger* and *Penicillium rubrum* infection. Spore suspensions of mentioned fungi was inoculated on two kinds of paper covered with alizarin crimson, cobalt blue, raw sienna, raw umber, burnt sienna, and burnt umber, the most used by conservators pigments for paper restoration. Generally, tissue paper with watercolors showed more fungal growth than acrylic covered paper, but surprisingly, most colorants, both watercolors and acrylic, showed lower fungi DNA concentration compared to the untreated paper. The aim of this investigation was to compare two kinds of paper for biostability, and

authors associate it with different pH and fabrication technology. The fact is that biodegradation of paper carrier and pigments are actually two different mechanisms. In mentioned [85] work bioinhibition properties of colored papers may be explained by a decrease in the nutritional properties of the paper surface, due to the possible toxicity of certain dyes, and / or in the case of acrylic, the presence of a synthetic polyacrylate binder, which covers the surface with the thin film and slows down for microflora spreading.

Sometimes it is difficult to distinguish watercolor and ink techniques since ink also very thinly and uniformly applied on paper. In the restoration of XVIII century ink landscape on paper it was found that artistic underpainting is a mixture of pigments (carbon black, brown bis-ter, sepia and other), the ink layer is the upper one [84]. The original drawing had some interlayers with adhesion paste. And when separated they revealed full fungal infestation with *Aureobasidium pullulans* which penetrated the surface of the picture. The restoration was successful after remove the source of nutrient paste and applying solution of sodium borohydride onto the local spots of fungi [84].

In the case of using pastel as a pigment, infection with undesirable flora, in particular fungi, occurs as follows. Fungal mycelia, for example of *Penicillium* sp., *Aspergillus niger*, *Trichoderma viride* as it was on pastel portrait surfaces of XIX-XX centuries restaured in Berovic [83], exploit a glucose hydrolyzed from starch glue or gum tricocanth or the other polysaccharides contained in pastel pigments. Of course fungal gifae use micro amounts of water in solid matrix to grow inside. Since vitality fungi produce metabolic products and they act on painted layer. This could be organic acids, mostly citric, oxalic, malic, or fumaric acids secreted in the metabolic tricarboxylic acid cycle, can react with a pastel pigment, changing it to its salts—this results in decolorization and changed optical properties [83]. And as in previous work the solution of sodium borohydrate was successfully used for bleaching and removing the fungal hyphae.

For the correct restoration actions, it is necessary to delve into the peculiarity of the execution technique, to determine what materials were used by the author to create a picture. For example engravings is usually done on paper with special etching or printing inks: mineral or organic, natural or synthetic. But the artist is allowed to enhance the effect by his tricks. So in restoration of engraving of XIX century on paper the gamboge glazing pigment was used by the author to emphasize his green and brown colors [84]. This transparent yellow pigment is a gum risin and contains a carboxylic group, and other elements such as phenolic compounds that should prohibit their growth. But much to the surprise fungi

flourished on the areas glazed with gamboges. Some of the fungi was *Aspergillus versicolor*. With respect for the age of the work, it was carefully processed firstly mechanically, and then was desinfected from verso.

As we can see, the degrading microflora can exist on almost any art object, whether it be stone and wooden monuments [49, 64, 65, 80], architectural [60] and archaeological art works [8, 10, 125, 126], fresco wall painting [11, 61, 91, 97], easel painting on canvas [5, 33, 88, 127, 128], board [9, 115], and paper [83, 84, 129]. Table 1 below shows the most abundant microorganisms degrading cultural heritage objects.

### Brown biodeterioration

#### *Wood Painting, Icons*

Tempera painting on wood (for example, iconography) is also susceptible to harmful microflora. Despite the fact that restorers in the twenty-first century have new knowledges and technologies for the conservation of cultural heritage objects, as well as galleries and exhibition halls in many countries of the world have premises, often equipped in the “smart home” style with clear regulation of temperature and humidity conditions, emergency situations can occur. Transportation to another exhibition or emergency in the public sector may disrupt the maintenance of art objects. Back in the eighteenth century, craftsmen knew that if the humidity regime was disturbed, paintings were easily exposed to mold [6]. Undesirable microflora can get into the craquelures and, using the organic components of art materials, form consortia, spreading deep into the art work [11, 48]. For instance the board itself is a natural material, and over time it can be biodegraded. When wood is prepared for painting, it is initially dried or “seasoned” for use, and all of the free water is removed. But the amount of water remaining is determined by the relative humidity of the surrounding atmosphere [130]. Therefore, it is very dangerous, in particular for tempera on wood, changes in humidity. So, starting in 1995, in the State Tretyakov Gallery in Moscow (STG), despite the compliance with temperature and humidity storage conditions (19 °C/55% humidity), problems with freezing and thawing of walls in the halls of ancient Russian art in the main historical building were observed in the winter-spring period. This temperature difference had a beneficial effect on the development of destructive microflora on the icons of the 16th–18th centuries exhibited in these halls. The problem was recorded and highlighted in Klindworth et al. [21]. The authors monitored the exhibits and internal communications of the halls of Painting of Ancient Rus’ (56, 57 and 61) of the main historical building of the STG, created a collection of cultivated microorganisms, including fungi of the genus *Aspergillaceae* (*Aspergillus*



*versicolor*, *A. creber*, *A. amoenus*); genera *Cladosporiaceae* (*Cladosporium halotolerans*, *C. parahalotolerans*) and *Pleosporaceae* (*Ulocladium chartarum*), *Cordycipitaceae* (*Simplicium lamellicola*) and *Microascaceae* (*Microascus paisii*). It has been previously shown that composition of tempera systems is complex [131] and that is why difficult to investigate due to various components. The investigation [21] shows the potential risk of the development of mold microflora on specially created models with paints, binding media and varnish coating with both natural and factory tempera pigments. To intense fungal infection can be subject the following components: egg emulsion (yolk and water), ocher and cinnabar on yolk emulsion, phthalocyanine blue on factory-made egg emulsion. Varnishes and plasticizers such as gum arabic are practically not susceptible to pathogenic flora, which justifies their use as covering agents. Among the potentially dangerous fungi, the authors of the cited work found *Aspergillus creber* in combination with a dominant bacterial representative *Stenotrophomonas maltophilia*. *A. creber* is known to be a xerophilic fungus surviving at low moisture content [132]. More than 100 isolates of *S. maltophilia* are also known capable of living and reproducing on a wide variety of substrates [133]. There are known clinical cases of co-infection with *S. maltophilia* and fungi of the genus *Aspergillus* [134]. The issue of wall freezing can be eliminated by shifting the so-called “dew point” outside the walls of the exhibition halls. However, when working with cultural heritage objects, such as the State Tretyakov Gallery building, it is impossible to change their façade or appearance. This issue was professionally resolved by the restorers of STG in collaboration with researchers [135, 136]: in problem locations, duplicate walls and screens were installed, the halls were kept in conservation for the next two years, after which the art objects were placed in their original place after restoration and conservation work.

### Ultramarine biodeterioration

#### *Aquatic environment*

Over the past 100 years, several shipwrecks have been excavated, raised and conserved [89]. Such grandiose works usually involve a whole team of researchers: archaeologists, historians, biologists, etc. For instance, the Viking-period Oseberg ship was built in AD 820, buried in a grave mound 14 years later, and excavated in 1904 in Norway [137]. The Swedish warship, *Vasa*, was raised in 1961 after 333 years when it was sank after floating about 1300 m in 1628 in Baltic Sea [138]. The *Bremen Cog*, a huge medieval merchant ship built in AD 1380, is the best preserved historic ship in the world. The discovery of the shipwreck in the Weser, Germany, in 1962 was a sensation [139]. King Henry VIII's warship

*Mary Rose* was discovered in 1971 and raised in 1982, and more over the author of this book is a marine archaeologist who was the consultant when this historical relic was raised [140]. And, of course, there are many other interesting examples. All of these historical objects have undergone a process of restoration and conservation and are now in excellent museum conditions. But before such preserving works are carried out, it is necessary to comprehensively assess the degree of biodegradation of a historical relic—to select optimal biocides against microorganisms. Waterlogged wood from shipwrecks is the primary object that often displays bacterial or soft-rot decay. There are two main bacterial groups that degrade waterlogged wood: erosion bacteria and tunnelling bacteria [89]. Erosion bacteria can degrade wood under very low oxygen concentrations, these are just underwater conditions, while tunnelling bacteria are widespread in nature, occurring in both terrestrial and aquatic environments and can tolerate a wide range of temperatures and humidity [141]. It was shown that the most abundant bacterial phyla of the Nanhai No. 1, 800-year-old shipwreck salvaged from the seabed and moved into the Marine Silk Road Museum, China, were Firmicutes (*Gracilibacillus* and *Alicyclobacillus*) and Proteobacteria (*Marinobacter*, *Halomonas* and *Azoarcus*). It's interesting that during excavation the upper deck of Nanhai No. 1 was found to be exposed to air, while the integral hull remained immersed in seawater. Since oxygen is crucial for fungal growth, mentioned above microbial analysis were specifically undertaken on wood exposed to air. And it was shown that *Fusarium* sp. is able to degrade of lignin and cellulose [89] in wood. It has been earlier shown that *Fusarium* sp. isolated from document collections were able to form biofilms, produce pigments, and decrease pH, which resulted in structural damage of the object [142]. To prevent further destruction of the archaeological subject, the wood was treated with specific biocides of the isothiazolinone series. The recommendation for storage of waterlogged excavated wood is a low temperature (the *Mary Rose* spraying system is approximately 5 °C) [143].

### Prevention, conservation, restoration, and control

The approach to the scientific restoration of biological destruction is determined by the type of object (stone, metal, wood, paper, canvas, fabric, etc.), artistic materials (type of pigment, its chemical composition), identification of the microbiological community (mosses, lichens, algae, bacteria, molds, etc.) and selection of targeted biocide. The scheme for determining destructive microflora on art work has an integrated approach combining interdisciplinary techniques [21]: (A). Preliminary diagnosis: (i) sampling (in most possible gentle way);

(ii) inoculation on a number of nutrient microbiological media to identify the morphological characteristics of cultivated microorganisms; (iii) characterization of isolates by microscopy; spectroscopy; (B). Molecular-based diagnosis: (i) PCR diagnosis of genomic DNA of original and cultured isolates; (C). Taxonomic and functional profiling: (i) 16/18S rRNA gene sequencing; (D) Biochemical analysis: enzymatic activity, etc. These methods are often enough to give restorers a timely answer—what is the destructive microflora inhabit the surface or interior of the object, so that they start the urgent conservation/restoration process. However, in a complex case, when working with a large-scale object of art for its complete diagnosis it is possible to gain access to modern analytical biotechnologies—high-throughput sequencing and multi-omics that can be successfully coupled with culture-dependent methods and specific biochemical assays [144].

Since it is necessary to work with cultural heritage objects as carefully as possible, special models simulating mock layers with art materials (pigments, primers, binders, plasticizers, etc.) should be created to test the optimal biocide and choose a scheme for preventing the development of biological damage and choose right restoration way of an art work [5, 61, 145, 146]. Mock layers can be tested in climatic chambers under special temperature and humidity conditions, which make it possible to predict the long-term use of these materials. The mechanical and physical characteristics of artistic restoration materials (crack formation, tearing force, glue creep, optical colorimetric changes, etc.) must also be measured. Microorganisms isolated from art works may exhibit distinctive growth character when cultivated on nutrient media or on mock layers, so they are inoculated onto models, and appropriate conclusions are drawn about their growth rate, the degree of penetration deep into the material, etc., with the help of FTIR, SEM, RS, XRD; XRF-diagnostics [147].

Extensive experience in biocides use against destructive microflora has been accumulated in relation to wall paintings and stone decor in Italy, Spain, India, Poland, France, Russia. The use of one or another biocide treatment depends primarily on the type of object itself and the degree of its biological damage. For monumental and easel art objects, the biocides used are strikingly different. As we know stone monuments and historic buildings are significant parts of the World Cultural Heritage [148]. All of them are facing irreversible damages, including deterioration of structural materials and in some cases ornamental features [149]. The bioreceptivity of stone monuments depends on the chemical and physicochemical natures of the substratum (sandstone for the Angkor monuments, Cambodia [150] and Beishiku

Temple, China [38] widely spread limestone for grave-stones located in Massachusetts, USA [51] and Coimbra monument, Portugal [92], marble for the Certosa of Pavia [54] and Titus Arch in Rome, Italy [50], granite for the Évora Cathedral, Portugal [61] and volcanic rock for the churches of Lalibela in northern Ethiopia [151]), ambient environmental conditions and microclimates. All of these materials will be inhabited by different preferred groups of microorganisms, which requires an individual approach to each object. If we talk about historical monument or wall painting in temples, grottoes, caves and suffers from strong biogenic contamination, before the biocidal treatment the following considerations should be taken into account for cleaning: algal, cyanobacterial layers and crusts should be completely dry before cleaning [152]. Laser technology with antibiotic prophylaxis [153] can also be applied, however, it should be remembered that some pigments, especially cinnabar, lead white and number of others, which are not protected from laser exposure by a layer of varnish, oil or tempera binder, may be subjected to photo-changes [154]. Also it should be kept in mind that melanins and carotenes of lichens and fungi are bio-pigments that can be burned into the crystal matrix by the heat of the laser and the resulting black stain is even more difficult to remove [152]. Biocides with various combinations of active ingredients, developed back in the mid-twentieth century, various azole derivatives (benzimidazole, imidazole, carbendazim) [155, 156], tin organic and mercury organic compounds [154] are still used. Such biocides as sodium pentachlorophenol (NaPCP), benzalkonium chloride (BAC), and fungicides related to guanidine bases continue to be used in Russia [154]. In particular, biocides based on polyhexamethylene guanidine have become widespread in restoration practice in relation to the preservation of cultural heritage [157, 158], since it demonstrates antibacterial and antifungal activity [159]. Most of all these chemical biocides listed above are quite enough toxic for restorers especially in the case of spray technology.

But the tendency nowadays, is to meet the demands of the cultural heritage field with ecological, economic and social aspects, and therefore the replacement of conventional biocides with new ones with improved biodegradability, is critical [160]. In paper [149] actively considered a biocleaning with selective microorganisms to remove harmful pollutants (for example, nitrates, sulfates and organic deposits). This is a modern eco-friendly technology for the conservation of stone heritage. Currently, so-called “green” biocides are being used more often contrary to chemical biocides [56, 161]. In particular, Biotin N treatment of Demetra and Cronos stone sculptures decorating the courtyard of the Buonconsiglio Castle in Trento (Italy)

demonstrated that the cyanobacteria, and most of the green algae and dematiaceous fungi, responsible for the green and black staining, had been efficiently removed [56]. Known methods for combating mold biofilms on fresco paintings. Investigation [61] in Royal Palace of Casas Pintadas (Evora, Portugal) demonstrates test of commercial antiseptic agents Preventol PN<sup>®</sup> [2, 3, 4, 5, 6-sodium pentachlorophenolate], Panacide<sup>®</sup> [4-chloro-2-[(5-chloro-2-hydroxyphenyl)methyl]phenol] and Linquad<sup>®</sup> [Alkylbenzyltrimethylammonium chloride] versus alternative “green” biocides from the BEVO-TECH series. BEVOTECH preparations [162] are a combination of plant extracts from the savannah region of Brazil’s Cerrado region, which have anti-inflammatory, antimicrobial, antifungal effects, and products of secondary metabolism of *Bacillus* sp. bacteria, capable of suppressing fungal growth, possibly due to the synthesis of cyclic lipopeptides [163–165]. Paper [61] shows that these drugs are not inferior in effectiveness to commercial ones, and due to their low toxicity, they are recommended by the authors for use.

A technologically different approach is applied to the restoration of easel art. One way to preserve an original painting is to transfer it to an additional duplicating base to prevent further damage. In Russia, the most popular and traditionally used restoration material for re-gluing is natural collagen sturgeon glue, obtained from the swim bladder of sturgeon fish. This method has successfully proven itself in restoration practice over the past two centuries [166]. Bee honey can also be added to sturgeon glue. This technology was developed and introduced by the restoration artist A.B. Aleshin, who founded the academic school of restoration of oil painting on canvas at the Academy of Arts in St. Petersburg (Russia) in the middle of the twentieth century [167]. Resins (turpentine and balsams) isolated from special tree species—can be used as natural adhesives in the duplication technique. Undoubtedly natural adhesives turn out to be indispensable in certain operations, however, they also exhibit a number of significant disadvantages: the rigidity and fragility of the formed adhesive film, the need to thoroughly clean the back side of the author’s canvas for subsequent gluing with the duplicating canvas, which leads to thinning of the threads, peeling of the soil from the canvas, and, due to the organic component, obvious bioavailability to mold fungi and bacteria when the temperature and humidity conditions are violated. In this regard, the use of synthetic restoration materials, which began to be actively developed back in the 70 s of the twentieth century, is currently gaining popularity. Thus, in restoration practice, copolymers based on acrylates and vinyl acetates are used. For example, BEVA 371 (Italy)—ethylene vinyl acetate, Paraloid B-72—copolymer

of ethyl acrylate with methyl methacrylate (Germany), acrylic resins Plexisol P-550-40 (Germany) and AK-211 (Russia), copolymer of butyl acrylate with methyl acrylate Lascaux Medium for consolidation (Switzerland) [168].

To duplicate large canvases, a combination of various natural and synthetic materials can be used. Thus, in paper [142], during the global restoration of the canvas “Panorama of Mesdach” (The Hague, the Netherlands), 1880, depicting a fishing village in the nineteenth century, 14 m high and 120 m long, four options of adhesives were tested: animal glue with wheat flour, beeswax with dammara resin, an aqueous dispersion of ethylene vinyl acetate mixed with acrylic resin (proprietary formula BEVA 371) and a mixture of two commercially produced acrylic copolymers (Plextol D541 and K360).

Restoration of the icon using the technique of oil painting on canvas “Virgen de Guadalupe” (Granada, Spain) was carried out in the work [5]. The study showed that separate infection of mock layers with fungi *Penicillium* sp. did not produce damaging ability, nor did individual infection with *Arthrobacter* sp. bacteria, while joint infection with these types of microorganisms gave a synergistic destructive effect. The authors note that any change in temperature or humidity in the environment of the painting can contribute to the favorable development of “dormant” microflora.

Graphic works on paper made, for example, with pastel and with traces of biological damage are recommended to be dry over the whole painted surface, as well as the backing and the frame. The next step is mechanical removal of fungal hyphae followed by the sterilization of the whole picture using gaseous formaldehyde [83], which provides disinfection of painted layer and porous of the base. Also in addition to mechanical removal of molds from image area the usage of aqueous solution of sodium borohydrate may be recommended for bleaching some penetrated underneath hyphae. This traditional technique has been successfully used in restoration of pastel portraits of 19-twentieth centuries [84].

One of the promising compounds that can be used to protect works of art from microbiological damage are alkyl nucleosides that have been recently developed [169–173]. Thus, in research, the authors propose compounds related to N<sup>4</sup>-derivatives of 5-methyldeoxyribocytidine, containing an extended alkyl substituent with a carbon chain length of C<sub>10</sub>–C<sub>12</sub>, which makes the molecule more hydrophobic [169]. Among previously obtained nucleoside biocidal compounds, N<sup>4</sup>-alkyl-5-methyl-2'-deoxycytidines demonstrate a higher inhibitory effect on microorganisms compared to cytidines or 2'-deoxycytidines containing the same N<sup>4</sup> substituent. The replacement of the 3'-hydroxyl group

with amino, amino-ethyl or dialkyl-amino groups significantly enhances the antifungal activity [170, 172].

Thus, the modern scientific approach to the preservation of cultural heritage objects is the study of artistic restoration materials in combination with effective biocides, which must meet the following requirements: act against a wide range of pathogenic microflora and be not toxic to humans and destructive to works of [136, 149, 169].

## Conclusions

For the first time, based on the classification of materials of cultural heritage objects, we have introduced the corresponding rainbow code. In that context, biodegradation principles in the colour index above may be a useful guide since it promoting public perception and understanding of conservation and restoration applications for preservation of cultural heritage.

## Abbreviations

ATR-FTIR	Attenuated total reflectance-Fourier-transform infrared spectroscopy
ESI-Q-ToF	Electrospray ionization/quadrupole-time-of-flight
MALDI-ToF	Matrix-assisted laser desorption/ionization-time-of-flight
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
XRC	X-ray crystallography
XRD	X-ray diffraction
XRF	X-ray fluorescence spectroscopy
STG	State Tretyakov Gallery
UV	Ultraviolet

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## Author contributions

DA wrote the entire manuscript, prepared figures, tables, graphical abstract. AZ proposed the main idea and supervised the whole process. The authors read and approved the final manuscript.

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## Declarations

### Competing interests

The authors declare that they have no competing interests.

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