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# Fungal Deterioration of Cultural Heritage Objects

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## Abstract

Significant percent of world cultural heritage artifacts is threatened by fungal infestation. Fungi can deteriorate different substrates via various physical and chemical mechanisms. Hyphal growth and penetration into the substrate can cause symptoms like discoloration, biopitting, cracking, exfoliation and patina formation. On the other hand, chemical mechanisms include acid secretion, release of extracellular enzymes, pigment production, oxidation/reduction reactions and secondary mycogenic minerals formation. These processes can lead to serious, both esthetic and structural, alterations which may be irreversible and could permanently impair artworks. Proper isolation and identification of autochthonous isolates, as well as employment of different microscopic techniques and *in vitro* biodegradation tests are pivotal in understanding complex biodeterioration mechanisms caused by microorganisms, including fungal deteriogens. Biodeterioration and biodegradation studies require multidisciplinary approach and close collaboration of microbiologists, chemists, geologists and different personnel responsible for the safeguarding of cultural heritage monuments and artifacts, especially restorers and conservators.

**Keywords:** alterations, biodegradation, cultural heritage, fungi, multidisciplinary research

## 1. Introduction

*Ars longa, vita brevis* – states the ancient Roman proverb, emphasizing that human need for artistic expression is as old as the civilization itself. Unfortunately, extant artworks are only a fragment of humanity's creations throughout history. Along with artistic creation, there is a need for protection of the artwork from external, frequently damaging influences. Since works of art are an essential part of the cultural heritage legacy of every nation, they ought to be protected for future generations. Biodeterioration is defined as any undesired alteration of the property of the material which is caused by living organisms and cultural heritage objects are frequently prone to this process [1]. Mentioned alterations can be induced by both macroorganisms (plants and animals) and microorganisms (bacteria, algae and fungi). Inadequate storage and irregular maintenance of artifacts in archives, museums and depots oftentimes favorize microbial, especially fungal, proliferation [2]. Since fungi are ubiquitous organisms, with pronounced metabolic activities, they

are capable of colonizing various types of microenvironments therefore constantly causing problems in cultural heritage collections around the world [3].

Fungal propagules - spores and mycelial fragments, are always present in the air, their concentrations being dependent on environmental factors [4, 5]. Namely, during their life cycle, fungi produce various types of sexual and asexual spores which are actively or passively released into the surrounding environment and dispersed by air currents to available substrates [3]. The successful colonization of available substrates requires propagules to be viable in addition to favorable growth conditions [4, 6]. It is known that due to their metabolic activities, numerous fungal species could cause both esthetic and physical damage to a variety of substrates, including stone, paint, paper, wood, textile and other materials of which cultural heritage artworks are made. Therefore, the application of adequate microscopic techniques, proper species identification and physiological characterization of autochthonous isolates are very important to appropriately assess potential threats to cultural heritage artworks, especially on those stored in inadequate conditions [3]. Consequently, biodeterioration and biodegradation studies require a multidisciplinary approach and a close collaboration of scientists (microbiologists, chemists, geologists etc.) and the specialists responsible for the safeguarding of cultural heritage objects, such as restorers and conservators. Therefore, this work addresses general mechanisms of biodeterioration caused by fungi and their role in the deterioration of different materials which constitute cultural heritage artworks.

## 2. Biodeterioration mechanisms

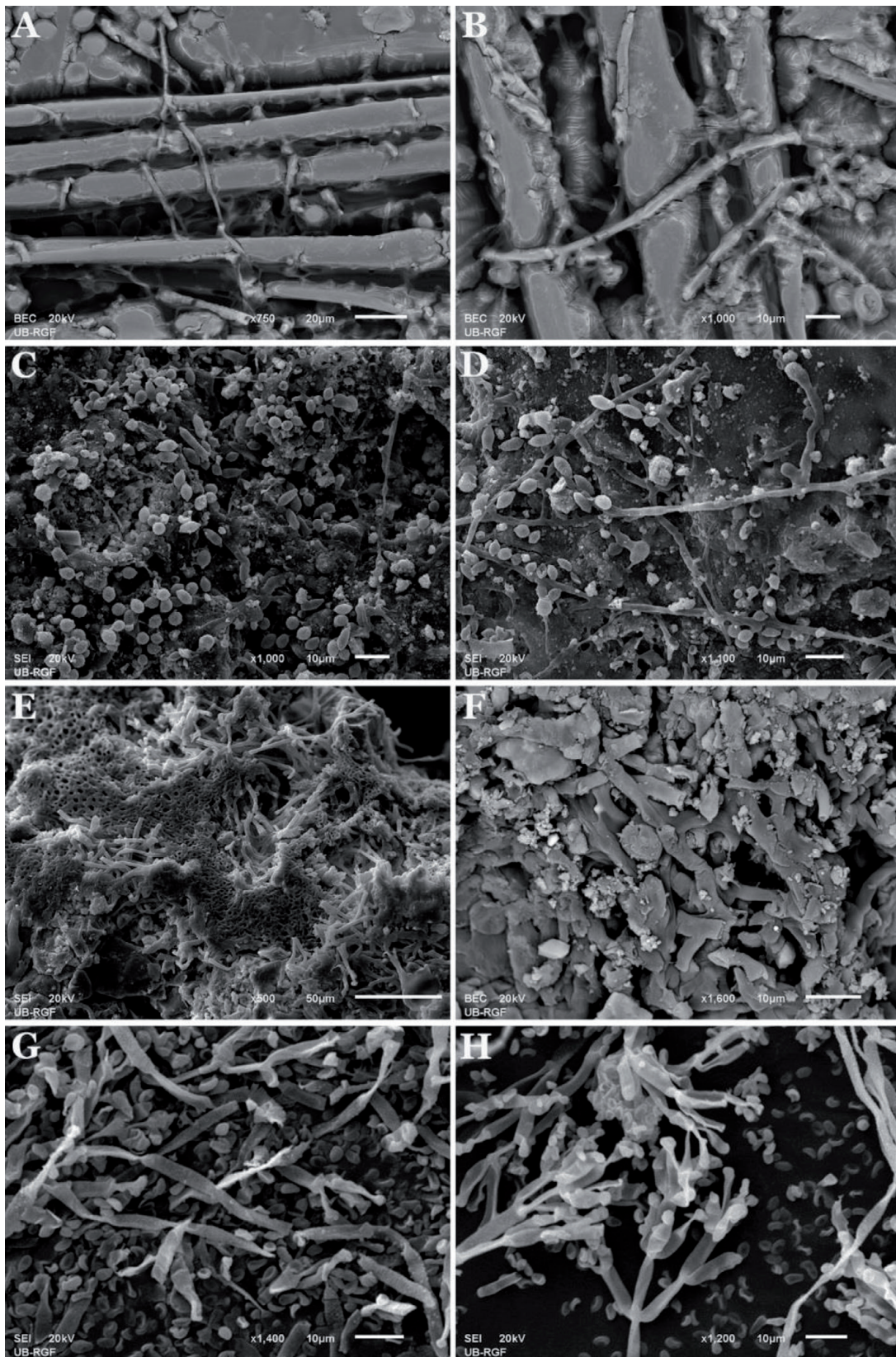
Fungi present on artworks can affect them in two ways – mechanically and chemically. The aforementioned processes, more often than not, are taking place simultaneously. Depending on the substrate's nature, exogenic and endogenic factors, the effect of one process can prove more prominent than the other [7, 8]. Notably, depending on its location, fungal colonizers can affect the substrate in two ways – from the surface to its interior and *vice versa* [7].

### 2.1 Physical processes

Physical processes are taking place under the influence of hyphal apical growth or by the formation of fruiting bodies on the surface and/or the inner layers of the colonized material. If the fungal growth is superficial, it results in the formation of spreading mycelium which covers the substrate and changes the original appearance, hence the esthetic value of the artifact [7]. Inner fungal growth might lead to further damage of the artworks and, especially if paintings are concerned, to the detachment of painted layers (exfoliation). Melanized micromycetes are well known inducers of mechanical deterioration, especially of stone substrates, since melanin provides mechanical rigidity to fungal structures, enhances the turgor pressure and facilitates hyphal penetration [8, 9]. In order to study mechanical deterioration, the application of different microscopic techniques is pivotal, especially *in situ* optical microscopy and scanning electron microscopy (**Figure 1**). The multimicroscopic approach is essential to ensure detailed information, not only about the deterioration status, but also to elucidate alterations that affect works of art, and to detect potential biodeterioration “culprits” [10].

### 2.2 Chemical processes

Mechanisms of chemical biodeterioration are much more complex and prominent than physical ones. Fungi can chemically alter the substrate via assimilation



**Figure 1.** Scanning electron micrographs depicting deteriorated surfaces of cultural heritage objects: A, B. deteriorated icon silk fibers with cracks and gaps formed by *Chaetomium globosum* hyphal growth; C, D. profusion of *Cladosporium* sp. hyphal network with conidial mass on deteriorated wall painting; E, F. Mycobionte of lichenized fungi from the surface of deteriorated limestone monument; G, H. anamorphic state of Xylariaceae infesting wooden iconostasis.

and dissimilation processes [11]. In case of the former, fungi utilize nutrients from the substrate by secreting various enzymes which catalyze the macromolecules' degradation. In contrast, dissimilation represents the production of various extra-cellular metabolites such as organic acids and pigments. These substances modify

or damage the colonized substrate. Since hyphae have high surface to volume ratio, these metabolites can quickly diffuse between the cells as well as from the cell into the substrate [7, 8]. Nowadays, various microbiological, biochemical and petrographical tests are employed to study chemical biodeterioration. *In vitro* tests provide rapid, cost effective estimation of fungal degradation capacity, which helps in evaluating a potential risk to cultural heritage artifacts [3].

**Acid production and acidolysis** are the most studied biodeterioration mechanisms, particularly on inorganic materials [8, 12]. Due to their metabolic activities, fungi produce organic acids such as gluconic, citric, oxalic, malic, succinic, itaconic etc. [13]. Once the spore germination occurs, organic acids are produced by respiration in mitochondria as intermediary products of the citric acid cycle. If the fungi grow on nutrient enriched substrates, these acids are formed in excess and excreted as secondary metabolites [6]. The secreted acids then react with different substances via cation solubilization and chelation reactions. The reaction of acids with different metals (i.e. K, Fe and Mn) results in the formation of organic salts and complex compounds [7]. It should be mentioned that many organic acids, especially the oxalic, are able to chelate different metals in the process called complexolysis. The oxalic acid is able to form complexes with diverse metals (Ca, Mg, Fe, Cu and others), consequently leading to secondary mycogenic minerals formation, calcium oxalate being the most well-known [14]. The aforementioned crystals are present in patinas on stone, frescoes, oil paintings, glass, wood and other materials [8]. It is ascertained that most of the fungi have the ability, in greater or lesser extent, to produce oxalic acid, and subsequently precipitate oxalates [3]. Furthermore, CO<sub>2</sub>, as a product of respiration, in the conditions of increased humidity is transformed into carbonic acid, which then solubilizes calcium carbonate and magnesium carbonate present in limestone, mortar and gypsum. As a result, water soluble bicarbonates are formed. Additionally, increased H<sup>+</sup> concentration favors the colonization of acidophilic fungi, which further facilitates the biodeterioration process [7, 8].

**Enzymes.** Fungi are able to digest organic matter, altering and weakening those materials, by the action of extracellular hydrolytic enzymes, such as lignocellulases, proteases, lipases, pectinases, chitinases, etc. [15, 16]. Enzymes that convert large, complex and often water-insoluble compounds (cellulose, hemicellulose, lignin, proteins and lipids) into low-molecular-weight soluble compounds, play an important role in the biodeterioration and biodegradation processes [7]. Although filamentous fungi primarily use simple sugars as a carbon source, they can be producers of lignocellulolytic enzymes to depolymerize wood or cellulose material for nutritional purposes [17]. Cellulolytic enzyme complex, which is responsible for degradation of cellulose to glucose monomers, comprises of: endoglucanase (hydrolyzes  $\beta$ -1,4-glycosidic bonds within cellulose fibers), exoglucanase (hydrolyzes  $\beta$ -glycosidic bonds and remove cellobiose units from the free ends of chains) and  $\beta$ -glucosidase (hydrolyzes cellobiose and cellodextrin to glucose) [18, 19]. Hemicellulases hydrolyze hemicellulose, which is made up of hexoses (mannose, glucose, galactose) and pentoses (xylose, arabinose) to monomeric sugars and acetic acid. The complex of enzymes that hydrolyze hemicellulose consists of at least eight enzymes: endo-1,4- $\beta$ -D xylanase, exo-1,4- $\beta$ -D xylocuronidase,  $\alpha$ -L arabinofuranosidase, endo-1,4- $\beta$ -D mananase,  $\beta$ -mannosidase, acetyl-acid esterase,  $\alpha$ -glucuronidase and  $\alpha$ -galactosidase [19]. Lignin degradation, characteristic for white-rot fungi, is catalyzed by nonspecific polyphenol oxidases: manganese oxidizing peroxidases, lignin peroxidases, and laccase. This process involves breaking inter-monomer bonds, demethylation, hydroxylation, side chain modification, and aromatic ring cleavage [20, 21]. Some fungal species inhabit art objects that are substrates rich in fibrillar proteins (wool, parchment, leather, silk, etc.). Proteolytic enzymes (proteases) degrade various protein fibers such as collagen (wool), fibroin

(silk) and keratin (parchment) [11]. Lipases catalyze the hydrolysis of triacylglycerols to glycerol and fatty acids. These enzymes might take part in the degradation of widely used painting constituents, linseed and shellac, derived primarily from unsaturated oleic, linoleic and linolenic acids [22].

**Pigment production.** Micromycetes produce pigments which vary in chemical composition and color and are species specific [7, 8]. They are present in hyphae, conidia, or are secreted into the substrate whilst their production is determined by the availability of nutrients and minerals, UV radiation, pH, temperature and other environmental factors [6]. Pigment secretion on/into the substrate leads to the appearance of different, frequently irreversible, colorations leading to the observable changes on cultural heritage objects. This diminishes the aesthetic value of the artwork and accelerates biodeterioration process [7, 11, 23]. Many fungi produce dark colored pigments – melanins, which are responsible for characteristic brownish color of mycelia and reproductive structures. These water insoluble, very stable and resistant, molecules are formed by oxidative polymerization of phenolic compounds (ortho-dihydroxy phenols) [24]. Dark colored melanins are characteristic for dematiaceous fungi (representatives of the former family Deamtiaceae) and are present in cell walls in both granular or amorphous form, while their amount increases with aging [6, 25]. On the other hand, the secretion of various water soluble exopigments into the substrate can also cause different aesthetic damage to artworks, especially on those made from organic materials. A vast number of different pigments are identified and grouped in three main families: the derivatives of toluquinone, of naphthoquinone and of beta-methyl-quinone [8].

### 3. Stone artifacts

A significant percentage of world cultural heritage objects is represented by stone artworks, such as architectural monuments, statues and tombstones, to name just a few [26]. Stone is considered an extreme environment for microbial growth and proliferation, mostly due to the intensive oscillations of diurnal and seasonal microclimatic parameters and low nutrient and water content [27]. Stone surfaces directly exposed to sunlight could achieve temperatures above 60°C, while being simultaneously susceptible to freezing–thawing cycles [28, 29]. Regardless, certain groups of microorganisms, the so called lithobionts, are able to colonize such environments. Primary colonizers are photoautotrophic organisms – cyanobacteria, algae and lichens, while hemolithotrophic and hemoorganotrophic bacteria and fungi are considered secondary. The latter are oligotrophic or poikilotrophic organisms which adapted to survive and grow in harsh or variable environmental conditions [29].

Microbial dwellers of stone surfaces frequently form biofilm, highly structured microbial consortium embedded in a mutual extracellular matrix. Biofilm formation starts with unspecific, reversible interactions, followed by stable interactions which are initiated as a consequence of the formation of specific molecules and structures (lipopolysaccharides, membrane proteins, flagellae). After initial adhesion, extracellular polymeric substances are produced and excreted, enhancing the adhesion and cohesion of cells [8]. Evolutionary advantages of biofilm are to provide protection, resistance to physical and chemical stressors, metabolic cooperation and mutually regulated gene expression [30]. All groups of microbial dwellers of stone are characterized with a high phenotypic plasticity, which is reflected in polymorphism – the change of growth or sporulation forms with regard to external conditions. Therefore, micromycetes, which colonize this environment, are able to form different somatic and reproductive structures - sclerotia, chlamidospores,

conidial clusters, perithecia and pycnidia [29, 31]. Moreover, stone interior is a specific microenvironment for the growth of certain microorganisms such as endolithic fungi. The microclimatic conditions in this environment are a little more favorable – water retention is higher and solar radiation and air current intensities are lower [27].

Among fungal colonizers of stone, microrcolonial fungi constitute a specific ecological group characterized by its slow growth and formation of irregular shaped cells, often packed in aggregates. They rarely form specialized reproductive structures and in turn, by active growth, form thick, pigmented cell walls which enable transition to the state of dormancy during prolonged, unfavorable environmental conditions. The ability to secrete extracellular polymeric substances and thick cell walls, enable water retention, nutrient absorption, desiccation reduction and cellular adhesion/cohesion. These organisms are able to survive for long periods without metabolic activities and their metabolic rates are low even during optimal environmental conditions [27].

Dematiaceous fungi are considered the most important agents of stone deterioration. All representatives intensively produce dark colored melanins which provide protection from excessive environmental radiation (UV radiation, x- and  $\gamma$ -rays) and chemical stressors. This group encompasses microcolonial fungi and black yeasts, species of genera: *Acrodictys*, *Aureobasidium*, *Capnobotryella*, *Coniosporium*, *Exophiala*, *Hormonema*, *Hortaea*, *Knuffia*, *Lichenothelia*, *Monodictys*, *Phaeococcus*, *Phaeococcomyces*, *Phaeosclera*, *Sarcinomyces* and *Trimmatostroma*, which are very important stone colonizers in arid and semiarid environments [27, 32]. Additionally, deatiaceous fungi include cosmopolitan filamentous species of genera *Alternaria*, *Cladosporium*, *Ulocladium*, *Epicoccum* etc. which are main colonizers of stone in more favorable conditions of temperate and humid environments [32]. They are especially important deteriorogens of restored stone artifacts [27].

Fungi can deteriorate stone via physical and chemical mechanisms. Physical mechanisms include hyphal penetration of the rock surface which causes its fragmentation, while chemical ones include secretion of acidic metabolites and pigments and oxidation of mineral forming cations. Although many microorganisms are able to produce acids, fungi are considered as the most potent ones in nature that degrade rocks and minerals. The production of various acidic metabolites leads to the biocorrosion - dissolution of the mineral substrate, resulting in the formation of various secondary mycogenic minerals [26]. *In vitro* acid production and formation of calcium oxalate and calcium carbonate minerals have been reported by autochthonous isolates from limestone monuments such as ancient Roman stela and Portuguese king tomb [26, 33]. Synthesis and excretion of extracellular pigments mostly affect the stone aesthetically, although studies concerning pigment production on stone monuments are generally scarce [26]. Due to the mentioned processes, symptoms such as biopitting, biogenic patina and colorations can occur [11]. The growth of dematiaceous fungi results in the presence of dark stains while microcolonial fungi are the main culprits of biopitting phenomena on limestone and marble artworks, [32, 34]. Sanctuary of Delos in Greece is an example of mentioned biopitting phenomena [35].

Lichenized fungi are important colonizers of stone substrates. These organisms have a high tolerance to variations of environmental factors, especially temperature, insolation and water availability, which is responsible for their cosmopolitan distribution and ability to colonize extreme environments [36]. These organisms are poikilohydric, i.e. they are capable of enduring cycles of desiccation and rehydration due to their ability of lowering their metabolic rate and enter cryptobiosis under conditions of low water availability [37]. Endolithic lichens are of special importance to stone deterioration, since they are capable of the deepest penetration

into the stone compared to other microorganisms [38]. Apart from the fact that hyphae of the mycobiont can penetrate the rock surface (**Figure 1E** and **F**), perithecia formation by some endolithic species can penetrate the surface from the inside out, which leads to biopitting [27]. Additionally, lichen growth could cause exfoliations, encrustations and disaggregation of the stone surface [11, 39]. Conversely, lichen morphology and its adhesive capability aren't always in correlation with its capacity to alter the substrate and physiological differences between the species are considered to be more significant [40, 41]. In fact, synthesis of different chemical deterioration agents is done by the mycobiont. Apart from carboxylic, lichens have the ability to produce lichen acids, semisoluble polyphenolic compounds which are able to form complexes with metal cations. The capability of lichens to absorb and maintain water enhances the duration of chemical reactions and therefore facilitates deterioration process [39, 41]. Lastly, some authors have reported the presence of orange-brownish patinas (*scialbatura*) on stone monuments made from limestone and marble. These colorations mainly consist of calcium carbonate and calcium oxalate minerals, sometimes intermixed with fragmented lichen thalli [42, 43]. Although this symptom is associated with deterioration, it is hypothesized that it may have a protective role to the monuments [27].

#### 4. Wall paintings

Wall painting, as the pictorial technique, encompasses all painting techniques aimed at beautifying wall surfaces. There is no universally accepted definition of fresco, as well as consensus on what techniques can be included in this type of wall painting, however, the term *al fresco* generally refers to paintings made on a fresh lime mortar with mineral pigments mixed with water [44]. On the contrary, in *fresco a secco* or *al secco* technique painting is done on a dry plaster with paints prepared by mixing mineral pigments with various organic binders [32, 45]. Although hallmarked as an extreme type of habitat, painted layer and lime mortar are also considered to be very suitable and bioreceptive substrates for fungal growth. This is due to the mineral composition and porous nature of lime mortar, and the fact that organic and inorganic components of the painted layer represent a suitable niche for the development of a wide range of heterotrophic microorganisms [46].

Fungal infestation of wall paintings can occur from several sources including contaminated indoor air as the main, but also soil and plants of immediate vicinity, visitors, contaminated conservation tools, and indoor hotspots as secondary sources [7]. Whether a certain fungus will be able to colonize the painted layer or mortar depends on the ecological and physiological requirements of a given species. If the requirements are met the process is further controlled by three main factors: nutrient availability, relative humidity, and temperature [32]. The origin of nutrients in fresco painting is related to (1) additives (chaff, wheat paste, barley flakes, animal hair, hemp and flax fibers, egg whites, oils, fats) mixed with mineral and complex fillers of chopped straw and lime mortar; (2) additives used in the preparation of mortar (liquid resins, tar, polymer latex, emulsions, bitumen, milk, olive and linseed oil, lard, animal blood); (3) binders of plant and animal origin mixed with mineral pigments; (4) casein, paraloid mixtures, fixatives and consolidants based on polymer components (cellulose acetates, polyvinyl acetate, polymethyl acrylate, etc.) used in restoration works [32, 45, 47–50]. These organic components determine the richness of the fresco mycobiota. Since the composition of the painted layer and mortar is predominated by inorganic components, its mycobiota differs greatly from the fungal communities established on other painted works of art [45]. Furthermore, heterogeneously pigmented zones of the painted



layer can be considered as selective substrates that condition the development of a specific mycobiota [51]. Using culture-dependent methods, the most commonly documented fungi on painted layer and mortar of wall paintings are Ascomycota of genera *Acremonium*, *Alternaria*, *Arthrinium*, *Aspergillus*, *Aureobasidium*, *Beauveria*, *Botrytis*, *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Curvularia*, *Dreschlera*, *Engyodontium*, *Epicoccum*, *Eurotium*, *Exophiala*, *Fusarium*, *Geomyces*, *Gliomastix*, *Phoma*, *Penicillium*, *Scopulariopsis*, *Sepedonium*, *Sporotrichum*, *Stachybotrys*, *Stemphylium*, *Trichoderma*, *Trichotecium*, *Ulocladium* and *Verticillium* [7, 45, 52–54]. Contamination by fungi from phyla Basidiomycota is rare (e.g. *Coprinus* spp.), while Zygomycota of genera *Mucor* and *Rhizopus* are isolated frequently but are considered only surface contaminants [45, 55].

Species of the genera *Aspergillus*, *Aureobasidium*, *Alternaria*, *Cladosporium* and *Penicillium*, are frequently listed as the most common wall paintings contaminants, as well as the primary fresco deterioration agents in temperate climates [56]. Many *Cladosporium* species are recognized as the main biological agents in the process of biodeterioration of wall paintings since they are able to not only induce brown discolorations, but also penetrate through the entire painted layer all the way to the mortar support [57] (**Figure 1C** and **D**). Many species of the genus *Penicillium* are known to develop and intensely sporulate in a period of only few days to a few weeks on periodically moist fresco paintings [58]. Furthermore, in addition to dominant members of fungal community, species from less represented genera can also significantly contribute to the damage of wall paintings. For example, isolation of *Phoma* species from the surface of painted layer indicates that given wall paintings are in an active process of decay [59]. *Chaetomium*, *Aureobasidium* and *Epicoccum* species, due to strong proteolytic activity, degrade protein binders of the painted layer, which results in the lifting and separation of the painted layer from the support. Likewise, it has been recently contemplated that species of the genera *Mucor* and *Rhizopus* might be more involved in process of biodeterioration of wall paintings than originally considered, as it was shown in in vitro experiments that they are able to degrade protein binders and epoxy resins [60].

Mechanical (1) and chemical (2) activity of fungi directly results in damages to structural and esthetic integrity of fresco paintings. (1) Hyphal penetration, together with formation of fruiting bodies and various modifications of mycelium, increases internal pressure thereby forming new cracks in the painted layer and mortar, as well as expanding the existing ones. Damages caused by mechanical activity are considered by some to be of greater importance compared to changes induced by environmental factors and fungal chemical activity [61]. Furthermore, aside from mechanical activity, damages as the result of change in substrate properties can also incur due to utilizing fresco components as a source of nutrients for fungal growth (2'1) and/or due to secretion and interaction of fungal metabolites with organic and inorganic components of the painted layer and mortar (2'2) [7, 62]. (2'1) Extracellular enzymes break down complex organic components into simpler molecules enabling their absorption and easier penetration of hyphae into the substrate which results in cracking and peeling of the painted layer and mortar. The main enzymes involved in this process are  $\beta$ -glucosidase, phosphatase, lipase, arylsulfatase, esterase, protease and endo-N-acetyl-PD-glucosamidase [63, 64]. (2'2) Excreted organic acids chelate metal ions present in mineral pigments and mortar, resulting in the formation of mineral salts and complex compounds that increase pressure in pores, which leads to cracking, peeling, and loss of fragments of the painted layer and mortar [65, 66]. Additionally, salts stimulate formation of surface irregularities that serve as suitable sites for the settlement of heterotrophic microorganisms, thereby increasing the bioreceptivity of fresco painting [67]. In these circumstances, there is an uncontrolled biofilm development and acceleration of chemical dissimilation activity through

oxidation, reduction and transformation of metal ions in pigments, primarily Fe and Mn, but also As, Pb, Cu, Zn and Hg, resulting in the alterations to the original color of the painted layer [53, 68]. Aside from organic acids, very stable and persistent fungal pigments (melanins, mycosporins, quinones, hydroxyanthraquinones and carotenoids) secreted onto the surface induce changes in the original coloration, which is a process that depends on the chemical composition of the pigment, environmental conditions and interactions with substrate components [7, 32].

## 5. Canvas oil paintings

One of the best-known pictorial techniques today, oil painting on canvas, emerged in the Middle Ages and has since been one of the most important art expressions, constituting outstanding works of art with important historic and cultural value [69]. Structurally speaking, these works of art are composed of the pictorial layer between the protective covering varnish and the ground (or preparatory layer) spread on a linen canvas. Compared to the other forms of artwork, oil paintings on canvas possibly provide the widest range of microhabitats and nutrients that may be exploited by a large variety of microbial species [70]. Materials that constitute the painting, i.e. the cellulose of the canvas support, organic adhesives (i.e. various animal, fish and plant glues) used in sizing the support, natural varnishes, and the oils used in binding the pigments (linseed, turpentine and other oils) are all composed of organic molecules of high nutritional content that are all easily degraded [32, 63]. These organic molecules encompass sugars, gums, and other polysaccharides, proteins and waxes, but also less chemically defined mixtures of biomolecules, such as egg yolk, bile, and urine, as well [45]. Organic glue pastes used to coat the back of paintings with linen canvas, i.e. “re-lining”, may also represent a rich nutrient source [71]. Furthermore, dirt, dust and other environmental contaminants (volatile hydrocarbons released from machinery, respiration and cigarette smoke) deposited on the surface of the oil paintings provide nutrients as well [63].

Given the wide range of organic molecules that are present in oil paintings, many different microorganisms may grow provided inadequate storage and favorable environmental conditions, primarily high relative humidity and temperature, are met [45]. These specific environmental conditions may start and/or accelerate the microbial growth, which otherwise would persist on the obverse and the reverse side of the painting in a dormant metabolic state [70]. Among multitudes of different microorganisms, fungi are notorious for their ability to inhabit and decay paintings due to their enormous metabolic activity and ability to grow at low  $a_w$  values [32]. However, to the best of our knowledge, despite being one of the most numerous objects exhibited and stored in museums and warehouses worldwide, relatively fewer numbers of studies to date have been engaged in describing the fungal communities dwelling on canvas oil paintings compared to the other forms of art. Using culture-dependent methods, the most commonly documented fungi on painted surface, canvas and wooden frame are Ascomycota of genera *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botrytis*, *Cladosporium*, *Drechslera*, *Epicoccum*, *Fusarium*, *Penicillium*, *Ulocladium*, *Scopulariopsis*, *Stemphylium*, *Trichoderma* and *Wardomyces*, with occasional observations of teleomorphs from *Chaetomium*, *Emericella* and *Eurotium* genera [32, 63, 69, 70, 72, 73]. Review of the above referenced literature data has highlighted fungi that were most frequently isolated from the infested oil paintings: *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *A. versicolor*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, and *Penicillium chrysogenum*. Aside from the Ascomycota, the only other documented species are Zygomycota of *Cunninghamella*, *Mucor* and

*Rhizopus* genera, but they are in most cases regarded as transients only, i.e. part of the surface dust deposits, and not partaking in the complex process of biodeterioration [32, 74]. Air mycobiota of the depots and exhibition rooms, where paintings are stored and exhibited, respectively, was shown to correlate well with fungal communities observed on the surface of the paintings indicating airborne origin of the infestations. Furthermore, higher density and diversity of the fungal communities on the obverse of the painting is a direct result of the higher amount of airborne fungal propagules deposited on the painted surface due to gravitational settling of propagules from the air [22]. It has to be noted, however, that there are discrepancies between results of the studies utilizing culture-dependent and -independent methods. Results of the limited number of studies on non-culturable fraction of the oil paintings fungal communities showed discrepancies in numbers, with unculturable or not viable part of the community being more dense and prevalent, as well as in composition: Ascomycota of genera *Alternaria*, *Ascoidea*, *Aspergillus*, *Blastomyces*, *Candida*, *Chaetomium*, *Coccidioides*, *Diplodia*, *Eutypa*, *Exophiala*, *Fusarium*, *Gaeumannomyces*, *Histoplasma*, *Marssonina*, *Microsporium*, *Neofusicoccum*, *Paracoccidioides*, *Parastagonospora*, *Penicillium*, *Penicilliopsis*, *Pestalotiopsis*, *Pichia*, *Rasamsonia*, *Lodderomyces*, *Neurospora*, *Sordaria*, *Talaromyces*, *Thermothelomyces*, *Thielavia*, *Trichoderma*, *Tuber*, and *Verticillium* were dominant, followed by Mucoromycota of genera *Phycomyces* and *Lobosporangium* and Basidiomycota of genus *Puccinia* [63, 75, 76].

Fungal induced deterioration of canvas oil paintings can occur on both the obverse and the reverse side. It usually starts on the reverse side as canvas components are more readily degraded than those found on the obverse side. In addition, support polymers and the glue sizing in the canvas act as supplementary substrates for fungal growth [22]. Canvas was shown to be one of the most susceptible painting materials (only surpassed by linseed oil), with the susceptibility depending on the percentage content of cellulose, lignin, and other organic components [74, 77]. The higher the percentage of cellulose and lignin, the more resistant it will be to fungal attack [78]. Due to their ability to produce cellulolytic enzymes responsible for cellulose fibers dissolution, fungi of the genus *Alternaria*, *Aspergillus*, *Cladosporium* and *Ulocladium* are considered to be the main agents of canvas deterioration [63, 69, 76].

On the other hand, the degree of deterioration on the obverse side depends on the oil paints and their mode of application. Varnish, added to provide protection against environmental attacks, is the least susceptible painting material to fungal attack, while many pigments are known to possess antifungal properties [74]. Fungal communities are found to be less dense and diverse in pictorial layers containing pigments with heavy metals (e.g. Pb, Cu and Hg), compared to those found in pictorial layers without such compounds [75]. On the obverse side, hydrolytic activities that the fungi undertake to sustain growth results in the detachment of the paint layer from the support, with further increase in the loss of material happening due to excretion of destructive metabolites, i.e. organic or inorganic acids and the additional production of extracellular enzymes: lipases, esterases, endo-N-acetyl-glucosaminidases and proteases [22]. Such activities lead to formation of structural impairments usually manifested as exfoliation of paint layers, cracking, peeling, formation of paint blisters, detachment of the paint layer from the support, deformations and loss of strength of support. Strongly linked to these damages are the esthetic impairments (preceding the structural damages or forming as a resulting consequence) manifesting as the change of the original coloration due to pigment alterations, biofilm formation on the painted surface or staining as the result of pigment excretion by fungi [75]. Fungi of *Aspergillus* and *Penicillium* genera degrade glues and oil binders, and dissolve paints, contributing

to chromatic alterations of the painted surfaces and detachment of the support, while *Aureobasidium pullulans* is considered by many to be the main biological agent of paint deterioration [1, 45, 69].

## 6. Wood and paper

Wood as a material has been widely used as a structural element for many types of constructions or for ritual, religious and decorative purpose. Historically, human usage of wood is embedded in wood cultural heritage reflecting past and present human life, culture, ideals, symbols and values. Wood cultural heritage objects can be classified as: moveable (musical instruments, frames, furniture, sculptures, iconic altar etc.), immovable (temples, churches, chapels, royal palaces, pagodas, wooden bridges etc.) and underwater (shipwrecks, foundation piles, wooden cargo or contents which were partially or totally underwater, periodically or continuously for at least 100 years), according to UNESCO [79]. Lignocellulose is the major component of wood biomass and consists of three types of polymers, cellulose (40–55%), hemicelluloses (24–40%) and lignin (18–35%) that are strongly intermeshed and chemically bonded by non-covalent bonds and by covalent crosslinkages [19]. Organic nature and optimal water content make the wooden substrate suitable for microbial attack [80]. However, microbial deterioration of these materials occurs only under poor conservation conditions: high humidity level, soil contact, poor ventilation, and rare maintenance [81]. Even though deterioration of wood cultural heritage is a process conducted by all groups of microorganisms, fungi have the most significant potential to affect this type of historic artworks [82]. Biodegradation and biodeterioration of wood materials is predominantly dependent on its moisture content, requiring a minimum of 20% of water. Despite dry wooden objects are considered to be resistant to fungal degradation due to low moisture content, occasional wetting, leaks and flooding can increase humidity, enabling conditions for fungal growth. The mechanism of biodeterioration implies the development of fungi on the surface (**Figure 1G** and **H**) or between internal structures, the production of extracellular enzymes, the structural change of basic biopolymers, which ultimately results in visible changes of the object [81]. Generally, fungi that attack wooden material can be distinguished as white-rot, brown-rot and soft rot fungi. White rot fungi are the only organisms that can completely depolymerize and degrade all lignin components as well as cellulose and hemicellulose. The largest number, of about 1500 species, belongs to the Basidiomycota and a smaller number belongs to Ascomycota. Most commonly found species are from genera *Bjerkandera*, *Donkioporia*, *Fomes*, *Irpex*, *Phanerochaete*, *Pholiota*, *Pleurotus* and *Trametes*. Brown rot fungi decompose cellulose and hemicellulose while lignin degradation is limited to the process of demethylation of methoxyl groups, partial oxidation and depolymerization in a non-enzymatic catalytic cycle of the Fenton type where the free radical reaction is initiated by hydroxyl radicals (OH•). Only 6% of the total number of species that have been confirmed to be able to decompose wood mass belong to this group, and almost all representatives inhabit coniferous wood (species from the genera *Antrodia*, *Aspergillus*, *Coniophora*, *Coriolellus*, *Fusarium*, *Gloeophyllum*, *Merulis*, *Paxillus*, *Poria*, *Postia*, *Serpula*, etc.). Soft rot fungi decompose cellulose and hemicellulose, while the process of lignin modification is limited to demethylation. It is typical for this group of fungi to attack wood mass with high levels of humidity and low lignin content, forming a microscopic cavities inside the wood, sometimes leading to discoloration and occurrence of cracking pattern similar to brown rot (species from the genera *Alternaria*, *Chaetomium*, *Daldinia*, *Humicola*, *Stemphylium*, *Xylaria*, etc.) [79, 82, 83]. Soft-rot decay has been described

frequently from construction timbers, ancient Egyptian wooden coffins, wooden structures of Buddhist temples, waterlogged archeological wooden material, which can be related to more tolerant growth conditions. In comparison to soft-rot species, other two groups of wood decay fungi have a relatively narrow spectrum of growth conditions (preferring moisture content between 35% and 50%) but were documented in wooden churches, historic timbers and various historic buildings [79]. Objects of wood cultural heritage in the outdoors have been more disposed to deterioration process than those in the indoor as they are exposed to the relative humidity of 70% or higher, which is stimulatory for fungal growth. Additionally, the physical contact with moisture-absorbing surface can also provoke fungal decay [79]. On the other hand, fungal degradation of waterlogged and buried wood is much slower than for that found in dry environment, but once excavated decay can occur rapidly [82]. Finally, it should also be emphasized that in some cases fungal decay can be observed in extreme conditions such as in wood with 17.4% of moisture content [79].

Additionally, paper, which is mostly produced by mechanical and chemical processing of cellulose fibers, originating from wood, is the most important material on which cultural achievements in the whole world are recorded and preserved. Since it is created as a product of the wood industry and consists of 90–99% of cellulose fibers, in the ecological sense, paper is considered to be a cellulose substrate. Books, documents, writings, old maps, photographs, etc. are objects made of paper that are most often kept in libraries, archives and museums. Apart from paper, cotton and linen are fabrics which main components are cellulose fibers. Also, paraments, defined as hangings or ornaments used for decorations of Christian churches' interiors are often tailored of cotton and linen. In that sense, it should be emphasized that, art objects made of cellulose fibers can be colonized by cellulolytic fungi. These fungi can degrade cellulose fibers via process of cellulolysis, defined as an enzymatic hydrolysis of cellulose polymer into glucose units. In that sense, the fungi capable for production of cellulolytic enzymes are frequently isolated from paper, especially from old books or documents kept in libraries, archives and museum depots. Among the frequently encountered species on the paper substrates are the members of genera *Chaetomium*, *Penicillium*, *Aspergillus*, *Eurotium*, and *Trichoderma* [84, 85]. Some authors reported the presence of *Fusarium* sp., *Humicola* sp., *Paecilomyces variotti* and *Trichoderma viride* on deteriorated art photographs which were part of the collection of the Museum of Contemporary Art (Belgrade, Serbia) [86]. Due to their ability to degrade cellulose fibers, these fungi are referred to soft rot fungi [87]. Ascospores and conidia of different cellulolytic microfungi ubiquitously present in the environment worldwide could easily be deposited on papers and other cellulolytic materials (books), and when optimal conditions are met, they could germinate, elongate and proliferate and consequently lead to fast book decay. Also, fungi can deteriorate the paper-based materials mechanically via hyphal penetration or through production and excretion of pigments and organic acids [88]. A specific and irreversible phenomenon in the form of brown to red spots on paper material has been described in the literature [89]. Since the color of these spots resembles the color of fox fur, the phenomenon is called “foxing”. The origin of this phenomenon on paper documents is explained by two theories - abiotic and biotic. According to the abiotic theory, “foxing” is a consequence of natural chemical processes, most often oxidation, which takes place on paper material, as well as a consequence of the deposition of certain compounds on the paper surface [90]. According to the biotic theory, “foxing” is caused by microorganisms, especially fungi that produce organic acids which deteriorate the paper, permanently damaging it [89]. Isolation of a large number of fungal species from the parts of the paper on which the symptoms of “foxing” are

observed, speaks in favor of biotic theory. The famous piece of art affected with foxing symptoms is self-portrait of Leonardo da Vinci's drawn in red chalk on paper and deposited in Royal Library in Turin. As a main culprit responsible for "foxing" spots on this famous piece of art, certain authors reported the fungus *Eurotium halophilicum*, which spores are documented near the foxing spots via SEM analyses, along with oxalates of fungal origin [91]. Additionally, they pointed out that tonophilic fungi can germinate on paper materials and also can metabolize organic acids, oligosaccharides and proteins, which react chemically with the material at a low water activity, forming brown products and, via oxidative reactions, leading to foxing spots.

## 7. Textile

Textile is defined as elastic material produced by spinning of natural or synthetic raw fibers and which are in final form composed of interlocked network of threads or yarns. Apart from synthetic fibers, materials used for textile production could be of plant or animal origin. Cotton, linen, hemp and jute are widely used fabrics of plant origin and hence they are composed of cellulose fibers. Many old-fashioned and vintage attires and garments, worn by our ancestors were woven from these fabrics and deposited in museum depots and exhibition rooms, or still worn during traditional festivities. In that sense, those fibers could be easily attacked by cellulolytic fungi, and mechanisms of biodeterioration are similar to those of the fungi capable of degrading paper-based materials. On the other hand, animal fibers include wool and silk [92]. Wool is a textile fiber obtained from various hairy mammals, but mostly from sheep, and main constituent of wool is a protein keratin. When compared with textile fibers of plant origin, wool is more resistant to fungal attack due to its specific cross-linked structure with disulphide bonds [93]. However, fungi capable for keratinolysis can attack wool fibers and cause wool degradation. Pioneer research by some authors demonstrated that fungi are the main "culprits" responsible for wool degradation, in much higher degree than bacteria, and members of genera *Aspergillus*, *Chaetomium*, *Fusarium*, *Microsporium*, *Penicillium*, *Rhizopus* and *Trichophyton* are among the most frequent wool colonizers [94]. In an *in vitro* study, other investigators showed that fungi do not grow directly on the wool fibers but rather between the fibers, and reported that proteolytic fungi *Cladosporium cladosporioides* and *Penicillium corylophilum* have the most intensive growth when inoculated on wool and also the highest impact on degradation and aging of wool fibers [93]. Although wool is very resilient to microbial attack, the silk is considered to be a natural fiber most resistant to the biodeterioration [92]. Silks are defined as fibrous proteins spun into fibers through activity of spiders and insects. The main producer of commercial silk is a domestic silkworm *Bombyx mori*. Chemically, raw silks are composed of highly crystalline polypeptide fibers, fibroin, linked to one another by a gum-like protein, sericin [95]. The amino acid composition of silk polypeptides results in a very stable  $\beta$ -pleated crystal structure, making fibroin totally insoluble in aqueous solvents and hence very resistant to enzymatic hydrolysis [96]. In that sense, scientific reports regarding the microbial deterioration of silk material are scarce. Still, some authors reported for the first time the mechanical deterioration of Japanese silk from Serbian museum collections caused by proteolytic fungus *Chaetomium globosum*. Scanning electron microscopy analysis of the analyzed scroll indicated that *C. globosum* hyphae are capable of the mechanical deterioration of silk, causing cracks and gaps in fibroin fibers and consequently lead to visible impairment of silk artifact itself (**Figure 1A and B**) [97].

## 8. Conclusion


Specific morphology and physiology of fungi enables them to colonize multifarious substrates, including cultural heritage artifacts. Due to their pronounced metabolic capacity, fungal deteriogens are able to significantly influence both aesthetical appearance and integrity of monuments, sculptures, murals, paintings, textile and documentary heritage. Nowadays, conversance of fungal biology is becoming crucial in proper assessment of contamination and colonization of artworks but also in their adequate storage and protection. Since mycology as a science gains more and more application in the conservation and restauration procedures, the investigations in this scientific filed become essential in cultural heritage safeguard.

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