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Review

Natural substances as biocides in the fungi treatment on artistic products to protect the environment and health of restoration workers

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Abstract
Cultural heritage artefacts, such as stone works, books, manuscripts, and parchments, are subjected to severe biodeterioration processes over time caused by fungi, algae, lichens, and complex communities of microbial biofilms. Fungal contaminations are widespread as active or dormant spores which are present in the air and on objects and can lead to irreversible biodeterioration processes. Highly toxic and hardly biodegradable compounds are commonly used in cultural heritage conservation and restoration practices, which can be harmful even to the treated materials. Therefore, sector operators may be exposed to both biological and chemical risks, and often the failure to use adequate protective equipment increases the exposure risk. The interest of many researchers has been recently orienting, in this sector also, towards the use of natural products, as a valid alternative both for operators' health and environmental protection. This review aims to provide an overview of the biological and chemical risks associated with the treatment of fungal biodeterioration of artistic works and suggests the use of natural substances as a possible alternative to chemical synthetic products for the safety of restoration operators.

Keywords
cultural heritage, biodeterioration, biodeteriogens, biological risk, essential oil, hydrolates, plant extracts

Introduction

Cultural heritage is the complex set of material testimonies (artistic or symbolic) inherited from previous generations, belonging not only to individual cultures but to all humanity (Jokilehto, 2005). Over time, cultural heritage undergoes several biodeterioration processes triggered by the growth of fungi (yeasts, mycelia, and molds), algae, lichens, and complex biofilms of microbial communities

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which represent an important aspect in the biodegradation processes of stone works and also of books, manuscripts, and parchments. Biodeterioration can be defined as “any undesirable change in the properties of a material caused by the vital activities of organisms” (Hueck, 2001). Contamination by fungi, which are known to be important bio-deteriogens, is a common phenomenon, especially when effective microclimatic control systems are lacking. Spores, whether active or dormant, are ubiquitous in the air and on object surfaces, and they are the source of numerous biodeterioration processes, which are often irreversible (Urzì and De Leo, 2010). These contaminations can spread quickly and invasively with high humidity and poor ventilation or if extraordinary events occur (floods, pipes ruptures, percolations, or air conditioning systems failures). These conditions could favor the massive development of fungi harmful for the conservation of artistic heritage and for human health (Saifeldin et al., 2014).

In the restoration and protection activities of cultural heritage from fungi, highly toxic and non-biodegradable compounds are commonly used, which are sometimes also harmful to the treated materials. Consequently, the sector operators could be exposed both to biological and chemical risks due respectively to pathogenic fungi and to chemical synthetic biocides. Furthermore, failure to use adequate protective equipment could increase the exposure risk (D’Angelo et al., 2012). In many fields, such as medical, industrial, food and restoration, the use of medicinal plant derivates (essential oils, hydrolates, aqueous, alcoholic, and hydroalcoholic extracts) as biocidal substances has recently grown up. The development of new materials and methods, which are eco-compatible, safe, and long-lasting alternatives to those currently in use for surface protection and corrosion and biodeterioration inhibition, represents a necessary response for the safety request not only of the protected asset but also of the operator and the environment.

In the last ten years, the data obtained from the available studies on the use of essential oils, hydrolates, and plant extracts as biocides in the cultural heritage treatment are very encouraging. The advantage of these natural products concerns the operators’ health safeguarding, in the reduction of the environmental impact (air, water, soil), and in waste reduction. This review aims to provide an overview of the biological and chemical exposition risk during the activities of prevention and treatment of the fungal biodeterioration of artistic works and suggests the natural substances as a possible alternative to chemical synthetic biocides to protect the restorer’s safety.

Biodeterioration

The biological agents that produce biodeterioration (such as fungi, bacteria, mosses, lichens, higher plants, and animals such as insects and rodents) are called biodeteriogens and can cause irreparable damages to artifacts (Lognoli et al., 2002). Biodeterioration is the consequence of the biocolonization process (formation of biofilm) which depends on the material’s “bioreceptivity”. This last term was used for the first time by Guillitte (1995) to highlight the ability of a given material to be colonized by living organisms. It depends on various parameters such as environmental conditions, material composition and conservation state, and possible surface treatments. Bioreceptivity must be considered, before any sustainable conservation procedure is performed, because it highlights the relationships between the surface, the specific environment, and the organisms that thrive there. In fact, a microbial community immersed in an extracellular polymeric matrix (biofilm), which adheres irreversibly to a surface, has a high resistance to environmental stresses and treatments (Lo Schiavo et al., 2020). These organisms develop in specific matrices based on their metabolic characteristics, because the chemical composition of the matrices influences the selection and growth of biodeteriogen populations in different ways. For example, organic materials (paper, wood, fabrics, parchments, etc.) provide nutritional components for the growth of specific bacterial and fungal microflora (Görs et al.,

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2007; Fazio et al., 2010), while inorganic materials (stones, marbles, metals) undergo the action of organisms that derive their nutritional source from occasional elements such as powders, resins, glues or previous biological colonization, such as cyanobacteria, algae, lichens and heterotrophic bacteria that form subaerial biofilms (Gorbushina and Broughton, 2009). The main biodeterioration cause is the combination of atmospheric pollutants and fungi present on the artifacts depending on the nature of the support material. The fungal biodeterioration mechanisms are related to: i) their ability to grow at low values of water activity, which allows them to colonize different materials and ii) their metabolism products, and molecules excretion (pigments, organic and inorganic acids, chelators, enzymes, and extracellular polymeric substances), that modify the chemical-physical material properties causing enzymatic hydrolysis of organic materials, biocorrosion, biomineralization and penetration into the manufactured goods (Gómez-Alarcón et al., 1994; Kumar and Kumar, 1999; Sterflinger, 2000; Tiano, 2002; Görs et al., 2007; Sasso et al., 2013). In fact, fungi are able to colonize, degrade and alter a great variety of materials used to create monuments and artifacts preserved in archives, museums, and historic buildings (Sterflinger, 2010; Fierascu et al., 2012):

- stone artworks (Kumar and Kumar, 1999);
- paper (books, manuscripts, parchments) (Keopannha, 2008);
- textile materials (Aranyanak, 1995);
- leather, wood, bone, ivory, horn, and plasters (Florian, 2002).

Several filamentous fungi, cellulase producers, can colonize wood artifacts (Fazio et al., 2010). On atmosphere-exposed stone substrates, fungi, jointly with cyanobacteria and algae (phototrophic partners), and heterotrophic bacteria, form specific microbial communities called "subaerial biofilms" (Gorbushina and Broughton, 2009). Stone monuments surfaces can be altered by the hyphae penetration through the porous matrix (Kumar and Kumar, 1999; Sterflinger, 2000) and by the production of organic acids and pigments (Gómez-Alarcón et al., 1994). The stone artworks colonization follows a very precise pattern that generally begins with first the colonizers autotrophic organisms (algae, mosses, and lichens). These organisms are able to grow on surfaces damaged by atmospheric agents using the stone mineral components, that progressively become more rough and porous. Materials consequently lose cohesion weakening the structural resistance of the work (Tiano, 2002). Such damage and alterations strongly depend on colonizing organisms, which are favored by micro and macro environmental conditions (Caneva et al., 2008). The metabolism of these first autotrophic colonizers generates a suitable microenvironment for the development of other heterotrophic biodeteriogens such as fungi. These, in turn, completing their life cycle, enrich the stone with organic matter and growth factors useful for the development of other microorganisms as well as the plant organisms, from cryptogams to higher plants. The biodeterioration phenomena on stone works include mechanical damage (breakage and loss of cohesion of the support), chemical alteration (excretion of metabolites), and aesthetic damage (formation of patinas and crusts) (Caneva et al., 2008; Salvadori and Municchia, 2016).

Fungi are the primary colonizers of paper works (books, manuscripts, parchments) stored in archives, museums, and historic buildings where poor ventilation and moist can create an environmental microclimate suitable to the germination and growth of airborne spores and spores present in the dust (Kaarakainen et al., 2009). The fungal spores may survive for a long time in a dormant phase. Their germination occurs under favorable temperature and humidity conditions (63-80% of relative humidity and temperature between 20-35 °C). However, these indoor fungal species may experience significant variations related to seasonality and environmental conditions (Gupta et al., 2021). Fungi commonly found on paper and books are slow growing species of Ascomycetes (*Eurotium*) and mitosporic xerophilic fungi (*Aspergillus*, *Paecilomyces*, *Chrysosporium*, *Penicillium*, *Cladosporium*, etc.), that thrive in materials with low water activity (aw 0.70–0.85). These fungi can cause allergic reactions

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in exposed workers (Polo et al., 2017). When humidity becomes higher to 65%, these fungi can be superseded from other species (*Chaetomium* spp., *Monoascus* spp., *Epicoccum* spp., *Trichoderma* spp. and *Stachybotrys* spp.) (Pinzari and Montanari, 2011; Montanari et al., 2012; Saifeldin et al., 2014). The major fungal species causing damages in art works are mentioned in supplementary Table S1 (Schmidt and Schaechter, 2012; Sterflinger and Piñar 2013; Saifeldin et al., 2014; Isola et al., 2022).

Resolving interventions against fungi require the adoption of emergency and monitoring procedures as well as appropriate intervention measures. Environmental control measures, such as relative humidity monitoring, and cleaning, can avoid the development of biocontaminants and the growth on the artifact. Although prevention is the best approach to conservation, in the event of an accident (floods, torrential rains, etc.) a fungicide treatment is required. Biodeterioration is a problem that must be managed with two objectives:

- accurately understanding the causes that lead to the uncontrolled growth of macro and/or microorganisms (diagnosis).
- developing appropriate methodologies to slow or remove unwanted biological growth (therapy).

The biodeteriogen control methods can be divided into direct and indirect. Indirect methods control microbial growth by acting on environmental factors (water, light, temperature, humidity, nutrients, pollutants, etc.). The direct methods directly target the biodeteriogenic agent and can be mechanical (spatula or brush chisel removal), physical (280-240 nm UV radiations), and chemical (biocides).

Effects on Workers

The presence of biodeteriogens may expose the sector operators to biological agents, while the restoration activities may expose them to physical and chemical agents, the latter represented by chemical biocides.

Biological risk

Although biological risk in the workplace is often present, it is frequently underestimated, particularly in work activities where biological agent exposure is an undesired but inevitable phenomenon. In fact, with the exception of sectors where the presence of biological agents is evident (health care, veterinary medicine, research, animal husbandry, waste disposal) several activities may appear to be exempt from this occupational risk. It is important to highlight that a proper assessment of the biological risk requires the analysis of various factors, which due to the difficulty of standardised measurements and exposure limit values are not always ponderable. In fact, both the biological agent characteristics (pathogenicity, infectivity, transmissibility, neutralization), and individual susceptibility (immunosuppression, drug use, lifestyle, etc.) must be evaluated. Furthermore, the different exposure scenarios, transmission routes, doses, and exposure duration are significant. As a result, it is therefore evident that the operator exposure modalities are the parameters on which to base workplace prevention and protection activities (Ciferri, 1999; Urzì, 2000; Palla et al., 2002; Suihko et al., 2007; Pinar et al., 2009; De Leo et al., 2012; Pangallo et al., 2012).

The biological risk exposure scenarios are various and diversified and include work activities in outdoor environments, that could expose workers to the presence of potentially harmful animals (rats, insects, reptiles), and indoor environments (underground excavations, archives, libraries). In these last cases, the climatic conditions (temperature, relative humidity) may provide an ideal habitat for the growth of species releasing spores and allergens, such as micro-fungi, bacteria, and viruses. Restoration workers, in particular, may be susceptible to respiratory diseases or, more rarely, dermatitis (Table 1).

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Table 1. – Pathologies, in restoration activities, related to fungi and bacteria exposure (Modified from: Johanning et al., 2014).

Organ / System	Patologies	Agents
Upper respiratory tract: nose, sinuses, throat	Rhinitis, sinusitis, laryngitis	Fungi, allergens, particulates, volatile organic compounds of microbial origin
Lower respiratory tract: lungs, bronchial system, alveoli	Bronchitis, asthma, bronchiolitis, allergic bronchopulmonary aspergillosis, extrinsic allergic alveolitis (or hypersensitivity pneumonitis)	Fungi, allergens, fine dust, fungal by-products
Upper and lower respiratory tract	Fungal rhinosinusitis, aspergillosis	Fungi, allergens, fine particles
Skin and mucous membranes	Urticaria, dermatitis, conjunctivitis	Fungi, allergens
Central nervous, immune, and endocrine systems, liver, kidneys	Cognitive and psychiatric disorders, hepatitis, nephritis, changes in the menstrual cycle, and thyroid function	Fungi, microbial products, mycotoxins, organic powders

In particular, several fungi can trigger allergies and respiratory diseases already after short exposures or, in the case of long or intense exposure, they may induce more serious pathologies (allergic bronchopulmonary aspergillosis or hypersensitivity pneumonitis) (Samson, 1985). Such pathologies, as shown in the Table 2, are mainly attributable to exposure to aerosols containing fungi, their propagules, or their by-products.

Table 2. – Fungal components and products relevant for pathologies attributable to aerosols exposure (Modified from: Nevalainen and Taubel, 2015).

Etiological Agents	**References**
Fungal origin fragments size less than the spores (< 1 µm)	Gorny et al. 2002 Gorny 2004 Méheust et al. 2014
β-D-Glucans (structural components linked to D-glucose polymers of the fungal wall) – induce a proinflammatory activity	Douwes et al. 2000
Fungal allergens (antigenic compounds) – stimulate the IgE-mediated immune response	Williams et al. 2016 Crameri et al. 2009
Volatile organic compounds of microbial origin, highly volatile chemicals (alcohols, aldehydes, amines, ketones, terpenes, and aromatic hydrocarbons) produced by the fungi growth as primary or secondary metabolites	Korpi et al. 2009 Schleibinger et al. 2008 Persoons et al. 2010 Inamdar et al. 2020
Mycotoxins (secondary non-volatile metabolites), varieties of chemical compounds bioactive or toxic by ingestion or inhalation	Amuzie et al. 2008 Wong et al. 2016

Hypersensitivity pneumonitis is characterized by the immune-complexes formation determined by antigens of microbial origin (Table 3) (Kleyn et al., 1981; Cormier et al., 1998; Bertorelli et al., 2000; Yoshikawa et al., 2006; Enríquez-Matas et al., 2009; Ficociello et al., 2019).

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Table 3. – Hypersensitivity pneumonitis and etiological agents (Modified from Mahmoudi, 2016).

Correlation between pathology and specific antigens of organic nature exposure		
Antigen	**Source**	**Pathology**
Thermophilic actinomycetes (*Saccharopolyspora rectivirgula / Micropolyspora faeni*, *Thermoactinomyces vulgaris*, *T. viridis*, *T. candidus*, *T. sacchari*), *Fusarium* spp., *Penicillium brevicompactum*, *Saccharomonospora viridis*, *Absidia cerymbifera*, *Wallemia sebi*	Plant compounds (moldy hay, grain, silage), foliage and dry herbs, fertilizer, moldy forage	Farmer's lung
Thermophilic actinomycetes (*Saccharopolyspora rectivirgula /Micropolyspora faeni*, *Thermoactinomyces vulgaris*, *T. viridis*, *T. candidus*, *T. sacchari*), *Monocillium* spp., Basidiospores of: *Pleurotus ostreatus*, *Hypsizygus marmoreus*, *Lycoperdon perlatum*	Compost for mushrooms, indoor mushrooms cultivation, peat	Mushroom worker's lung
Penicillium expansum, *P. cyclopium*, *P. chrysogenum*, *Acremonium* spp., *Alternaria* spp.	Moldy wood and wood dust	Woodworker's lung
Aspergillus spp., *A. clavatus*	Moldy barley	Malt worker's lung
Aspergillus spp., *Penicillium* spp.	Greenhouse soil	Greenhouse worker's lung
Penicillium casei, *P. roqueforti*, *P. cyclopium*, *P. chrysogenum*, *P. camemberti*	Cheese molds, salami molds	Dairy workers' lung, Salami seasoners' lung

In Italy, the Ministerial Decree of June 10, 2014 recognizes organic dust syndrome as an occupational disease, an acute non-allergic pulmonary inflammatory reaction attributable to exposure to fungal spores. Its symptoms arise without any previous sensitization and it is caused by inhalation of toxins produced by fungi or other contaminants of organic dust (Langley, 2011; Piecková, 2012; Rando et al., 2012).

It is also known that some fungal species producing mycotoxins frequently colonize indoor environments. Throughout the past few years, attention has been paid to the exposure to mycotoxins in indoor work environments. Mycotoxins are secondary fungal metabolites that contain a large set of organic compounds (alkaloids, cyclopeptides, coumarins, phenols, and terpenoid). Mycotoxins are therefore a risk factor that is currently unknown and most likely underestimated. Indeed, mycotoxins are not very volatile and human exposure is predominantly linked to the contaminated food ingestion. However, the possibility that a potential exposure route could be inhalation, carried by spores or contaminated substrates, cannot be excluded (Sorenson et al., 1981; Dvorackova and Pichova, 1986; Wangia et al., 2019). Their presence depends on environmental factors and microclimatic conditions. Cases of pulmonary mycotoxicosis have been reported in agricultural and textile workers.

Lougheed et al. (1995) were perhaps the first to associate cases of lung disease with the presence of aflatoxin B1 due to the exposure to a work environment contaminated by *Fusarium* species (Saad-Hussein et al., 2013; Ferri et al., 2017; Masciarelli et al., 2020). Although *Penicillium* and *Aspergillus* species are the most prevalent fungal contaminants in indoor environments, there are also less common fungal species such as *Stachybotrys* and *Chaetomium* that are being investigated for their effects on health, due to chronic long-term exposure (Table 4) (Jarvis and Miller, 2005; Fromme et al., 2016).

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Table 4. – Mycotoxins and fungi producing mycotoxins in indoor environments are significant to occupational purposes (Modified from Fromme et al., 2016).

Mycotoxins	Some fungal species producing mycotoxins	Toxicity
alternariol, alternariol monomethyl ether, altenuene (and other less frequent)	*Alternaria* spp., *A. alternata*	Genotoxic, teratogenic, carcinogenic
Atranoni A-K	*Stachybotrys chlorohalonata*, *S. chartarum* (chemotype A)	Pro-inflammatory action
Beauvericin	*Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Fusarium* spp.	Cytotoxic and immunotoxic
Chetoglobosine, chaetomina, chaetoviridine	*Chaetomium* spp. *C. globosum*	Cytotoxic
Enniatine	*Fusarium* spp.	Cytotoxic and immunotoxic
Fumitremorgine	*Aspergillus fumigatus*	Tremors
Gliotoxin	*Aspergillus* spp., *A. fumigatus*	Cytotoxic and immunosoppressive
Mycophenolic Acid	*Penicillium* spp., *P. brevicompactum*, *P. roquefortii*	immunosoppressive
Penitrem A	*Penicillium* spp.	Tremors
Roquefortine C	*Penicillium roquefortii*, *P. chrysogenum*	Neurotoxic
Roridine (E e L-2)	*Stachybotrys chartarum* (chemotype S)	Cytotoxic and immunosoppressive
Satratozin (G, H, F)	*Stachybotrys chartarum* (chemotype S)	Cytotoxic and immunosoppressive
Sterigmatocystin	*Aspergillus versicolor*	Carcinogenic
Trichotecenes	*Stachybotrys chlorohalonata*, *S. chartarum* (chemotype A), *Memnoniella echinata*	Cytotoxic
Verrucarin	*Stachybotrys, Fusarium,* and *Myrothecium*	Cytotoxic and immunosoppressive
Verrucologen	*Aspergillus fumigatus*, *Penicillium* spp.	Tremorgenic

Experimental studies on the mycotoxins inhalation have shown the induction of a rather wide spectrum of adverse reactions: mucous, membrane irritation, endocrine and immune system alterations, renal failure, fever, asthenia, nausea. The presence of mycotoxins in indoor environments has also been linked to Sick Building Syndrome, particularly in relation to some *Stachybotrys chartarum* strains able to produce different mycotoxins (roridina E and L-2, satratozina F, G, and H, isosatratoxin F, G, and H, verrucarine B and J, tricoverrole A and B, tricoverrins A and B, atranone B) (Hardin et al., 2003). These fungal species, for grow, require substrates with a high cellulose content (fabrics, paper, glue, bookbinding), a low nitrogen content, and a relative humidity higher than 55% (Gorny et al., 2002; Brasel et al., 2005).

The presence of fungi producing mycotoxins should be underestimated even in underground excavation environments, museum deposits, archives, and libraries where dust, poor air exchange, and humidity can favour the presence of pathogenic fungi. Other frequently encountered pathologies are allergic and non-allergic dermatitis, in rare cases manifestations of scabies due to the manipulation of fibrous materials (fabrics, paper, etc.), and conjunctivitis caused by microorganisms present in the dust or by rubbing eyes with dirty hands.

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Chemical risk

To stop the degradation caused by microorganisms and organisms or to delay their appearance, restoration interventions imply the use of biocides, composed of active ingredients, co-formulates, and solvents (Marconi et al., 2019).

Biocide (Table 6) or "biocidal product means any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action" and "any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on any harmful organism by any means other than mere physical or mechanical action" (Regulation (EU) 528/2012).

The biocide choice has to be made evaluating:

- pathogenic organism/s;
- the artifact's constituent materials;
- the artifact's preservation state (see Table 5).

Table 5 – Most used biocides in Italy (supplemented and modified from Bartolini et al., 2007; Coutinho et al., 2016; Favero-Longo et al., 2017; 2018; Pinna, 2017; Kakakhel et al., 2019; Lo Schiavo et al., 2020; Franco-Castillo et al., 2021).

Commercial product	Composition	Action spectrum
Preventol RI 80 (CTS)	N-Alkyl-N-benzyl-N,N-dimethylammonium chloride (benzalkonium chloride) approx. 80% + isopropyl alcohol (2%) in water	Fungi, bacteria, and algae
Biotin T (CTS), Rocima™ 103	N-Decyl-N,N-dimethyldecan-1-aminium chloride (Didecyl-dimethyl ammonium chloride) (40–60%) + 2-octyl-4-isothiazolin-3-one (OIT) (7–10%) + isopropyl alcohol (15–20%) + formic acid(2.0–2.5%)	Lichens, fungi, bacteria, and algae
Lichenicida 464	4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) (25– < 40%) + 3-iodo-2-propynyl N-butylcarbamate (IPBC) (12.5– < 15.0%) + 2-octyl-4-isothiazolin-3-one (OIT) (0.06– < 0.10%) + benzyl alcohol (40– < 60%)	Lichens
Biotin R (CTS)	3-iodo-2-propynyl N-butyl carbammate (IPBC) (10–25%); 2-octyl-4-isothiazolin-3-one (OIT) (3–5%); in diethylene glycol butyl ether	Fungi, bacteria, and algae
Sinoctan PS	4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) + 3-iodo-2-propynyl N-butylcarbamate (IPBC)	Fungi, bacteria, mosses, lichens, and algae
Algophase	2,3,5,6-tetrachloro-4-(methylsulfonyl)pyridine (TCMSP) (30%) + N-methyl-2-pyrrolidone	Lichen, algae

Generally, the action mechanism of biocides consists in blocking cell wall synthesis and causing damage to DNA and RNA, therefore they are often also toxic to humans and the ecosystem (Guardiola et al., 2012; Kakakhel et al., 2019; Galvão de Campos et al., 2022). Potentially, restorers may be exposed to these compounds but there is a lack of data on biocides exposure and the possible health impacts on this category of workers (Varnai et al., 2011). Chemicals' potential adverse effects on human health are linked, in addition to their intrinsic toxicity, to factors such as their chemical/physical properties, their concentration of use, the exposure routes and duration, the environmental conditions (humidity, temperature, absence adequate air changes), the interaction with other substances, and the

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receiving organism's capacity to absorb, metabolize and excrete, according to individual susceptibility (age, weight, sex, genetic factors, any pathologies present, etc.). In particular, the exposure levels to which a restoration worker may be subjected depend on the type of artifact to be restored, its state of deterioration, the techniques used (brush, pack, injection, spray, or fumigation treatments), the quantities of chemical products used, and the possibility of transporting the artifact to an adequately equipped for safety purposes restoration laboratory.

The biocides commonly used by restorers are based on quaternary ammonium salts, carbamates, and isothiazolinones. Quaternary ammonium salts are inexpensive cationic biocides with relatively low toxicity, but with a short-lasting effect, such as alkyl-dimethyl-benzyl ammonium chloride (benzalkonium chloride) (Table 6), a powerful disinfectant-germicide effective on bacteria Gram+, Gram-, fungi, and microflora in general. Benzalkonium chloride is therefore used for the cleaning and disinfection of surfaces in glass, ceramics, stone materials, metals, synthetic and natural rubber, textile fibers, and paper, and for the room disinfection. Being soluble in water (ethyl, methyl, and isopropyl alcohol too), it is used above all in an aqueous solution or in formulation with other products (animal glues or mush for re-lining of paintings). However, benzalkonium chloride is corrosive to the eyes, skin, and the respiratory tract. In short-term exposure, aspiration into the lungs can lead to chemical pneumonitis. In a fire event, furthermore, thermal decomposition produces toxic and corrosive fumes of ammonia, chlorine, and nitrogen oxides.

Isothiazolinones (isothiazol-3-ones), such as 2-octyl-4-isothiazolin-3-one (OIT), and 4,5-dichloro-2-octyl-4-isothiazolin-3-one (DCOIT), are widely known as humans strong skin sensitizers due to their irreversible reaction with proteins' cysteine residues (Alvarez-Sánchez et al., 2003; 2004). Due to their strong bactericide, fungicide, and algaecide properties, they are present in a wide range of commercial products for everyday life, therefore they are used in many industrial sectors (textiles, paints, glues, detergents, and leather tanning) where several cases of allergic contact dermatitis have been detected on workers (Schwensen et al., 2020; Silva et al., 2020). Several studies on cell lines investigated the potential cytotoxic effects of these substances. Kim et al. (2021) performed *in vitro* studies on a blood-brain barrier (BBB) model treated with OIT, suggesting that constant exposure to OIT may represent a risk of vascular or neurological diseases. In a previous study by Arning et al. (2008) the effects of OIT and DCOIT on the human hepatoblastoma cell line Hep G2 and on the glutathione (GSH) / oxidized glutathione (GSSG) ratio within the cell determined by the enzyme glutathione reductase were tested. The obtained results showed that the DCOIT effects are greater than those of OIT. DCOIT significantly reduces the GSH content in HepG2 cells in a dose/time dependent manner and simultaneously increases the GSSG level. This causes a significant variation in the GSH/GSSG ratio which can be the reason for necrosis and subsequent cell lysis. Often, isothiazolinones are found in mixtures with carbamates (organic compounds characterized by the functional group –NHCOO–) such as 3-iodo-2-propynyl N-butyl carbamate (IPBC) (Table 6) (Aronson, 2016). Cases of allergic contact dermatitis have been attributed to this compound (Badreshia and Marks, 2002; Jensen et al., 2003; Schöllnast et al., 2003; Davis and Johnston, 2007; Henriks-Eckerman et al., 2008). Several cases of contact dermatitis have also been attributed to 2,3,5,6-tetrachloro-4- (methylsulfonyl) pyridine (TCMSP), an active ingredient present in the Algophase (Table 6) used as an antimicrobial (Sesseville et al., 1996; Le Coz et al., 1998; Huh et al., 2001; Gushi et al., 2003; Inoue et al., 2008). The UK Advisory Committee on Pesticides has listed TCMSP as a skin irritant and a severe eye irritant (Advisory Committee on Pesticides, 2004).

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Natural substances use in the restoration

In recent decades, there has been a growing demand for natural and eco-friendly biocides with long-term efficacy and no adverse effects on cultural heritage or operator health. Natural or biologically derived products that can act as biocides can have different origins:

- natural molecules with biocidal activity (zoosteric acid, capsaicin, extracellular enzymes, hydrolase, usnic acid, parietin, or bacterial extracts) (Bruno et al., 2019; Cappitelli et al., 2020).
- microorganisms (biocleaning: predation processes operated by bacteria, removal of biofilms by fungi, bacteriophages and viruses with antialgal activity; biomineralization: carbonatogenesis or autotrophic or heterotrophic calcite production by bacteria) (Le Métayer-Levrel et al., 1999; Klaassen et al., 2008; May et al., 2011; Charola et al., 2011; Zammit et al., 2011; Hu et al., 2013; Krakova et al., 2015; Páramo-Aguilera et al., 2015; Silva et al., 2017; Jurado et al., 2020).
- essential oils and plant extracts alone, incorporated in sol/gel matrices or applied by a polyphasic approach to enhance their effectiveness (Veneranda et al., 2018; Jurado et al., 2020; Palla et al., 2020; Sparacello et al., 2021).

Essential oils, known since ancient times for their antimicrobial-repellent properties (Petrovska, 2012), may be used to counteract biological colonization (Bakkali et al., 2008; Nerio Quintana et al., 2009) by regulating intermediate metabolism, activating or blocking enzymatic reactions, having direct effects on enzymatic synthesis, or by altering the membrane structure (Bakkali et al., 2008; Reichling et al., 2009).

Many researchers are currently interested in using low environmental impact biocides such as plant extracts as part of an eco-friendly approach to restoration. As an alternative to the use of synthetic products, the use of natural and non-destructive treatments such as essential oils, hydrolates, and plant extracts, seems to be a valid alternative for the health protection of environment and restoration operators (Satish et al., 2007; Axinte et al., 2011; Afifi, 2012; Fierascu et al., 2012; Fierascu et al., 2013). Tables 6 and 7 show the most significant studies.

Conclusions

In cultural heritage conservation, the risk prevention management of operators is particularly articulated. In fact, despite knowing the hazard sources and risks connected with the conservation operations, defining risk profiles and quantifying them for potentially correlated pathologies is not entirely clear. This context necessitates research into organizational and procedural strategies, as well as the application of appropriate protective equipment, to prevent and decrease risks, enhance working conditions, and ensures the safety of people involved in the restoration and preservation of cultural heritage.

The use of natural products in the control and restoration of artifacts attacked by fungi fits perfectly into a world that is globally shifting its attention to green approaches in various productive sectors in order to safeguard human health and the environment. Recent studies have shown that essential oils, hydrolates, and plant extracts are effective against fungi that deteriorate building materials. Therefore, the application of essential oils or their active components could be a good option for contaminated walls or other surfaces disinfection. However, there are scarce studies on their use in the conservation of cultural heritage (Gatenby and Townley, 2003; Rakotonirainy and Lavédrine, 2005; Chung et al., 2018; Fidanza and Caneva, 2019).

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Table 6 – Efficacy on fungal strains of essential oils (EOs) and hydrolates.

Source of essential oils (EOs) and hydrolates	**Fungal strains**	**Results**	**Authors**
Pimpinella anisum, *Syzygium aromaticum*, *Cuminum cyminum*, *Allium sativum*, *Laurus nobilis*, *Citrus sinensis*, *Origanum vulgare*	*Aspergillus niger*, *Aspergillus clavatus*, *Penicillium* sp., *Fusarium* sp.	EOs of *P. anisum* and *S. aromaticum*, extremely effective antifungals, less *L. nobilis* and *C. sinensis*	Borrego et al. 2012 - air samples and documents National Archives of Cuba; Historical Archives of the Museum of La Plata, Argentina
Abies sibirica, *Carum carvi*, *Mentha piperita*, *Eucalyptus globulus*, *Thymus pulegioides*, *Syzygium aromaticum*, *Citrus bergamia*	*Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp.	*S. aromaticum*, higher antifungal activity, followed by *A. sibirica* and *C. carvi*; *T. pulegioides* inhibition especially on *Penicillium* sp.; *S. aromaticum* better than chemical treatments	Levinskait et al. 2013 - stone material of the walls of the historic city of Vilnius in Lithuania
Melaleuca alternifolia, Lavandula angustifolia, Rosmarinus officinalis, *Origanum vulgare*	*Cladosporium cladosporioides*, *Paecilomyces variotii*, *Curvularia lunata*	*M. alternifolia, L. angustifolia*, *R. officinalis* and *O. vulgare* completely inhibited the tested fungi	Mansour 2013 - stone sarcophagus Behbeet el-Hager, El-Gharbieh, Egypt
Origanum vulgare	*Aspergillus* spp., *Penicillium* spp.	strong antifungal potential, equal or greater than benzalkonium chloride	Stupar et al. 2014a - frescoes of the Holy Virgin Church in Serbia
Ocimum basilicum	*Aspergillus* sp., *Penicillium* sp., *Mucor* spp.	hydroalcoholic extracts better than EOs	Fierascu et al. 2014 - paper artifacts in Romania
Thymus capitatus	*Candida albicans*, *Fusarium oxysporum*, *Aspergillus niger*	The best the harvests of June and July against *F. oxysporum* e *A. niger*	Casiglia et al. 2015 - Sicilian historical-artistic craftsmanship
Origanum vulgare *Thymus vulgaris*	*Fusarium* sp., *Scopulariopsis* sp.	The under-study EOs vapors temporarily reduced or halted the growth of *Scopulariopsis* sp. e *Fusarium* sp.	Lavin et al. 2016 - Historical Cartographic Research of Geodesy, Buenos Aires; Museum of Historical Archive, La Plata, Argentina
Origanum vulgare, *Thymus vulgaris* (EOs in an organic solvent, individually or synergically)	Black fungi	EOs better than benzalkonium chloride	Devreux et al. 2016 - bench with a female bust, located in the Zitella area of the Vatican Gardens
Origanum vulgare	*Aspergillus* spp.	EOs of *O. vulgare* showed greater antifungal activity than the commercial biocide Sanosil S003	Savković et al. 2016 - Sculpture, brick and stone walls, silk icon, archivial papers, Serbia

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Table 6 – Efficacy on fungal strains of essential oils (EOs) and hydrolates (continue)

Source of essential oils (EOs) and hydrolates	**Fungal strains**	**Results**	**Authors**
Melaleuca alternifolia	*Penicillium chrysogenum*, *Aspergillus* spp.	EOs *M. alternifolia* activity against *P. chrysogenum,* no vs *Aspergillus* spp.	Rotolo et al. 2016 - colonized artworks
Origanum majorana, *Cinnamomum camphora*, *Syzygium aromaticum*, *Ocimum basilicum*	*Aspergillus niger*, *Alternaria alternata*	*C. camphora* and *S. aromaticum* EOs efficient on both fungi.	Elsayed and Shabana 2018 - oil paintings and other cultural heritage objects
Cinnamomum zeylanicum	*Aspergillus niger*, *Penicillium funiculosum*, *Trichoderma viride*	Vapors of EOs treatment without altering the characteristics of the tissues	Matusiak et al. 2018 - Four fabric objects (Museum of Archeology and Ethnography, Lodz, Poland)
Origanum vulgare, *Thymus vulgaris*	*Aspergillus flavus*	EOs better than synthetic biocides (benzalkonium chloride and Nipagin-M)	Palla et al. 2020 - wooden works bio- deterioration
Azadirachta indica, EOs and Neem seeds ethereal extracts (ethyl ether)	*Aspergillus flavus*	Neem seed EOs result more effective than Neem seed extracts	Gupta et al. 2021 - old manuscript of the National Research Laboratory India
Emulsion of 0.3% *Cinnamomum zeylanicum* EOs and 99.7% *Citrus aurantium* var. *amara* hydrolate	*Aspergillus niger*, *Aureobasidium pullulans*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Alternaria alternata*, *Penicillium citrinum*	Sprayed on the painting's back and left to act for 24 hours without altering the painting's chemical-physical characteristics	Minotti et al. 2022 - ancient oil paintings restoration ("The Silence" by Jacopo Zucchi kept at the Uffizi Museum in Florence)
GELYD (*Monarda fistulosa* e *Citrus aurantium* var *amara* hydrolates added with Gellan hydrogel	*Aspergillus sydowii*, *Penicillium chrysogenum*, *Cladosporium cladosporioides*	with *M. fistulosa* and *C. aurantium* var *amara* hydrolates, fungicidal action only with *C. aurantium* for paper cleaning and bioattack preventing.	Di Vito et al. 2017; 2018
Citrus limon, *Mentha spicata*, *Phoeniculum vulgare*, *Origanum majorana*, *Rosmarinus officinalis*	*Saccharomyces cerevisiae*, *Candida albicans*, *C. lipolytica*, *Lodderomyces elongisporus*	*C. limon* and *M. spicata*; *P. vulgare*, *O. majorana*. *R. officinalis* no effect	Sakr et al. 2012 - paintings in the royal tombs of Tanis in Egypt 840 BC

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Table 7. - Efficacy on fungal strains of plant extracts (EOs essential oils, HE Hydroalcoholic Exstract, CE Choloform Exstract).

Plant extracts	Fungal strains	Results	References
Calamintha nepeta *Allium sativum*	*Penicillium chrysogenum* *Aspergillus* sp.	*C. nepeta* shows very low activity against fungal strains; *A. sativum* exhibits high activity against both strains at all concentrations tested	Rotolo et al. 2016 - colonized artworks
Anethum graveolens *Cymbopogon citrates* *Juniperus oxycedrus*	*Fusarium oxysporum* *Aspergillus niger* *Alternaria alternata*	*C. citrates* shows more significant inhibitory activity on all fungal species tested; *A. graveolens* only retarded the fungi growth; *J. oxycedrus* was found to be the least effective; *A. alternata*, more resistant strain; *F. oxysporum* most sensitive strain	Afifi 2012 - stucco ornaments in the Mihrab of Mostafa Pasha Ribate, Azdomor Al Salehy, Egypt
Synadenium grantii *Codiaeum variagatum*	*Cladosporium cladosporioides* *Paecilomyces variotii* *Curvularia lunata*	*S. grantii* and *C. variegatum* extracts reduced all organisms tested growth; *S. grantii* CE is the most effective	Mansour 2013 - stone sarcophagus Behbeet el-Hager, El-Gharbieh, Egypt
Ocimum basilicum hydroalcoholic and aqueous extract	*Aspergillus* sp. *Penicillium* sp. *Mucor* sp.	HE showed better antifungal activity, followed by EOs, and finally by aqueous extracts. The HE has longer-term effects than EOs; HE more safe, efficient and less expensive as fungicidal than EOs	Fierascu 2014 - paper artifacts, Romania

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The use of essential oils as new biocidal agents in cultural heritage conservation has the advantage of a low environmental permanence, but some of them, at high doses, are toxic or even neurotoxic for humans, therefore their use cannot be separated from the use of adequate protective equipment (gloves, mask, goggles, and overalls). Essential oils are also very expensive, unlike hydrolates, which are by-products of their production. Vegetable extracts, on the other hand, not only have a high biocidal activity, but are also inexpensive and simple to prepare from cultivated and wild officinal plants found in the territory. Because of their strong antibacterial activity, minimal mammalian toxicity, and biodegradability, hydrolates and plant extracts could be effective alternatives to synthetic biocides (Saxena and Mathela, 1996). However, further studies are needed to develop adequate use methods of these substances in the conservation of cultural heritage and, particularly, in the fungi treatment (Stupar et al., 2014b).

It would also be desirable to develop good preventive conservation practices on all cultural heritage artifacts including good cleaning methods, custody, dusting, periodic control, and prevention of any possibility of physical, chemical, and biological damage. Preventive conservation plays a crucial role and can take on great importance in every country with a rich cultural heritage. A diagnosis made in time and adequate preventive measures can solve and/or eliminate many problems.

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