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Review

Antifungals on paper conservation: An overview

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ABSTRACT

Since its invention, paper has become one of the main carriers of our cultural, scientific, political, economic and historical information. Given the importance of this material, its preservation is a matter of great interest. Paper can be deteriorated due to physical, chemical and biological agents. Within microorganisms, fungi are the major paper biodeteriogens. Throughout history, several methods have been used to prevent and stop fungal deterioration on paper based materials. In this work we present a review of the main chemical and physical methods used to avoid fungal paper biodeterioration until nowadays and also of some new approaches tested recently. The advantages and disadvantages of these methods are discussed as well as their health effects. Studies regarding antifungal compositions, methods of application, performance and effects on the treated materials are also presented with the aim of providing a clear set of conclusions on the topic.

Contents

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1. Introduction

A great portion of our History is registered in the form of documents or works of art made of paper, making the preservation of this material a matter of great importance. Paper, like all other materials inevitably degrades over time. Its deterioration rate can nevertheless be accelerated by endogenous factors like acidity, metal ions, lignin or degradation products, and exogenous factors like heat, humidity, UV light, oxygen, pollutants or biodeteriogens (Strlic et al., 2005). Biodeteriogens are organisms characterized by their ability to use a substrate to sustain their growth and reproduction (Pinzari et al., 2006) and biodeterioration is defined as any unwanted alteration in a material caused by the vital activities of those organisms (Allsopp et al., 2004). The prevention and treatment of the biodeterioration of paper is one of the main concerns in the paper conservation field. The combination of the high bioreceptivity of paper with inappropriate storage conditions or water related emergency circumstances makes this material very susceptible to microbial deteriogens, mainly fungi. Bioreceptivity, as defined by Guillitte (1995), is the ability of a material to be colonized by living organisms. The bioreceptivity of paper to fungi is considered high due to this its hygroscopicity and composition (cellulose, hemicelluloses, lignin, adhesives, sizings), which represents an abundant carbon source to these heterotrophic organisms. Bacteria can also deteriorate paper materials, but since fungi require less moisture to develop, the environmental conditions normally existent in libraries, archives or museums are more prone to the growth of fungi than that of bacteria.

The physical and chemical forms of cellulose also influence the bioreceptivity of paper. Native cellulose is mainly crystalline with some amorphous sites, whereas the cellulose present in a paper sheet, by having already undergone physical and chemical processing, contains a larger number of amorphous sites along the polymer. These sites are more susceptible to biodeterioration (Gallo et al., 1998; Allsopp et al., 2004) and therefore have a higher bioreceptivity. Also, the removal of lignin in papermaking processes, while contributing to the increase of the quality of paper, amplifies its bioreceptivity, since lignin increases the resistance of cellulose to microorganisms (Allsopp et al., 2004).

When paper is affected by fungi, besides the developing fungal structures, several excreted metabolic products also accumulate in the material. According to Florian (2002) the majority of these metabolic products will continue their deleterious effects, even after the fungus is dead. Some of the excreted products, like glycerine, may increase the moisture content of the contaminated spot and act as conidia activators on a subsequent contamination. Excreted lipids easily undergo autoxidation and form free-radicals and peroxides, which contribute to the formation of brown discolourations on the substrate (Florian, 2002). Coloured pigments formed by some metabolic processes will also interfere with the readability of the object (Florian, 2002; Abdel-Kareem, 2010).

The decomposition of cellulose by cellulolytic fungi is due to the production and secretion of a complex of extracellular enzymes – cellulases – which hydrolyse the cellulose macromolecule into small water soluble sugars which can then be processed by the fungi (Gallo et al., 1998). The deterioration caused by these enzymes and also by excreted organic acids, will cause a gradual loss of mechanical strength in paper (Valentin, 2007; Abdel-Kareem, 2010). On a final stage of deterioration, paper cannot be handled without disintegration and loss of information.

Fungi can also decompose other materials present on paper, like fillers or sizings, which can be rich in proteins and sugars. Pinzari et al. (2006) reported that generally, more than the type of paper fibres, it is the type of sizing and fillers that strongly influences the intensity and results of fungal action on paper. The biogenic formation of calcium oxalate crystals promoted by fungi can also change the calcium carbonate alkaline reserve present in paper. Moreover, the precipitation and growth of these crystals among cellulose fibres can be a source of chemical and mechanical damage (Pinzari et al., 2010).

Besides all these deterioration effects, handling mould contaminated paper objects can constitute a serious health risk, because many of these microorganisms can be pathogenic/toxinogenic (Bennett and Klich, 2009; Pinheiro et al., 2011). Even when the fungi is already dead, the fungal structures can still contain active allergenic and toxic compounds to humans (Florian, 2002).

According to the literature (Pitt and Christia, 1968; Arai, 2000; Raschle, 2001; Florian, 2002; Allsopp et al., 2004; Valentin, 2010), by keeping the water activity of paper under 0.60, and the temperature under 20 °C, with good air circulation, the growth of fungi can be avoided. Nevertheless, the technology needed for an efficient climate control is not available to all the heritage repository institutions in the world, especially in developing countries. Microbial infestations can occur even in rooms with climate control, in local microclimates with higher water availability generated by ineffective ventilation and surface temperature dishomogeneity, as well as in emergency situations like floods, leaks or aqueous fire suppression. In these situations control and/or recovery measures need to be carried out (Craig, 1986; Valentin, 2010; Pinzari et al., 2011; Sterflinger and Pinzari, 2012).

The main goal of this work is to make a review on the main chemical and physical antifungal methods used in paper based collections until nowadays. By describing their major advantages and disadvantages, the data gathered in this manuscript will be useful to provide a basis for decision making on the prevention and treatment of fungal deterioration of paper.

2. The use of antifungal methods on paper conservation

Since ancient times there has been a great concern about the inhibition of biodeterioration of paper items. Tsuen-hsuin (1985) cites the 6th century author Chhi Min Tao Shu, recommending that “Between the fifteenth of the fifth month and the twentieth of the seventh month, book rolls must be unrolled and rolled three times. This should be done on a clear day in a spacious house which is aired and cool, and books should not be exposed directly to the sun, for it will turn the paper brownish. Rolls heated by the sun quickly attract insects, and rainy and humid days should especially be avoided. If books were cared for in this way, they would last for several hundred years”.

The great majority of antifungal methods used to prevent and/or stop biodeterioration caused by fungi in paper conservation have been adapted from other scientific fields (Nittérus, 2000a), like material protection, agriculture or medicine. These methods can go from limiting the access to water by the fungi, to the application of chemical products in the gaseous or liquid state, or physical methods like extreme temperatures, radiation or current. Generally, the physical methods do not have a long term action – since they leave no residues, their microbicidal action is only immediate. However, the majority of chemical compounds, even in the gaseous state, leaves residues that can prolong its antimicrobial effect during a limited period of time.

Limiting the access to water, by lowering the water activity on the substrate, may be the simplest and harmless way to stop fungal growth. This can be achieved by drying, but when dealing with great amounts of humid or wet paper, due to this material’s hygroscopicity, this is usually a slow process and time is a key factor on the growth of microorganisms.

Chemical microbicides can be introduced already in the manufacture process of paper, as an addition to sizing products, directly

to the paper pulp, or as a coating, to prevent the microbial development (Paulus, 2004). These compounds can also be added to glues used in paper items. Traditionally, in the preparation of starch paste – an adhesive easily spoiled by microorganisms when fresh and eaten by insects when already applied in documents – fungicides and insecticides were added occasionally (Jenkinson, 1922; Freitas, 1937). Nevertheless, the majority of antifungal methods have been applied in the disinfection of already formed paper objects, like documents and artworks.

Although antifungals can be applied locally on a single object, most of the antifungal methods for paper items have been used as massive disinfection treatments for collections. These massive treatments have been applied after a mould outbreak, as a preventive measure before incorporation in an institution, or as a periodic curative and preventive procedure. Occasionally, the same product was used to eliminate both insect pests and mould.

A proper antifungal method for materials should have a broad activity spectrum, good chemical stability, low cost, should not be toxic to humans, and should have no adverse effects on the treated material.

There are several factors that can affect the antimicrobial activity of a microbicide, namely the period of contact, concentration, temperature, pH, presence of organic soiling matter and type of microorganism (Russell, 2003).

Below we describe in alphabetical order the most frequent antifungal methods used for paper items until the present time and also some new approaches tested recently.

2.1. Chemical methods

Antifungal chemical substances act on the fungi through the interaction between their active ingredients and specific target sites on and in the microbial cell (Paulus, 2004). Their efficacy can be related to the chemical structure and chemical and physical properties (Paulus, 2004). The majority of microbicides used for material protection can be characterized according to their mechanism of action as being membrane-actives or electrophilically actives (Paulus, 2004).

Membrane active microbicides include: alcohols, phenols, acids, salicylanilides, carbanilides, dibenzamidines, biguanides, quaternary ammonium salts, and azole antifungals. These microbicides act by coating the cell wall adsorptively (forming a thin film on its surface), and causing changes in the outer membrane and along the cell wall. By losing their integrity, these barriers will allow the microbicide to access the cytoplasmic membrane, where it will cause its lethal effects (Paulus, 2004).

Electrophilically active microbicides include: aldehydes, compounds with a vinyl group in α,β-position to an electronegative group, compounds having an activated halogen atom in α-position and/or vinylogous to an electronegative group, compounds with an activated N–S bond as a structural toxophoric element, and organometallic compounds (Paulus, 2004). The higher the electrophilicity of the microbicide, the higher will be its antimicrobial efficacy. These compounds are attracted to components in the microbial cell with high electron density, such as nucleophilic components. By reacting through electrophilic addition or substitution, electrophilically active microbicides can lead to the inactivation of enzymes (Paulus, 2004).

The chemical antifungal methods organized by chemical classes are described below and summarized in Table 1.

2.1.1. Alcohols

Alcohols are membrane-active microbicides whose antimicrobial efficacy is universally acknowledged. This efficacy increases with the chain length (Paulus, 2004) and is mainly caused by coagulation and denaturisation of proteins in the microbial cell (Bacílková, 2006). Aqueous alcohol solutions are more effective against microorganisms than pure alcohol. The higher efficacy is reached with concentrations between 50% and 90% (v/v), depending on the kind of alcohol (Bacílková, 2006).

Alcohols can be effective against vegetative forms of bacteria, fungi and viruses, but their effect against sporulating microorganisms has not been yet proven, and so, they are only considered disinfecting and not sterilizing agents (Bacílková, 2006).

A comparison between the effects of ethanol, isopropanol and butanol vapours on the inactivation of two species of fungi and on solubility of four types of writing inks has been performed by Bacílková (2006). The results show that the butanol vapours eliminated the fungi tested on concentrations between 80 and 96%, and isopropanol and ethanol were effective on a range between 30 and 90%. For the solubility assay, all the alcohols vapours caused feathering of three of the tested inks.

As ethanol is the most used alcohol for treating mould contaminated heritage objects, a more detailed review follows.

2.1.1.1. Ethanol. Ethanol (C_2H_6O) or ethyl alcohol has been widely used as a disinfectant. The first study concerning the effects of ethanol on bacterial cultures dates from 1880 (Bacílková, 2006). Ethanol acts on fungi by affecting the permeability of their cytoplasmic membrane, causing the leakage of cytosol constituents and ultimately leading to the disintegration of the cell (Nittérus, 2000b; Bacílková, 2006).

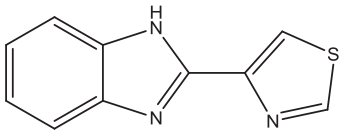
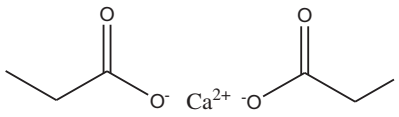
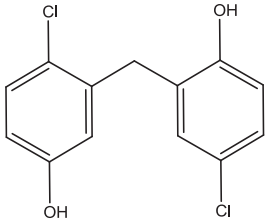
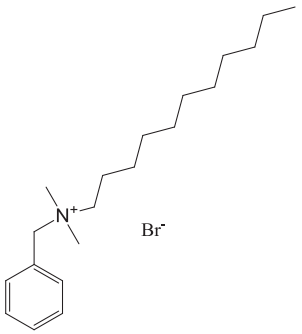
The microbicide effect of ethanol depends on its concentration and formulation composition (Paulus, 2004; Bacílková, 2006). The higher effect is reported for ethanol concentrations between 50% and 80% in aqueous solutions, reaching a maximum of efficiency at 70% (Nittérus, 2000b). While the ethanol turns the cell wall more permeable, the water in the solution carries the alcohol into the cytoplasm (Florian, 2002). Higher concentrations have shown to be non-efficient because of the rapid denaturation of lipid structures which will form a protective coagulate around the cell, preventing further penetration of the alcohol (Nittérus, 2000b). Besides, high concentrations will also act as a wetting agent on the conidial cell, enhancing conidial activation (Florian, 2002). Lower concentrations do not exhibit direct killing or cytolytic properties but through a continuous contact with the microbial cell can lead to the inhibition of cell growth (Nittérus, 2000b).

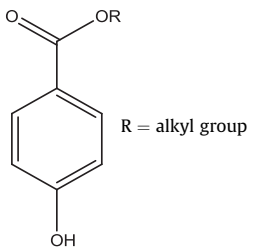
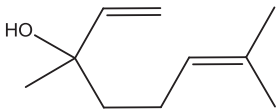
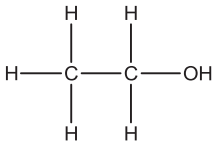
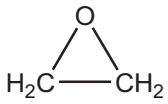
Ethanol disinfectant solutions are usually applied by spraying, brushing, swabbing, immersion, or by exposing the infected papers to the vapours of this compound.

Bacílková (2006), reports the efficiency of vapours of ethanol solutions on paper samples inoculated with *Aspergilus niger* and *Penicillum notatum*. According to this author, concentrations between 30 and 90% can inhibit fungal re-growth for at least 14 days after the evaporation of the alcohol. Nevertheless, Bacílková (2006), regards that for thicker materials, like books, the treatment will not be as effective.

The effectiveness of 70% ethanol solution applied by spray or immersion on paper inoculated with the fungi species *Aspergillus flavus* and *A. niger*, *Chaetomium globosum*, and *Trichoderma viride*, was described by Nittérus (2000b). As reported by this author, this ethanol solution does not have a sporicidal effect, since a re-growth of the fungi occurs within 14 days after the treatment. Additionally, ethanol immersion shows higher efficiency than spraying (Nittérus, 2000b), maybe due to a longer contact with the solution before evaporation. An interesting observation was also made: *A. niger* did not grow on untreated reference samples but it did grow on spray treated samples, which can indicate some spore activation properties of the ethanol solution (Nittérus, 2000b).

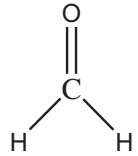
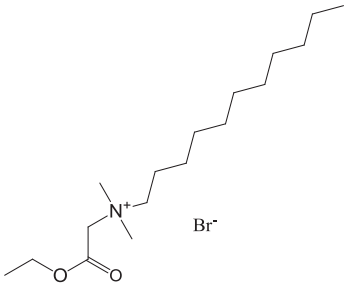
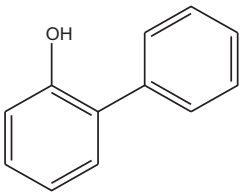
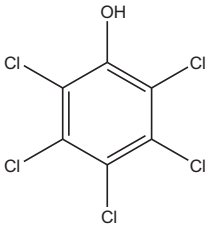
Table 1
Summary of the antifungal chemical methods reviewed, in alphabetical order.

Antifungal compound	Advantages	Disadvantages	Effects on paper	First report of use	Use restrictions[a]	LD50 for rats oral route (mg/kg bw)[b]	References
Azole antifungals e.g. Thiabendazole: 	Can be used for aerial disinfection	Fungistatic rather than fungicidal Can leave fatty or powdery films on treated materials	Thiabendazole: Slight decrease on brightness	1944	Different restrictions for each essential oil component (see references)	Different values for each azole compound e.g. Thiabendazole: >2000	Fabbri et al., 1997; Rakotonirainy et al., 1999; Lamb et al., 2000; US-EPA, 2002; Gollapudy et al., 2004; Paulus, 2004; US-EPA, 2005; Pérez-Rivera et al., 2009; Giavini and Menegola, 2010; EC, 2012
Calcium propionate 	Also has deacidifying properties Low toxicity	Fungistatic but not fungicidal	Increase of pH Increase of polymerization degree Minor increase on lightness and yellowness	1930's (1980 on heritage materials)	EU: N/A USA: N/A	3920	Dersarkissian and Goodberry, 1980; Zappalà, 1990; US-EPA, 1991; Florian, 2002; Paulus, 2004; Suhr and Nielsen, 2004; Neves, 2006; Zotti et al., 2007; Şifa, 2008
Dichlorophen 	Can be used in alkaline pH Strongly effective against fungi	Causes irritation on skin and eyes	Increase of the deterioration rate Decrease of whiteness Harmful effects on inks	1929	EU: R USA: N/A	1250	Triolo et al., 1968; Kowalik, 1980; Block, 2001; McBain and Gilbert, 2001; Cox et al., 2004; Paulus, 2004; Yamarik et al., 2004; Escalada et al., 2011; EC, 2012; Gupta and Aggarwal, 2012
Dimenthyl-lauryl-benzyl-ammonium bromide 		Low-level disinfectant with no sporicidal activity	Decrease of physical properties, pH, whiteness, and alpha cellulose content	1986: on heritage materials	EU: R USA: R	N/A	Strzelczyk and Rozanski, 1986; Bello-Gonzalez et al., 2008; EC, 2012

Esters of p-hydroxybenzoic acid  	Low toxicity pH range of activity	Mainly fungistatic and bacteriostatic	Minor increase on yellowing Slight decrease on tensile strength Considerable raise on pH Slight increase on the percentage of deformation	1920's (1990 on heritage materials)	EU: N/A USA: N/A	2100−>8000	Gustafson et al., 1990; Soni et al., 2002; Russell, 2003; Paulus, 2004; SCCP, 2005; Soni et al., 2005; Zhang et al., 2005; Neves, 2006; Zotti et al., 2007; Neves et al., 2009
Essential oils e.g. Linalool:  	Low toxicity	Fungistatic rather than fungicidal Can act as insect attractants Linalool: undergoes autoxidation on air, forming hydroperoxides	Decrease of pH Possibility of causing oxidation by the hydroperoxides formed	N/A	Different restrictions for each essential oil component (see references)	Different values for each essential oil e.g. Linalool: 2790	Sikkema et al., 1994; Florian, 1998; Karpouhtsis et al., 1998; Sköld et al., 2002; Rakotonirainy and Lavedrine, 2005; Abad et al., 2007; Rakotonirainy et al., 2007; ECHA, 2012
Ethanol  	Evaporates and does not leave toxic residues	Can act as a conidia activator Fungistatic, not fungicidal	Loss of gloss, increase in opacity and slight deformation (especially on transparent papers) Possibility of dissolving media, adhesives, and seals	1880	EU: R USA: N/A	10,470	Valentin and Garcia, 1999; Nittérus, 2000a; Florian, 2002; Bacílková, 2006; EC, 2012; ECHA, 2012
Ethylene oxide  	High antimicrobial efficacy Great power of penetration Can be used for mass treatments	Raises the susceptibility of objects to future microbial attack Classified as a category 1 carcinogen	Decrease of mechanical properties and polymerization degree Oxidation Yellowing	1928 (1933 on heritage materials)	EU: R USA: R	330	Cotton and Roark, 1928; Flieder, 1965; Strassberg, 1978; OSHA, 1984; Ballard and Baer, 1986; Craig, 1986; Valentin, 1986; Valentin et al., 1990; Hengemihle et al., 1995; Florian, 2002; Ponce-Jimenez et al., 2002; Rakotonirainy et al., 2003; US-EPA, 2004; Tateo and Bononi, 2006; Mendes et al., 2007; EC, 2008; IARC, 2008; EU-OSHA, 2009; ECHA, 2012

(*continued on next page*)

Table 1 (*continued*)

Antifungal compound	Advantages	Disadvantages	Effects on paper	First report of use	Use restrictions[a]	LD50 for rats oral route (mg/kg bw)[b]	References
Formaldehyde 	Also has sporicidal effect Can be used for mass treatments	At low RH undergoes polymerization and precipitates on materials Low power of penetration It is carcinogenic Causes irritation of the eyes, nose and throat, and contact dermatitis	Cross-linking of cellulose Loss of flexibility Enhance of iron gall ink corrosion	1889	EU: R USA: R	460	Gallo, 1963; Heim et al., 1968; Valentin and Garcia, 1999; Paulus, 2004; IARC, 2006; US-EPA, 2008a; EC, 2012; ECHA, 2012
Lauryl-dimethyl-Carbethoxymethyl ammonium bromide 	Can be used for mass treatments (nebulisation)	Undergoes acidification with time. Irritates the mucous membrane.	Minor darkening and acidification of paper Depolymerisation of cellulose Decrease of adhesion of inks to paper		EU: R USA: R	N/A	Heim et al., 1968; Triolo et al., 1968; Strassberg, 1978; Kowalik, 1980; EC, 2012
Ortho-phenylphenol 	Has a broad spectrum of activity Efficient at low concentrations Can be used in alkaline pH Can be used for mass treatments	Fungistatic but not fungicidal Can leave a very irritating and suffocating odour in the air	Changes in colour Depolymerization of adhesives Accelerated ageing	N/A	EU: N/A USA: N/A	2733	Triolo et al., 1968; Strassberg, 1978; Haines and Kohler, 1986; IARC, 1999a; Rakotonirainy et al., 1999; Valentin and Garcia, 1999; Paulus, 2004; ECHA, 2012
Pentachlorophenol 		Carcinogenic, highly toxic	Acidic hydrolysis	1936	EU: SR USA: SR	80	Miller and Aboul-Ela, 1969; Strassberg, 1978; IARC, 1999b; Valentin and Garcia, 1999; Paulus, 2004; Fernández Freire et al., 2005; EC, 2008; US-EPA, 2008b; Gupta and Aggarwal, 2012

Thymol	Can be used for mass treatments (Thymol chambers)	Not fungicidal, only fungistatic Poses a genotoxic risk for humans	Decrease of mechanical properties Yellowing Deterioration of iron gall ink Dissolution of inks	Since the ancient Egypt	EU: N/A USA: N/A	980	Flieder, 1965; Strassberg, 1978; Craig, 1986; Daniels and Boyd, 1986; Haines and Kohler, 1986; Gustafson et al., 1990; Isbell, 1997; Karpouhtsis et al., 1998; Šegvić Klarić et al., 2007; Buyukleyla and Rencuzogullari, 2009; Napoli et al., 2010; US-EPA, 2010; Coimbra et al., 2011; Numpaque et al., 2011; ECHA, 2012
Titanium dioxide (TiO_2 in crystalline form)	May prevent the formation of biofilms	Fungistatic rather than fungicidal Requires UV radiation to exert its microbicidal activity Classified as possibly carcinogenic to humans	Minor chemical degradation of cellulose Acceleration of fading of organic colourants	1985	EU: N/A USA: N/A	>5000	Matsunaga et al., 1988; Blake et al., 1999; Huang et al., 2000; Chawengkijwanich and Hayata, 2008; Gavriliu et al., 2009; Harding et al., 2009; IARC, 2010; Afsharpour et al., 2011; Markowska-Szczupak et al., 2011; ECHA, 2012

[a] Use restrictions concern only the use of the substance as a preservative or biocide for material protection: R = restricted use; SV = severely restricted use; N/A = information not available. This information was gathered from the documents currently available online in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Environmental Protection Agency (EPA) websites, for USA regulations; and in the European Community Regulation on chemicals and their safe use (REACH), and the European Database of Export and Import of Dangerous Chemicals (EDEXIM) websites for EU regulations.

[b] LD50 for rats (mg/kg bw) oral route: Median lethal dose of each compound to kill half the members of a tested population of rats by oral route, measured by mg/kg of body weight.

According to Bacílková (2006), alcohols, ethanol included, can cause changes on paper, namely extraction of soluble components, specially by immersion application. Other changes, especially on transparent papers, include loss of gloss, increase in opacity and slight deformation (Bacílková, 2006). Ethanol can also dissolve media, adhesives, and seals, and so, solubility tests have to be performed previously to treatment. Even with vapour application, ethanol can cause feathering of ink lines (Bacílková, 2006).

Valentin and Garcia (1999) report that in order to raise ethanol's microbicide activity, it is recommended to add 0.1% of ortho-phenylphenol to the 70% ethanol solution. These authors classify this mixture has the most effective and least toxic of the chemical antifungals (Valentin and Garcia, 1999).

As ethanol is highly flammable it has to be applied and stored away from heat sources. Besides, inhalation can cause respiratory tract irritation and direct contact may cause skin irritation and dehydration, so it must be handled using protective clothing and masks or in a fume hood.

2.1.2. Alkylating agents

Alkylating agents can combine with amino, carboxylic, sulfhydryl and amino groups in proteins and enzymes in the microbial cell. These compounds are able to inactivate bacteria, fungi and viruses (Russell, 2003).

2.1.2.1. Ethylene oxide. Ethylene oxide [$(CH_2)_2O$], is a colourless flammable gas that was discovered in 1859 (Ballard and Baer, 1986), although its insecticidal potential was only reported in 1928 (Cotton and Roark, 1928). In 1933, ethylene oxide (EtO) began to be used in disinfection of museum textiles and documents. By that period its microbicidal properties also became recognized (Ballard and Baer, 1986).

The microbiologic inactivation properties of EtO are related to its powerful alkylation reaction of cellular constituents of organisms, like nucleic acids and functional proteins, causing their denaturation. This process affects the normal cellular metabolism, leading to non viable microbes (Mendes et al., 2007). Due to its effective bactericidal, sporicidal, and virucidal activity EtO is also used as an alternative sterilization treatment for moisture and heat sensitive materials in different fields like in the disinfection of medical devices (Mendes et al., 2007).

Thanks to its high efficiency on the elimination of both insects and microbes this biocide became a very popular fumigant for libraries, archives and museums. This is also related with its great power of penetration and the possibility of use at room temperature and in the gaseous form, allowing the treatment of heat and moisture sensitive objects (Cotton and Roark, 1928; Ballard and Baer, 1986). EtO was generally commercialized as a mixture with other gases, like CO_2 (10% EtO, 90% CO_2) and dichlorofluoromethane (12% EtO, 88% dichlorofluoromethane). These dilutions reduce the explosiveness, flammability and toxicity of the EtO (Craig, 1986). The procedures for EtO fumigation varied almost according to each institution. The fumigations could be performed in vacuum chambers, chambers with normal pressure, or pressurized chambers with pressure varying between 20 mm Hg and 400 mm Hg (Flieder, 1965; Craig, 1986). The temperatures used in the treatment ranged essentially between 20 °C and 37 °C, and the exposure times between 3 and 24 h (Flieder, 1965; Craig, 1986). For example, in France, the standard conditions for the treatment of documents with EtO in 2003, were T = 25 °C; RH = 50%; 20 h; and 500 g m^{-2} concentration (Rakotonirainy et al., 2003). Strassberg (1978) refers that the exposure time depended on the gas mixture proportion and the nature of disinfestation, e.g., the elimination of mould requiring twice the exposure time as for elimination of insects.

Before removing the treated materials from the fumigation chamber, air washing cycles should be performed in order to remove as much of the residual EtO as possible, since this compound and also some of its derivatives, such as ethylene chlorohydrin (produced by reaction with chlorine ions) and ethylene glycol (formed by reaction with moisture in the materials) are highly toxic (Tateo and Bononi, 2006; Mendes et al., 2007). In fact, exposure to EtO represents a carcinogenic, mutagenic, genotoxic, reproductive, neurological and sensitization hazard for people (Ballard and Baer, 1986) and is classified as a category 1 carcinogen by IARC – International Agency for Research on Cancer (IARC, 2008).

In each air cycle wash, the chamber is evacuated and air, nitrogen, or carbon dioxide is introduced. Accordingly to the Environmental Protection Agency of the United States (US-EPA, 2004), four to six air washing cycles should be carried out to meet the permissible exposure limit (PEL) to EtO of 1 ppm as an 8 h time-weighted average (TWA) imposed by the Occupational Safety and Health Administration (OSHA, 1984). In a study comparing the relative capacity of different library materials to offgas residual ethylene, it is demonstrated that some of the tested materials needed more than 6 air washing cycles to reach 1 ppm due to their lower desorption rates (Hengemihle et al., 1995). In the air over wood pulp offset paper and over newsprint paper, even after 8 and 13 air washing cycles, respectively, there was still an EtO concentration of circa 2 ppm. Over photographic film, after 25 air washing cycles, a concentration of 38 ppm EtO was still observed under the tested conditions (Hengemihle et al., 1995).

EtO fumigation treatments have shown to affect the physical and chemical properties of treated paper (Valentin et al., 1990). Flieder (1965) indicates that EtO treatments can diminish the folding endurance (c. 40%), raise the copper number (4–19%), diminish the polymerization degree (2–8%) and slightly yellow the paper. Ponce-Jimenez et al. (2002) reports a slight decrease in the pH of paper after sterilization with EtO.

Moreover, Craig (1986) and Valentin (1986) describe that after sterilization of archive materials with ethylene oxide, they become more susceptible to microbial attack. According to Craig (1986) this effect could be caused by the removal or disruption of the natural flora equilibrium, which would turn the materials more susceptible to colonization by any active microorganism with which it comes to contact. Florian (2002) points out that this higher susceptibility may be due to the deposition of small amounts of ethylene glycol on the materials which would raise hygroscopicity and act as an activator of conidia settled thereafter.

EtO is listed in Annex III to the Rotterdam Convention, which includes pesticides and industrial chemicals that have been banned or severely restricted for health or environmental reasons, and is also included in Annex I to Regulation (EC) No 689/2008 of the European Parliament and of the Council concerning the export and import of dangerous chemicals (EC, 2008). Although already banned in several countries, the use of EtO is still permitted in some, with restrictions, namely on the occupational exposure levels. For instance, the value of PEL for the European Union countries varies between 0.56 and 5 ppm and in the USA this value is 1 ppm (EU-OSHA, 2009).

2.1.2.2. Formaldehyde. Formaldehyde (CH_2O) was first synthesized in 1859 but it was only in 1867 that it was conclusively identified. This compound has been produced commercially since 1889 (IARC, 2006). Besides being used for synthesis of several more complex organic compounds and materials, formaldehyde is also used as a microbicide. It is an electrophilically active microbicide with the ability to react with several different amino acids present in the microbial cell, including purine and pyrimidine groups of both DNA and RNA, a unique characteristic of this compound (Paulus, 2004). It is highly reactive and also considered sporicidal, by attacking the spore both at the surface and internally, due to its penetrating power (Paulus, 2004).

Formaldehyde is a gas at room temperature, and was frequently applied as a gaseous fumigant, although it was also often used in solution as a preservative in the preparation of glues, like starch paste.

As a fumigant, formaldehyde could be applied in a hermetically closed oven, at a temperature of 30 °C, in a proportion of 250 g m^{-3}, with a similar quantity of water also being vaporized in order to prevent dehydration and cracking of sensible materials, like parchment or leather. The objects would remain in the oven for 24–48 h, depending on the extension of fungal attack (Heim et al., 1968). Gallo (1963) suggested a 12 h fumigation treatment with relative humidity above 60% and temperature above 18 °C. Also, the treated materials should be afterwards exposed to air for several hours or days, since formaldehyde would polymerize and settle as a superficial film on materials and so its elimination was rather difficult (Gallo, 1963).

According to Valentin and Garcia (1999), this compound, when applied by fumigation, has a low power of penetration and a limited fungicide effect. Rather than to prevent dehydration, these authors indicate that this treatment is generally applied at high RH levels in order to keep formaldehyde from undergoing polymerization and subsequent precipitation on treated materials, forming a white deposit (Valentin and Garcia, 1999).

Formaldehyde has been reported to cause adverse effects on archival and library materials, like: cross-linking of cellulose, loss of flexibility on paper and protein containing materials, like parchment, leathers and silk, and also enhancing the corrosion of iron gall ink (Gallo, 1963; Valentin and Garcia, 1999).

Regarding the toxicity of formaldehyde to humans, the International Agency for Research on Cancer (IARC), classifies formaldehyde as carcinogenic, besides causing several other effects like irritation of the eyes, nose and throat, and contact dermatitis (IARC, 2006). The occupational exposure limits for this compound vary with each country and can be found in the literature (IARC, 2006). According to the European Council regulations, formaldehyde should no longer be placed in the European Union market as a preservative for fibre, leather, rubber and polymerized materials since 01/07/2012 (EC, 2012).

2.1.3. Azole antifungals

Azole antifungals are membrane active microbicides that can be classified as imidazoles or triazoles depending on whether they contain two or three nitrogen atoms, respectively, in the azole ring (Paulus, 2004; Pérez-Rivera et al., 2009). The first azole with antifungal activity was reported in 1944, but it was not until 1958 that it begun to be marketed (Pérez-Rivera et al., 2009). In the last decades, intensive research has led to the establishment of azole compounds as antimycotics for human and veterinary uses and also as fungicides for agrochemical proposes (Paulus, 2004).

These compounds act by inhibiting the ergosterol biosynthetic pathway, which leads to changes of sterol composition in the plasma membranes of fungi (Gollapudy et al., 2004; Paulus, 2004). This results in alterations in the membrane fluidity or in membrane disruption, causing a delay, or ultimately an arrest of fungal growth (Lamb et al., 2000; Paulus, 2004).

Fabbri et al. (1997) tested several azole antifungals compounds, chitin syntase inhibitors and antimicrobials for the control of fungal growth in different kinds of paper. The results obtained show significant variations on the efficacy of these compounds depending on the fungal strain and on the kind of paper used. Overall, the best inhibiting effect on fungal growth was obtained with two of

the azole antifungals – miconazole and econazole at concentrations of 1 mol/m^3. In this study the secondary effects on paper were not evaluated.

Rakotonirainy et al. (1999) studied the aerial disinfection of emptied storerooms in libraries and archives after a fungal contamination using different aerosolized compounds: three azole antifungals – econazole, thiabendazole, and imazalil (enilconazole) and also orthophenylphenol. The aerosol method used – thermal fogging – revealed to have a very good penetration and homogeneity power, having also the advantage of not causing any change in relative humidity and temperature values in the room. Regarding the antifungal compounds, none of them exhibited a fungicidal action, only a fungistatic one. Even tough, thiabendazole revealed the best aerial disinfection and surface decontamination properties. Also, this compound does not cause the deposition of a fatty film on surfaces unlike the other tested compounds but a powdery film that can be removed afterwards. Regarding the effects of thiabendazole on paper, slight changes in the brightness values have occurred, although the level of oxidation and fibre strength were practically unaltered after artificial ageing (Rakotonirainy et al., 1999). Nevertheless, the authors recommend removing all the documents from the room during the treatment with thiabendazole since it has not been yet tested on parchment, leather or other materials.

Azole antifungals have showed in numerous studies to possess an embryotoxic potential, including teratogenic effects in laboratory animals and therefore should be avoided for use by pregnant women (Giavini and Menegola, 2010).

The legal restrictions of use of these antifungals will depend on the specific azole compound and on the country and can be found in the literature (US-EPA, 2002, 2005; EC, 2012).

2.1.4. Essential oils

In the last few decades, the increasing resistance of microbes to the available antifungal compounds has led to the search of alternative natural products derived from plants commonly used for their empirical antifungal properties in traditional medicine. Natural products can be used either as pure compounds or as standardized plant extracts, like essential oils (Abad et al., 2007).

Essential oils are mainly composed of terpenoid compounds (Karpouhtsis et al., 1998). Aromatic terpenes are cyclic hydrocarbons, which due to their hydrophobic character accumulate in the lipid bilayer of the microbial cell membrane according to a partition coefficient that is specific for each compound. This accumulation leads to alteration of the membrane structure and function, namely exerting negative effects on the proton motive force (Sikkema et al., 1994).

Although thymol is also a component of essential oils, it will be more thoroughly discussed below in Section 2.1.5 Phenol Derivatives.

Rakotonirainy has exploited the use of alternative essential oils as antifungals for heritage objects (Rakotonirainy and Lavedrine, 2005; Rakotonirainy et al., 2007). The antifungal activity of essential oils of armoise, clove, boldo, eucalyptus, ravensare, lavender, tea tree, thuya, wormseed and their main components linalool, linalyl acetate, eugenol, $\alpha + \beta$ thujone and cineole were tested in the vapour phase. The vapours of linalool showed the highest antifungal efficiency against the fungal strains tested, although its action was fungistatic rather than fungicidal at the tested concentration (415 ppm) (Rakotonirainy and Lavedrine, 2005). The assessment of the potential damaging effects of linalool vapours on two types of paper showed that this compound did not affect the papers' brightness and degree of polymerization, but it did reduce the pH values (Rakotonirainy and Lavedrine, 2005).

Linalool applied by vapours and spraying was also tested on silver–gelatine photographs and bookbinding leathers (Rakotonirainy et al., 2007). This compound caused oxidation of the photographs, which was revealed by changes in the optical density of the materials. On the leather test pieces, linalool caused a decrease on the temperature and enthalpy of denaturation, which was more pronounced after the artificial ageing tests (Rakotonirainy et al., 2007).

These results may be explained by the fact that linalool undergoes autoxidation on air exposure, forming mainly a hydroperoxide (7-hydroperoxy-3,7-dimethyl-octa-1,5-diene-3-ol) (Sköld et al., 2002). This reaction is enhanced by temperature, and the hydroperoxides formed can oxidize the silver on the photographs (Rakotonirainy et al., 2007). The possible reactions between this oxidation product and leather need further research.

Tests on linalool allergenic potential revealed that the oxidized compound caused sensitization to allergic contact dermatitis, while the pure compound gave no reactions (Sköld et al., 2002). Since linalool undergoes autoxidation just by being exposed to air, it can pose an allergenic risk to sensitive people.

Florian (1998) in a review regarding the use of natural products for insect and fungi control, states that some medicinal plants, like coriander, although may inhibit some fungal species, can also enhance the growth of other ones. Also, other varieties of plants that inhibited the growth of selected fungal species are at the same time classified as insect attractants (Florian, 1998).

2.1.5. Phenol derivatives

There are several phenol derivatives used for their disinfectant and preservative properties. These compounds are membrane-active antifungals, causing mainly damages in the plasma membrane of fungi (Russell, 2003).

Like acids, phenol derivatives dissociate hydrogen ions in solution and are able to form salts. Their antimicrobial effect only occurs in the undissociated state. When the compound is dissociated, its negative charged anions will be repelled by the negatively charged surface of the microbial cell, and therefore cannot exert its antimicrobial effects. This means that in order to keep the antimicrobial properties of these compounds to a maximum, one has to be aware of its pKa value – the pH at which 50% of the phenol is in the dissociated state (Paulus, 2004). At a pH below its pKa, the compound exists as a neutral species, while above that value it will attain a negative charge. In this way, for example, if a pKa of a phenol is 5.0, it will be more effective at pH values below 5.0.

2.1.5.1. Dichlorophen. Dichlorophen (4-chloro-2-[(5-chloro-2-hydroxyphenyl)methyl]phenol), also known by the trade names Preventol GD or Panacide, is a chlorinated bisphenol that was first prepared in 1929 (Block, 2001). Besides being used as a fungicide for paper, cardboard, textiles, adhesives, and as a slimicide in paper manufacture, it is also used for treating fungal infections of the skin, as a germicide in soaps and cosmetics and as an anthelmintic (Block, 2001; Cox et al., 2004). This compound is a weak acid with pKa values for the two hydroxyl groups of 7.66 and 11.60 that can affect a multitude of microbial intracellular targets (McBain and Gilbert, 2001; Escalada et al., 2011). Its inhibitory processes are concentration-dependant and vary from action as a potassium proton antiporter and respiration uncoupler, to competitive inhibition of NADH-binding by malate dehydrogenase (McBain and Gilbert, 2001). Accordingly to Paulus (2004), dichlorophen is strongly effective against yeast and filamentous fungi. Being poorly soluble in water, dichlorophen is usually applied in organic solvents or in the solid state.

Triolo et al. (1968) studied the stability of paper samples treated with dichlorophen in a 15 mol/m^3 ethanolic solution. They observed that this compound caused a slight increase in the

deterioration rate of the paper. The white grade also dropped 15.5% after ageing in comparison to the non treated samples that suffered only a 5% decrease (taking as a reference the non treated and non aged paper). On the other hand, the pH values remained practically the same.

Kowalik (1980) refers as well that dichlorophen does not change the paper's pH, although it may cause harmful effects when applied to documents written with ink (those effects are not specified), and represents a potential danger to paper caused by the release of chlorine. Thus, dichlorophen was suggested only for unwritten paper or for impregnating blotting paper to interleave damp books (Kowalik, 1980).

Dichlorophen can cause mild skin irritation and severe eye irritation (Paulus, 2004). According to Yamarik et al. (2004) no reproductive or developmental toxicity data were available for dichlorophen and the overall available data were insufficient to support safety of this compound. In the European union, dichlorophen shall no longer be placed in the market as a biocidal product since 2009, and as a preservative for film, fibre, leather, and other materials since 2011 (EC, 2012).

2.1.5.2. Ortho-phenylphenol. Ortho-phenylphenol ($C_{12}H_{10}O$), also known by the trade names Preventol O, Topane or Dowicide, is a membrane-active microbicide with a broad spectrum of activity, covering bacteria, yeasts and fungi (Valentin and Garcia, 1999; Paulus, 2004). This compound exhibits antimicrobial action even at low concentrations (between 10 and 50 ppm, pH 5–8) and its inhibition effect increases with increasing pH (its pKa is 11.6) (Paulus, 2004).

For being less toxic than thymol, the use of ortho-phenylphenol (OPP) started to be recommended in substitution of that compound in fumigation chambers (Haines and Kohler, 1986). It was also applied directly in infested documents, by interleaving or wrapping with paper soaked in a 10% aqueous solution. Due to the low volatility of OPP, its fumigation activity would last for six months or more (Strassberg, 1978). Moreover, OPP was used as a preservative in restoration products, synthetic adhesives and animal glues (Valentin and Garcia, 1999).

Considering OPP's antifungal activity, Haines and Kohler (1986) report that this compound applied by fumigation is unable to render all the fungus colonies tested unviable, and describe it as a non effective method of treating mould infected books and papers. According to Rakotonirainy et al. (1999) OPP applied by thermal fogging, showed a fungistatic but not a fungicidal effect on spores, germ tubes and mycelium on all the tested strains. Besides, it can leave a very irritating and suffocating odour in the air persisting for at least 15 days (Rakotonirainy et al., 1999).

OPP has been reported to cause alterations on paper materials, like changes in colour, depolymerization of adhesives and accelerated ageing (Triolo et al., 1968; Valentin and Garcia, 1999).

OPP's sodium salt (SOPP), with the trade names Preventol ON or Dowicide A, was also seldom used as an antifungal compound for paper items, but its marked colouration could produce severe stains on paper (Valentin and Garcia, 1999).

According to the International Agency for Research on Cancer (IARC, 1999a) OPP is not classifiable as to its carcinogenicity to humans (Group 3), although SOPP is considered as possibly carcinogenic to humans (Group 2B).

2.1.5.3. Pentachlorophenol. Pentachlorophenol (C_6HCl_5O), with the trade names Dowicide 7, G, EC-7 or Preventol P, is a chlorinated phenol that was first synthesized in 1841 but was not manufactured in large quantities until 1936 (Miller and Aboul-Ela, 1969). It is a highly lipophilic weak acid with a pKa of c. 4.75, and is considered a broad-spectrum microbicide (Fernández Freire et al., 2005).

According to Valentin and Garcia (1999), this compound was widely used as a fungicide for books, textiles and wood, although it attacks metals and pigments, and degrades paper and wood. Strassberg (1978) also states that by being an acid, pentachlorophenol may adversely affect archival materials.

Pentachlorophenol is considered carcinogenic by the International Agency for Research on Cancer (IARC, 1999b) and is listed as a priority pollutant due to its slow and incomplete biodegradation (Fernández Freire et al., 2005). For these reasons the use of this compound is banned as plant protection product and severely restricted as a biocide in the European Union and in several other countries (Valentin and Garcia, 1999; Paulus, 2004; Fernández Freire et al., 2005; EC, 2008; US-EPA, 2008b).

2.1.5.4. Thymol. Thymol (2-Isopropyl-5-methylphenol) is a natural monoterpene phenol. Already used in the ancient Egypt for the preservation of mummies (Napoli et al., 2010) this compound is present in the essential oils of aromatic plants like thyme, oregano or savory (Numpaque et al., 2011). As a membrane-active microbicide, it alters the permeability of the microbial cell and causes alterations in the hyphal morphology, resulting in reduced hyphal diameters and lyses of hyphal wall, permitting the loss of its macromolecules (Šegvić Klarić et al., 2007; Numpaque et al., 2011).

Thymol has poor solubility in water and a pKa of 10.6 (Coimbra et al., 2011). It is used mainly in its crystalline form as a sublimable solid, in fumigation chambers. Other methods of treatment include placing thymol impregnated paper interleaving books or inside picture frames, providing a continuous contact of the objects with the antifungal vapours, thus prolonging its effect (Strassberg, 1978; Daniels and Boyd, 1986). It can also be applied directly in to the paper in a 10% solution in denatured ethanol (Strassberg, 1978).

In 1983 thymol was nearly the exclusive fumigant used in archives in the United Kingdom (Craig, 1986). The conditions by which thymol was applied, varied almost according to each institution. The concentration used ranged from 1 to 90 g m^{-3}, and the time of exposure from 24 h to 3 weeks (Craig, 1986).

The efficiency of thymol as an antifungal agent for paper has been tested by several authors. According to Craig (1986), filter paper samples inoculated with four cellulolytic fungi species, and treated with thymol by fumigation (20 g m^{-3}), with a constant temperature of 73 °C for 72 h, showed no fungal growth, in comparison with control non treated samples that exhibited a strong fungal development. Nevertheless, following the treatment, after other 3 days at room temperature, all the treated samples showed fungal regrowth, which indicates that thymol did not kill the spores (Craig, 1986). Flieder (1965), reported that for inoculated filter paper samples treated in a room with 33 g m^{-3} of evaporated thymol for 14 days, with a temperature of 26.7 °C, the antifungal treatment was totally ineffective.

Haines and Kohler (1986) and also Gustafson et al. (1990) stated that thymol was not totally fungicidal for the fungal species tested, since there was not a total elimination of the fungal spores.

According to Flieder (1965), the same thymol treatment described above, caused changes in the physical–chemical characteristics of filter paper samples. Namely, the folding endurance decreased considerably (27–63%), and the mechanical resistance slightly dropped (c. 10%), although the chemical properties (copper number, polymerization degree and pH) remained alike and there was practically no yellowing (Flieder, 1965).

Daniels and Boyd (1986) reported the yellowing of prints sealed in frames containing thymol vapours. His research revealed that the yellow colour was probably due to the formation of a polymeric form of thymol, through a photochemical reaction.

The experiments conducted by Isbell (1997) by exposing different paper samples with different mediums to thymol vapours

(three-tenths of an ounce of thymol, to four cubic feet) during one week, revealed an apparent degradation of paper, binders and iron gall ink. Even tough, the mechanism of degradation was not clarified.

Strassberg (1978) states that thymol may attack glues in books, dissolve inks, leave residues on parchment and on paper, and by direct application or interleaving it can cause discolouration on documents.

Concerning its toxicity to humans, thymol has proven to have a genotoxic effect, meaning that it is capable of damaging our genetic material (Karpouhtsis et al., 1998; Buyukleyla and Rencuzogullari, 2009).

US-EPA (2010) reports that additional mutagenicity data is required in order to review the registration of thymol, besides adding that this compound is extremely irritating to the eye and skin.

2.1.6. Photocatalysts

Photocatalysis is the type of reaction that takes place on the surface of a semiconductor in the presence of a very specific range of radiation (Pyrgiotakis and Sigmund, 2008). This chemical process has been used to decompose several pollutants and biological contaminations (Pyrgiotakis and Sigmund, 2008). Titanium dioxide has been the most used and studied compound for these purposes, namely for heritage materials protection and therefore it is further described below.

2.1.6.1. Titanium dioxide. Titanium dioxide (TiO_2) is a natural occurring compound that exists more commonly as tetragonal crystallites of anatase or rutile, which are represented as octahedral structures of (TiO_2^{6-}) (Gavriliu et al., 2009). Crude TiO_2 or titanium white has been used as a pigment from the beginning of the 20th century and is still applied in contemporary art nowadays. Soon it gained other functions, like providing whiteness and opacity in food, medicines or toothpastes, among others. It is an excellent semiconductor photocatalyst, which effectively transforms light energy into chemical energy (Markowska-Szczupak et al., 2011). TiO_2 has been used as a self-disinfecting compound on surface coatings and in purification of water and air (Huang et al., 2000; Chawengkijwanich and Hayata, 2008; Afsharpour et al., 2011). The knowledge about the microbicidal effect of TiO_2 is rather recent, since the first published studies on this subject date from 1985 (Matsunaga et al., 1988).

The microbicidal activity of titanium dioxide is due to its photocatalytic properties. When illuminated with UV light of less than 385 nm, TiO_2 generates strong oxidizing power, producing reactive species like hydroxyl radicals and superoxide ions, which can decompose and mineralize organic compounds, causing fatal damage to microorganisms (Blake et al., 1999; Huang et al., 2000). Nevertheless, the sensitivity of fungi to this kind of treatment is significantly weaker than that of bacteria (Gavriliu et al., 2009; Markowska-Szczupak et al., 2011). According to Markowska-Szczupak et al. (2011), this occurs due to the chemical composition, structure and thickness of the fungal cell walls, when compared to bacteria, namely the presence of chitin rather than peptidoglycan on cell walls. The studies performed so far show that TiO_2 irradiated with UV can only act as a fungistatic and do not affect the conidia (Gavriliu et al., 2009; Markowska-Szczupak et al., 2011).

Recently, TiO_2 has been studied as a layered nanocomposite for protection of paper documents and works of art from UV radiation, pollutant gases, mould and bacteria (Afsharpour et al., 2011). In this study, TiO_2 nanoparticles dispersed in ethanol (0.2% w/v) were applied by spraying Whatman paper samples coated with a 0.7% Klucel G (hydroxypropylcellulose) ethanolic solution. The antifungal properties of TiO_2 were tested on *A. niger* and *Penicillium* sp. Although the experimental procedure and results of the antifungal assay are not described in this article, the authors present their conclusions, stating that the TiO_2 coating may prevent the formation of biofilms, rather than killing the microorganisms (Afsharpour et al., 2011). A biofilm is a community of microbial cells that becomes attached to a surface and/or to other cells within an extracellular polymeric matrix. One of the main features of cells growing in biofilms is their increased resistance to antimicrobial agents, besides their significant increased tolerance to chemical, biological or physical stresses (Harding et al., 2009).

In the study by Afsharpour et al. (2011), the effects of the titanium oxide nanocomposite on the physical and chemical properties of paper samples were evaluated before and after artificial ageing. The results showed that the TiO_2 nanoparticles alone caused a minor chemical degradation of cellulose and accelerated the fading of organic colourants. This effect was not observed with the nanocomposite with the Klucel G layer. As follows, hydroxypropylcellulose may be protecting the paper from deterioration catalysed by TiO_2 (Afsharpour et al., 2011). According to Chawengkijwanich and Hayata (2008), a TiO_2 surface when illuminated, forms hydroxyl radicals and reactive oxygen species. These unstable chemical species can react with cellulose, which can justify the results obtained. On the other hand, both the nanoparticles alone and on top of the Klucel G layer protected the paper samples from losing tensile strength (Afsharpour et al., 2011).

Concerning the health hazards, the International Agency for Research on Cancer, considers titanium dioxide as possibly carcinogenic to humans – Group 2B (IARC, 2010). Research on the application of ultrafine titanium dioxide particles on healthy skin revealed that the compound only penetrates the outermost layers of skin. Nevertheless, there were no studies available about the penetration of TiO_2 on compromised skin (IARC, 2010).

2.1.7. Quaternary ammonium compounds

Quaternary ammonium compounds (Quats) were discovered by August Hofmann in 1851. These membrane-active microbicides with cationic properties are attracted by the negatively charged surface of the microbial cell, forming an electrostatic bond with the negatively charged sites on the cell wall. The permeability of the cell wall is altered, and the active ingredients of the microbicide are able to penetrate into the cell membrane, leading to damage and ultimately death of the cell (Paulus, 2004).

Quats are considered sporistatic microbicides, since they inhibit spore germination and outgrowth without actually killing the spore (Paulus, 2004). Besides, Quats also have surfactant properties (Paulus, 2004).

Some Quats are considered to be highly toxic with a detrimental effect on the immune system (Nittérus, 2000a).

2.1.7.1. Dimethyl-lauryl-benzyl ammonium bromide. Dimethyl-lauryl-benzyl ammonium bromide is a Quat used in medicine as a low-level disinfectant with no sporicidal activity (Bello-Gonzalez et al., 2008).

Strzelczyk and Rozanski (1986) have tested this compound, with the trade name Sterinol, for the disinfection of paper affected by mould. They have reported that a 15 min warm bath containing 0.75–1.0% of a commercial 10% aqueous solution of dimethyl-lauryl-benzyl ammonium bromide (Sterinol) was effective on the tested objects. Nevertheless, they recommend that after the disinfection treatment, the objects should be thoroughly washed with tap water and distilled water to remove the remaining Quat, since this compound has the ability to cumulate in the paper (Strzelczyk and Rozanski, 1986). If this Quat remains in the paper, it

can negatively affect the paper's physical properties, pH, whiteness, and alpha cellulose content (Strzelczyk and Rozanski, 1986).

2.1.7.2. Lauryl-dimethyl-carbethoxymethyl ammonium bromide. Lauryl-dimethyl-carbethoxymethyl ammonium bromide is a Quat used in archives and libraries for elimination of mould. A 5% concentration solution in denatured alcohol at 70° could be applied by nebulisation using an air compressor and a spray gun or an atomizer in a proportion of 5 ml of solution per cubic metre of air (Heim et al., 1968; Strassberg, 1978).

Triolo et al. (1968) studied the effects of lauryl-dimethyl-carbethoxymethyl ammonium bromide on paper. This compound, commercialized by the trade name Cequartyl BE in a 50% aqueous solution, was diluted to a 3% aqueous solution (0.08 M), in which the paper samples were immersed for 5 min without any subsequent washing. The artificial ageing tests performed at 105 °C for 3 days, showed a minor darkening of the samples (2.3%) and a negligible acidification of the paper samples after ageing. Nevertheless, a strong acidification of the treatment solution occurred with time, which was not reflected on the paper's pH, maybe due to a tampon effect provided by the paper's alkaline reserve. Not even neutralization with NaOH could stabilize the pH of the solution, which continued to slowly hydrolyse, probably due to the scission of a covalent bonding on the molecule (Triolo et al., 1968).

Besides, paper samples immersed in 1.5% and 0.5% solutions exhibited considerable depolymerization with ageing, showing the deteriorating effects on cellulose of this compound (Triolo et al., 1968). The negative effects of lauryl-dimethyl-carbethoxymethyl ammonium bromide on cellulose were also pointed out by Strassberg (1978) and Kowalik (1980).

Due to its surfactant properties, Quats can also affect the adhesion of inks used on paper (Triolo et al., 1968; Strassberg, 1978).

Heim et al. (1968) advice for the use of masks during the fumigation with lauryl-dimethyl-carbethoxymethyl ammonium bromide, since it irritates the mucous membrane.

In the European Union, products containing as active substances quaternary ammonium compounds (benzylalkyldimethyl (alkyl from C8-C22, saturated and unsaturated, tallow alkyl, coco alkyl, and soya alkyl) chlorides, bromides, or hydroxides)/BKC, shall no longer be placed in the market since 2011 for film, fibre, leather, rubber, and polymerized materials preservatives since 2011 (EC, 2012).

2.1.8. Salts and esters of acids

Acids, like phenol derivatives, only exert their antimicrobial effects in the undissociated state, and so, the pKa values must be taken into account when using these compounds.

2.1.8.1. Calcium propionate. Calcium propionate [$Ca(C_2H_5COO)_2$] is a salt of propionic acid, that has been commonly used as a food preservative since the late 1930's (Paulus, 2004). Its ability to inhibit moulds but not yeasts to the same extent, have made it a common option for bread preservation since it does not impede the fermentation. This salt is more often used than the acid itself due to its higher solubility in water (Suhr and Nielsen, 2004).

The pH of the substrate is of major importance in the use of these kind of preservatives (weak acids), since their antimicrobial effectiveness is much stronger in the undissociated form than in the dissociated one. The pKa of propionic acid is 4.87 and the maximum pH for propionate activity is 5.0–5.5 (Suhr and Nielsen, 2004).

The use of calcium propionate in conservation of heritage objects was first studied, to our knowledge, by Dersarkissian and Goodberry (1980), who tested its antifungal efficacy on leather and paper. Latter, Zappalà (1990) studied also calcium propionate's ability to deacidify paper, and considered it a good cellulose deacidifier and stabilizer.

The study performed by Dersarkissian and Goodberry (1980), consisted on testing calcium propionate for its antifungal properties on paper and leather against *A. niger*. Since the authors could not achieve any fungal growth on the paper samples, the antifungal properties of calcium propionate were only tested on the leather samples. A 5% calcium propionate aqueous solution was applied using a cotton swab on samples with fungal development. Only after the fourth application the fungal growth was totally stopped, as no fungus was visible 10 days after the treatment. However under ideal growing conditions, the fungus slowly restarted its development. The authors suggest that a modest increase in concentration should greatly enhance this compound effectiveness, and highlight its low cost in comparison with the other tested compounds (Dersarkissian and Goodberry, 1980).

Zotti et al. (2007) also evaluated the antifungal properties of calcium propionate on paper, both in aqueous and in ethanolic solutions, and compared it with a commercial solution of *p*-hydrobenzoates (parabens). Their results show that a saturated ethanolic solution of calcium propionate (0.35% w/v), albeit having a lower concentration that the aqueous solutions (3% and 5% w/v) exhibits a much higher efficiency against the fungal species tested. The authors point out that this superior effectiveness is due to the ability of ethanol to act as a vehicle for the calcium propionate molecule through the fungal cell wall (Zotti et al., 2007).

Neves (2006) studied the antifungal and deacidification potential of calcium propionate and also its effects on Whatman #1 filter paper. Calcium propionate was used at a 5% concentration dispersed in an 85% (w/w) ethanol/water solution. The fungal species tested were *Cladosporium* sp. and *Penicillium corylophilum*. Neves compared the fungal growth on paper samples treated with the calcium propionate solution, treated with the solvent mixture alone, and non treated. Two days after the inoculation all the tested paper samples showed fungal growth, though on the non treated ones the growth was wider. However, eight days after inoculation, the paper samples inoculated with *P. corylophilum* and treated with the solvent mixture and the calcium propionate dispersion revealed a higher fungal development than the control samples (Neves, 2006). Since ethanol can act as a conidia activator (Florian, 2002), the samples treated with this solvent may have had a larger number of activated spores and therefore a more intense fungal development.

In the above mentioned studies, the efficiency of calcium propionate was never related to the pH at which it was applied. Since propionic acid has a pKa of 4.87, its efficiency will be much higher in the acidic range. In the study reported by Neves (2006), e.g., we can presume that the compound was applied in an alkaline solution, since it raised the pH of filter paper which had already a nearly neutral pH. This factor could justify, at least in part, the poor antimicrobial results achieved. Although, adding an acidic compound to paper would not be reasonable in a paper conservation practice.

Regarding the effects of the calcium propionate solution on Whatman #1 filter paper, Neves (2006) reported a minor increase on the lightness and yellowness ($\Delta L^* = 0.43$ and $\Delta b^* = 0.37$, respectively) after the treatment, an increase on the pH values, a maintenance of the tensile strength, and an increase on the polymerization degree (maybe due to the ability of the calcium ions to form gels with polysaccharides improving the viscosity of solutions) (Neves, 2006).

Concerning the health hazards of calcium propionate, Paulus (2004) reports that even when administrated at large doses in a diet, propionic acid is excreted in the urine and there is no risk of

accumulation in the human body. The United States Environmental Protection Office indicates that the tests for the teratogenicity (ability to cause birth defects) and reproductive toxicity for calcium propionate are negative (US-EPA, 1991). Nevertheless, the research performed by Şifa (2008) indicates that this compound has chromotoxic effects, and advises caution on the use of this compound as a food preservative.

2.1.8.2. Esters of p-hydroxybenzoic acid. The esters of *p*-hydroxybenzoic acid, commonly known as parabens, are one of the most common antimicrobial agents in pharmacy and cosmetics' industries, due to their low toxicity, pH range of activity, good stability and minimum secondary effects (Soni et al., 2005; Zhang et al., 2005).

These compounds were firstly synthesized in the early 1920's in order to replace salicylic and benzoic acids which were only effective at low pH values (Paulus, 2004).

Parabens are mainly fungistatic and bacteriostatic, being more effective against yeast and moulds than bacteria (Russell, 2003; Paulus, 2004). At low concentrations they inhibit selectively the proton motive force across the microbial cell membrane, and at high concentrations they affect the permeability of the membrane, causing leakage of intracellular constituents (Russell, 2003).

The antimicrobial efficacy of parabens increases with increasing chain length, while the water solubility correspondingly decreases (Paulus, 2004). Besides, parabens can become inactive by micellization with non-ionic surfactants (Paulus, 2004).

The most widely used esters of *p*-hydroxybenzoic acid are methyl, ethyl and propyl esters (Russell, 2003; Paulus, 2004).

The pKa values of methyl-*p*-hydroxybenzoate (methyl-paraben) and propyl-*p*-hydroxybenzoate (propyl-paraben) are 8.5 and 8.1, respectively, and so they are still active in slight alkaline media (Paulus, 2004).

The use of parabens as antifungal agents on the conservation of heritage objects was published for the first time in 1990, on a study about alternatives to thymol on fumigation chambers (Gustafson et al., 1990). On this research, butyl-paraben and propyl-paraben were tested separately, applied in the gaseous form, and compared with other antifungal compounds. The results showed that the parabens, as well as the majority of the chemicals tested, did not exhibit a positive antifungal effect, and so did not provide an alternative for use in thymol cabinets (Gustafson et al., 1990).

The direct application of parabens in solution on paper materials started to be studied by Neves (2006) in her graduation thesis, and was published later in 2009 (Neves et al., 2009). In this study, several concentrations and proportions of methyl-paraben and propyl-paraben were tested against two fungal species – *Cladosporium* sp. and *P. corylophilum*. The mixture of 0.5% methyl-paraben and 1% propyl-paraben in an 85% ethanolic solution was the minimum inhibitory concentration for those species of fungi. The addition of 5% calcium propionate improved the characteristics of this mixture as, besides being a fungistatic compound, it has also deacidification properties (Neves et al., 2009). The effects of this parabens/calcium propionate mixture on paper were tested on Whatman #1 filter paper, before and after application. The results obtained revealed a minor increase on yellowing and a slight decrease on the tensile strength, while considerably raising the pH and moderately increasing the percentage of deformation (Neves et al., 2009).

Zotti et al. (2007) tested the antifungal properties of parabens on fungi isolated from foxing spots (name commonly given to brownish-red spots on paper), and compared them with aqueous and ethanolic solutions of calcium propionate. The parabens tested were methyl-paraben and propyl-paraben as part of a commercial spray formulation, whose concentrations and brand name are not revealed in the study. The fungi strains tested were *Penicillium spinulosum*, *Trichoderma pseudokoningii*, *Geomyces pannorum* and *Aureobasidium pullulans*. The results obtained revealed an intermediate fungistatic activity of the parabens commercial spray, between the water based and the alcohol based calcium propionate solutions. Since there is no indication of the quantity of parabens applied on the paper samples, these results can hardly be compared with the ones obtained in other studies.

Regarding health aspects, parabens are completely absorbed from the gastrointestinal tract and hydrolysed to form *p*-hydroxybenzoic acid which is excreted via the urine (Soni et al., 2002; Paulus, 2004).

In the past few years, the detection of parabens in some breast tumour tissue samples has engaged a controversy on the possible oestrogenic hazards of these compounds. Some studies conducted thereafter claim that the previous ones fail to consider the metabolism and elimination rates of parabens, which are dose, route, and species dependent (Soni et al., 2005). The Scientific Committee on Consumer Products – European Commission, reports that "viewing the current knowledge, there is no evidence of demonstrable risk for the development of breast cancer caused by the use of underarm cosmetics" (SCCP, 2005). According to the same institution, several studies have proven parabens to be practically non-toxic, not carcinogenic, not genotoxic and not teratogenic (SCCP, 2005).

2.2. Physical methods

Physical antifungal methods are the ones that do not require the application of chemical compounds. They leave no residues *pers se*, which can be a positive characteristic, but for the same reason they only exert a momentary action.

The physical antifungal methods are described below and summarized in Table 2.

2.2.1. Dehydration

Water is one of the main limiting factors to fungal growth, and dehydration consists in lowering or even ceasing the availability of water to microorganisms. Already in the early civilizations, drying of meat and fish was used as a food preservation method (Hugo, 1995).

In heritage conservation, dehydration is considered by many as the best way to stop fungal growth on wet materials. It should be performed quickly to prevent microbial development, but on the other hand, slow drying is recommended to prevent dimensional changes on heritage objects.

Dehydration can be performed using dehumidifying equipment and/or wrapping or interleaving wet objects with dry absorbing materials.

Florian (1997, 2002) recommends drying objects under constraint to prevent distortions and dimensional changes, for example, drying water-soaked books without removal from the shelves.

However, large volumes of wet absorbing materials, like shelves full of books will respond slowly to a dry environment due to its self-buffering effect (Garside and Knight, 2011), and will remain wet for several days, which can allow the development of fungi.

2.2.2. Gamma irradiation

Gamma radiation (γ-radiation) is the electromagnetic radiation of highest energy and shortest wavelength. Among other functionalities, this kind of radiation is often used as a sterilizer in several fields like medicine, pharmacy or agriculture. It is usually obtained from Cobalt 60, a radioisotope that emits gamma rays continuously and therefore the irradiation cabinet must be

Table 2
Summary of the antifungal physical methods reviewed, in alphabetical order.

Antifungal method	Advantages	Disadvantages	Effects on paper	First report of use	References
Dehydration	Does not leave toxic residues	High volumes of wet paper respond slowly to a dry environment and meanwhile fungi development can occur Fungistatic rather than fungicidal	Physical deformations	General: Since early civilizations	Hugo, 1995; Florian, 1997, 2002; Garside and Knight, 2011
Freezing	Does not leave toxic residues Can prevent soluble compounds in the materials from bleeding and migrating	Dry conidia can resist freezing The concentration of solutes in the freezing process can increase some deterioration chemical reactions (ex. pH dependent and lipid oxidation) Fungicidal effect is time and temperature dependent	Ice crystals increase the porosity and thickness of paper materials, which can alter its absorbance and mechanical properties	1000 BC	Flink and Hoyer, 1971; Takenaka et al., 1996; Florian, 1997, 2002; Gunde-Cimerman et al., 2003; Archer, 2004; Basset and Draïs, 2011
Gamma irradiation	Does not leave toxic residues. Can be used for mass treatments Can act as a fungicidal, depending on the radiation dosage	Can induce fungi to produce more coloured metabolic products Can turn cellulose more susceptible to further infestations	Diminishment of mechanical resistance, pH and polymerization degree, raise of copper number and yellowing	1896 (1960's on heritage materials)	Ben-Arie and Barkai-Golan, 1969; Pavon Flores, 1976; Hanus, 1985; Butterfield, 1987; Tomazello et al., 1995; Adamo et al., 1998; Adamo et al., 2001; Florian, 2002; Adamo et al., 2003; Magaudda, 2004; Bank et al., 2008
High frequency current	Also dries the materials, which is an advantage in a case of wet documents. Does not leave toxic residues. Can be used for mass treatments	Mediocre antifungal activity Uses high temperature and physical pressure Soiled paper may ignite	Increasing of carbonyl groups on cellulose Diminishment of tear resistance.	1947	Flieder, 1965
High temperature		Can lower the moisture content on materials	Can act as a conidia activator Temperatures above 100 °C need to be reached in order to kill spores	Accelerates the rates of chemical	deterioration
	Thevelein et al., 1979; Strang, 1992; Hugo, 1995; Strang, 2001; Brokerhof, 2002; Florian, 2002; Pinniger, 2003; Ackery et al., 2004				
Low-oxygen environments		Does not leave toxic residues Can prevent deteriorating chemical reactions that are oxygen dependent Can also kill insect pests	Only causes a decrease on fungal growth, does not stop it		Atmospheres high on CO_2 can change the acid/base balance on the materials
1990: on heritage materials	Hocking, 1989; Valentin et al., 1990; Florian, 1997				
Refrigeration, 4 °C	Does not leave toxic residues Slows down chemical deterioration rates	Only slows down but does not stop fungal growth Some fungal species can produce heavy coloured pigments, more conidia and polyols under this kind of stress Can activate conidia	Solubilisation and migration of water soluble compounds		Florian, 1997, 2002
Ultraviolet radiation	Does not leave toxic residues Can act as a fungicidal, depending on the radiation dosage	Poor penetration power Has cumulative degrading effects on the treated materials	Causes oxidation of cellulose, leading to yellowing, bleaching and brittleness of paper	1903	Gallo, 1963; Nyberg, 1987; Hugo, 1995; Florian, 2002; Bukovsky and Trnkova, 2003a, b; Paulus, 2004; Belloni et al., 2006

correctly shielded from the outside in order to prevent health hazards. The antimicrobial effect of γ-radiation was shown by Mink already in 1896 (Hugo, 1995).

The microbicidal activity of this highly penetrating ionization radiation is due to its ability to generate radicals capable of cleaving carbon–carbon bonds thus destroying cellular DNA and turning microbial cells non-viable (Bank et al., 2008).

The disinfection of archival materials with γ-radiation was firstly studied in Russia in the early 1960s (Tomazello et al., 1995; Magaudda, 2004). Since then, several studies have been performed in order to determine the minimum radiation level required to eliminate the more common fungal species identified in graphic documents, and also evaluate the effects γ-radiation has on paper materials at short and long term.

On the literature reviewed, several minimum lethal radiation dosages are reported, which is not surprising since different fungal species were tested (Pavon Flores, 1976; Butterfield, 1987; Tomazello et al., 1995; Adamo et al., 2001; Adamo et al., 2003).

Pavon Flores (1976) concluded that the lethal dose of radiation required for all the fungal species tested was 18 kGy (kGy) at a rate of 5 kGy h^{-1}, although lower dosages were already lethal for many species. She also observed a remarkable increase in the pigmentation intensity on some fungal species after irradiation, which could represent a problem when real heritage documents are treated by this method. The effects of γ-irradiation on paper samples were tested as well, under normal and nitrogen atmospheres in order to clarify the role of oxygen in the deterioration reactions. According to the author, the mechanical resistance of the papers did not suffer considerable modifications after the irradiation, but after the artificial ageing some changes were noticed, mainly in the folding endurance. On the other hand, the copper number and the polymerization degree were severely affected both before and after artificial ageing. This chemical deterioration was mainly observed on the papers with high cellulose content, by comparison with papers with high lignin content, like newspaper, revealing a protective effect of this aromatic compound. Moreover, the nitrogen atmosphere did not have any protective effect on the deteriorations of papers (Pavon Flores, 1976).

These results on the paper deterioration can be justified by the effect γ-radiation has on organic materials. By the same mechanism it works on microorganisms – through the formation of radicals that deteriorate organic matter – it can also act on other organic materials, like cellulose.

Some authors indicate a synergistic effect between γ-radiation and heat, referring that this effect could allow a twenty-fold decrease in minimum effective radiation dosage (Ben-Arie and Barkai-Golan, 1969; Hanus, 1985). Nevertheless, no studies were performed in order to verify what were the effects of this combination on paper materials, since the deterioration reactions could also be synergistic.

Butterfield (1987), assuming 10 kGy gamma irradiation as the lethal dose for most fungi, and using a 156 Gy h^{-1} rate, tested its effects on paper samples. His results point out that irradiation causes similar deterioration as artificial ageing. Although, the combined treatment of irradiation followed by artificial ageing acts synergistically, since the deteriorating effects of this combination are more severe than the sum of the effects of the two separate treatments (Butterfield, 1987). These results point out that heat can act as a catalyser on the deteriorating reactions caused by radiation.

Tomazello et al. (1995) evaluated the lethal activity of γ-radiation on fungi spores existent on naturally contaminated papers and also the effect of pre-treatments with variable temperature and humidity levels on the minimum lethal dose of radiation. By increasing the doses of radiation (2–20 kGy at 4.103 kGy h^{-1}), the number of fungi decreased, although at higher doses between 17.5 and 20 kGy there were still viable fungi on the paper samples. The fungi contaminated samples subjected to a drying on oven at 50 °C pre-treatment showed more resistance to radiation when compared with the samples pre-treated with moist heat and samples at room conditions (Tomazello et al., 1995). Since dormant conidia are more resistant to extreme conditions than activated conidia or fungi in vegetative stages and the maintenance of dormancy is considered to be the low water content in the spore (Florian, 2002), it is possible that the moist heat and the room conditions could have activated the spores existent on the paper and thus turned them more susceptible to radiation. On the other hand, the same conditions that turned the fungi more susceptible to radiation (moist heat), conversely improved tremendously the resistance of bacteria (Tomazello et al., 1995). Since bacteria and fungi coexist in natural contaminated objects, this kind of pre-treatment would not be advisable and besides, moist heat is known to have deteriorating effects on paper.

Adamo et al. (1998) evaluated the effects of gamma irradiation treatment on pure cellulose paper samples, at room temperature, soaked with water, and soaked and frozen. Before analysis, the soaked and frozen samples were either dried in a ventilated oven or dried in vacuum by sublimation (freeze-dried). Irradiation was performed at doses between 0 and 10 kGy, using a dose rate of 2.8 kGy h^{-1}. The results achieved are similar to the ones obtained by Butterfield (1987), concluding that the alterations caused by radiation are similar to those caused by artificial ageing and that the combination of both treatments has a synergistic effect on the deterioration of cellulose, revealed by a drastic fall in the degree of polymerization. Besides, a very slight decrease in pH and a significant yellowing were observed on the irradiated samples, especially after ageing (Adamo et al., 1998). Regarding the variations between sample sets, the freeze-dried ones show significantly lower mechanical resistance than the other ones (Adamo et al., 1998), one possible explanation is described in the "Freezing" chapter.

Different environmental conditions during irradiation, like nitrogen and vacuum atmospheres, and saturation of samples with water, were tested by Adamo et al. (2001). The radiation doses administered varied between 2 and 5 kGy at a dose rate of 14.7 kGy h^{-1}. The results showed that in general, different environmental conditions tested did not reduce the deleterious effects caused by γ-radiation on paper. Regarding the antifungal essay, according to the authors, only the saturated samples showed an additional antifungal activity by comparison with the altered atmosphere variants, maybe due to the radicals also formed in the water which could provide an extra germicidal power to the treatment (Adamo et al., 2001).

The exposure to γ-radiation can also turn cellulose more susceptible to further infestations (Adamo et al., 2003). The depolymerization caused by irradiation breaks the cellulose polymer into smaller units which can be more easily available to biodeteriogens.

Summarizing the results obtained by the several studies reviewed, we can conclude that the antifungal activity of γ-radiation is dose dependent and varies with the fungal species and their stage of development. Exposure to this radiation can induce fungi to produce more coloured metabolic products and after irradiation the treated paper will become more susceptible to further biodeterioration. Gamma radiation can cause severe depolymerization of cellulose and significant yellowing, which is highly enhanced by artificial ageing. On the other hand, the mechanical resistance and pH of irradiated papers remain practically unaffected. This antifungal treatment does not leave any toxic residues but has cumulative degrading effects on the treated materials.

2.2.3. High frequency current

The high frequency current method started to be used by the Russians in 1947 for drying wet documents. During this process, they realized that the method was also destroying the fungi spores on documents, and so they started to used it also as an antifungal treatment (Flieder, 1965). This method acts through an extremely fast increase in temperature (a few seconds) in a homogeneous way, from the centre to the periphery of documents.

One of the high frequency current method procedures, as described by Flieder (1965), consists on placing the documents in a cabinet, press them strongly between 2 capacitor plates, where a current of 1.5–1.6 A and a grid current of 0.30–0.32 A passes through them. The temperature is kept between 90 and 100 °C, for 12–15 min.

It has also an insecticidal effect, and no chemicals remain in the materials treated. Nevertheless, its antifungal effect is described as mediocre. Leather, parchment, seals and documents with elements in relief cannot be treated due to the pressure and temperature.

Also, the documents have to be thoroughly cleaned previously, because the dust may ignite. Analysis on paper samples reveal an increase on the copper number (c. 40%) and a diminishment of tear resistance (c. 20%) (Flieder, 1965).

2.2.4. Low-oxygen environments

The use of modified atmospheres, i.e., low oxygen, carbon dioxide or nitrogen is common in the food industry. According to Hocking (1989) atmospheres with high percentage of carbon dioxide are more effective in controlling fungal growth than those which exclude oxygen by replacement with nitrogen. Also, the production of mycotoxins can be inhibited in modified atmospheres, but again high concentrations of CO_2 are more effective than reduced O_2 content (Hocking, 1989).

Valentin et al. (1990) have studied the microbial control potential of low oxygen and low relative humidity environments. They used parchment samples inoculated with *A. flavus* or exposed to environmental contamination. The low O_2 environments (5%, 1% and 0.1%) were achieved by addition of nitrogen. The results showed that even though the reduction of O_2 caused a decrease on fungal growth; it was the reduction of RH that highly diminished the microbial development (Valentin et al., 1990).

Fungi need oxygen to growth, but some fungal species are efficient scavengers capable of near normal growth in oxygen concentrations below 1% (Hocking, 1989).

Low oxygen atmospheres besides reducing biodeterioration caused by fungi, can also eliminate insect pests, and in long term storage can prevent chemical reactions that are oxygen dependent like photooxidation or combustion in case of fire hazard. Nevertheless, atmospheres high in CO_2 can change the acid/base balance on treated materials (Florian, 1997).

2.2.5. Ultraviolet radiation

Ultraviolet (UV) radiation is electromagnetic radiation with wavelength between 10 and 400 nm. It is used in many scientific and industrial fields according to the wavelength range. For sterilization purposes, UV light is used in the range of about 260 nm, since this is the wavelength that causes the highest damage in DNA molecules (Paulus, 2004). UV radiation is commonly used to sterilize air, as in inoculating chambers in mycological laboratories, or water, as a sanitizing treatment for recreational water.

The microbicidal effect of UV radiation was discovered in 1903, by Barnard and Morgan, which by using a continuous arc current, found that the maximum bacterial effect occurred in the 226–328 nm range (Hugo, 1995).

UV radiation causes degradation of the genetic material of microbes by forming dimmers between adjacent thymine nucleotides in DNA chains, which will inhibit the correct replication and transcription of this nucleic acid (Paulus, 2004). Due to its low energy content, this kind of radiation has poor penetration power (Hugo, 1995).

The exposure of mouldy books to sunlight due to the disinfecting properties of UV radiation has been performed as common sense and suggested for treating small, localized outbreaks (Nyberg, 1987), although, according to Florian (2002), the main process behind the inhibition of fungal growth by this method is in fact dehydration.

According to Gallo (1963), UV radiation is rather inefficient in disinfecting textiles and books due to its low penetration power.

Belloni et al. (2006) studied UV radiation as a sterilizer for cellulose-based cultural heritage, using as UV source an excimer laser operating at 308 nm. The sterilizing properties of the laser were tested against a mycelial actinomycete and the results show that after 75 s of irradiation, the survival rate decreased three orders of magnitude.

However, it is well known that UV radiation due to its high energy can cause damage to most of the heritage materials. Cellulose can be damaged significantly by UV radiation of high energy (about 280 nm) which is the within the range used to kill microorganisms (Florian, 2002; Bukovsky and Trnkova, 2003a). The oxidation of cellulose caused by UV radiation, leads to yellowing, bleaching and brittleness of paper (Bukovsky and Trnkova, 2003a,b).

2.2.6. Temperature extremes

Temperature influences the rate of metabolic activity of microorganisms and thus their rate of growth. Each species of fungi has its own optimum growth temperature, and also its minimum and maximum limiting temperatures for development. Temperature is related to the equilibrium moisture content (EMC) of microbial cells, and also the EMC of the substrate, which are limiting factors for fungal development. According to Florian (2002), the growth of fungi can occur between −7 °C and 30 °C. Even tough, before activation, conidial cells are much more resistant to temperature extremes than on the subsequent developmental stages (Florian, 2002).

2.2.6.1. Freezing. Freezing has been used in food preservation since as early as 1000 BC in ice cellars in China (Archer, 2004).

The lethal effects on microorganisms caused by freezing are time and temperature dependent and can originate from physical and/or chemical damages. The physical damages are related to the formation of extracellular or intracellular ice formation, causing the rupture of membranes and organelles (Florian, 2002; Archer, 2004). The chemical damages occur as the water freezes and the solutes become concentrated in the unfrozen fraction of water in which the microorganisms tend to concentrate. This process leads to a concentration of extracellular and intracellular solutes, causing lethal pH and ionic changes (Florian, 2002; Archer, 2004).

The formation of ice also lowers the water activity on the substrate, and thus limits the microbial activity (Gunde-Cimerman et al., 2003).

In the field of heritage conservation, freezing, besides being used to kill insect pests, and in long term preservation of photographic materials, it is also used, like refrigeration, as an intermediate temporary methodology to prevent fungal growth until the materials can be properly dried. However, low enough freezing temperatures can actually kill microorganisms, rather than just slowing down their growth as in refrigeration. Also, freezing can prevent soluble compounds in the materials from migrating and bleeding.

Generally, the organisms are killed more quickly when are held at or near their freezing temperatures, with cycles of freezing/thawing and when the rates of freezing and thawing are slow (Florian, 2002). At temperatures between 0 and −4 °C, only a small quantity of water in materials is in fact frozen and in the fine capillaries of organic materials, the water may not freeze even at much lower temperatures and can still support microbial activity (Florian, 1997). The microbial growth can be stopped in storage at −20 °C but at this temperature the ice may also cause damage of the water-soaked materials (Florian, 2002).

It is important to take into account that dry conidia are much more resistant to freezing than hydrated spores and vegetative hyphae, due to their low water content which does not form internal ice crystals (Florian, 2002). These cells can still be viable after thawing and become activated when conductive conditions arise.

The increasing concentration of solutes in the materials during the freezing process, besides contributing to the inactivation of microbes, can also contribute to increase some deteriorating

chemical reactions on the substrate, like pH dependent reactions and lipid oxidation which produces high energy radicals (Takenaka et al., 1996; Florian, 1997).

The formation of ice crystals increases the volume of water in about 8.5% (Florian, 1997). In water-soaked materials, the formation of ice crystals during freezing will also cause a volume expansion. The spaces created by the crystals will remain unaltered after the material is dried, and on materials such as paper the thickness and porosity will become higher (Florian, 2002). The increasing in porosity will change some of the paper's characteristics, like absorbance and mechanical properties, and the dimensional changes can compose a problem, for instance on bound volumes.

On the thawing process, the moisture in materials will become high, which can activate dry conidia and also cause migration and bleeding of soluble components on the material. Moreover, the water evaporation forces on the drying process can cause dimensional changes and distortions.

In order to prevent these deteriorating processes from happening, the frozen heritage materials can be dried by sublimation, a method where the water passes directly from the solid to the gaseous state. This process of freezing and subsequently drying by sublimation is called *freeze-drying*. This method is reported to be more lethal to fungi than freezing followed by thawing, due to its ability to remove also bound water from the microbial cells, nevertheless, more resistant cells may still survive (Florian, 1997).

According to Florian (1997) the process of sublimation can be carried out in a vacuum chamber maintained at freezing temperature. The frozen material is associated with a heat source for sublimation. In the system, a condensing surface is maintained at a lower temperature than the frozen material and so the sublimed water vapour will diffuse from the material to the condenser and then thawed. This is a slow process but it can be accelerated by using vacuum. Flink and Hoyer (1971) tested this procedure on documents affected by a flood and subsequently frozen, using a heat source at 45 °C and a pressure of 26.7 Pa (vacuum pressure). According to their results, in none of the objects did ink running occur. The average drying time for documents 2–3 cm thick was 1.5–2 days, while a photographic album 10 cm thick took 4.5 days to dry (Flink and Hoyer, 1971).

The freeze-drying method, besides requiring technology mostly not available in cultural heritage institutions, such as museums, archives or historical houses, also presents some risks to the paper based objects. Since the sublimation front moves from the outside to the inside of the object, the outer layers are continuously being dried and may lose some of their bound water and become impermeable and case-hardened (Florian, 1997). It is the bound water that provides plasticity to organic polymers like cellulose or collagen, and so, its lost will mean embrittlement of paper, parchment, leather and some adhesives. One possible example of this effect is presented on an article about gamma-irradiation (Adamo et al., 1998), in which a set of paper samples is irradiated while frozen and then freeze-dried. In comparison with other set of samples irradiated while frozen and dried in a ventilated oven at 35 °C, this first one exhibits a much lower mechanical resistance.

Basset and Draïs (2011) have developed an alternative to lyophilisation in drying frozen graphic documents. Firstly the excess ice on the surface of the object is removed with a hair dryer at 35 °C and a spatula. Afterwards the object is wrapped on blotting paper, sealed in a hermetic bag and immersed in 70 °C water. The blotters are replaced in every 20 min. After the object is thawed, it is dried using fans and interleaving the book's pages with blotting paper. According to the authors, a book 15 cm thick can be thawed in 3 h and dried in 48 h with no additional damage caused, although the amount of handling that the book is subjected to when changing the blotting is an inconvenience. In this study it is not revealed if soluble media migrates with thawing and if any pressure is applied to prevent physical deformations during the drying process.

2.2.6.2. High temperature. Heat has been used for ages as a method of sterilization. Already Aristotle has recommended to Alexander the Great that his troops should boil the water before drinking in order to avoid illness (Hugo, 1995).

The use of high temperatures to eliminate insect pests from heritage objects has been studied by several authors (Strang, 2001; Brokerhof, 2002; Ackery et al., 2004). According to the literature, insect pests are killed by being exposed to 55–60 °C for 1 h (Strang, 1992; Pinniger, 2003).

Fungi, on the other hand can resist to higher temperatures. For instance, thermophilic fungi can still grow at 50–60 °C (Rajasekaran and Maheshwari, 1993). The methods used to sterilize media and equipment in laboratory practice use temperatures above 100 °C (autoclaving is performed at 121 °C, 100 kPa) in order to kill both vegetative forms and spores of microorganisms.

According to Florian (2002), herbarium samples are usually dried at temperatures around 50 °C, which kills germinating conidia. On the other hand, dry conidia have longer viability and are more resistant to heat than hydrated conidia or the vegetative stages of fungi, and so, they can survive a heat treatment that apparently has stopped fungal growth (Florian, 2002). Besides, heat can also act as a conidia activator, allowing an immediate development of the fungus once the conductive conditions arise (Thevelein et al., 1979).

Heat can be an auxiliary method for drying wet heritage materials, since it helps to reduce their moisture content, but the exposure to high temperatures can accelerate deterioration rates and cause dimensional changes on heritage materials, namely on paper.

2.2.6.3. Refrigeration. Refrigeration (c. 4 °C) is universally used in domestic short term food preservation. In heritage conservation field, refrigeration is mainly used for temporary storage of water soaked materials, in order to slow down fungal growth, while gaining time for preparing dehydration or other antifungal procedures.

Long term storage at this range of temperatures is not advisable, since moisture in materials remains high and the fungal growth, although slowed down, still continues. The fungi being under this kind of stress can produce more conidia, melanin pigmented hyphae staining the substrate sometimes irreversibly, or polyols, which can easily undergo autoxidation and form free radicals and peroxides (Florian, 2002). Moreover, some conidia can become activated by this range of temperatures, and as soon as they are exposed to a proper growth temperature, they will immediately germinate (Florian, 1997, 2002).

Paper materials by remaining wet under refrigeration, can also suffer dissolution and migration of water soluble media, adhesives, sizing and grounds. On the other hand, chemical deterioration rates will remain lower at this range of temperatures.

3. Concluding remarks

Biodeterioration by fungi is a major problem in paper conservation, not just because of the damages it causes, but also due to the lack of safe preventive and curative available options.

The maintenance of low RH and temperature levels together with good air circulation are well known procedures to prevent fungal development. However, not all heritage depository institutions have the means to achieve proper environmental conditions. Also, water related hazards can occur, originating fungal outbreaks. For these reasons, curative procedures are performed in various situations.

Throughout the history of cultural heritage conservation several toxic chemical compounds have been used to prevent and control fungal deterioration. Nowadays, this fact represents a health hazard to people who have to deal directly with these materials due to the toxic residues left by the treatments (Hollinger and Hansen, 2010). More recently, a growing concern about environmental and health issues has led to the investigation on new antifungal alternatives, with lower toxicity, which are summarized in this manuscript.

In the literature reviewed it can be found that the efficacy of an antifungal compound or method may vary with several factors, like its kind of mechanism of action, the concentration/intensity used, and the period of contact/action. Also, the susceptibility of fungi to a particular antifungal compound or method can depend on the fungal strain, on its stage of development, or if it is or not part of a biofilm.

According to these studies, regarding the chemical antifungal compounds (fungistatic or fungicide), calcium propionate, esters of *p*-hydroxybenzoic acid (parabens) and ethanol, are the ones that combine minor health effects with less negative impact in paper properties. Among these three, parabens are the ones with best antifungal properties. Concerning the physical methods, dehydration seems to present the best conjugation between antifungal effect and minor alterations on the properties of paper materials. Nonetheless, one has to bear in mind that with the evolution of knowledge and research, a compound that is considered safe nowadays in terms of health and effects on materials, may not be so in the future.

All antifungal methods reviewed in this work have strengths and weaknesses. They present different degrees of efficacy, toxicity and some of them are safer to use in heritage materials than others. Development and evaluation of innovative antifungal methods in order to achieve and recognize better approaches than the ones already existent is still an active research field.

Knowing the pros and cons of the available methods to antifungal treatment of paper allows conscious decisions adapted to different situations. The collection of data presented in this manuscript is intended to be an aid to this decision-making effort. The information compiled in this review can be of great value when a fast intervention is needed, as in an emergency situation.

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References

Abad, M., Ansuategui, M., Bermejo, P., 2007. Active antifungal substances from natural sources. Arkivoc VII, 116–145.

Abdel-Kareem, O., 2010. Fungal deterioration of historical textiles and approaches for their control in Egypt. E-Preservation Science 7, 40–47.

Ackery, P.R., Testa, J.M., Ready, P.D., Doyle, A.M., Pinniger, D.B., 2004. Effects of high temperature pest eradication on DNA in entomological collections. Studies in Conservation 49, 35–40.

Adamo, A.M., Giovannotti, M., Magaudda, G., Zappala, M.P., Rocchetti, F., Rossi, G., 1998. Effect of gamma rays on pure cellulose paper as a model for the study of a treatment of "biological recovery" of biodeteriorated books. Restaurator 19, 41–59.

Adamo, M., Brizzi, M., Magaudda, G., Martinelli, G., Plossi-Zappala, M., Rocchetti, F., Savagnone, F., 2001. Gamma radiation treatment of paper in different environmental conditions - chemical, physical and microbiology analysis. Restaurator 22, 107–131.

Adamo, M., Magaudda, G., Nisini, P.T., Tronelli, G., 2003. Susceptibility of cellulose to attack by cellulolytic microfungi after gamma irradiation and ageing. Restaurator 24, 145–151.

Afsharpour, M., Rad, F.T., Malekian, H., 2011. New cellulosic titanium dioxide nanocomposite as a protective coating for preserving paper-art-works. Journal of Cultural Heritage 12, 380–383.

Allsopp, D., Seal, K., Gaylarde, C., 2004. Introduction to Biodeterioration, second ed. Press Sindicate of the University of Cambridge United States of America.

Arai, H., 2000. Foxing caused by Fungi: twenty-five years of study. International Biodeterioration & Biodegradation 46, 181–188.

Archer, D.L., 2004. Freezing: an underutilized food safety technology? International Journal of Food Microbiology 90, 127–138.

Bacílková, B., 2006. Study on the effect of butanol vapours and other alcohols on fungi. Restaurator 27, 186–199.

Ballard, M.W., Baer, N.S., 1986. Ethylene oxide fumigation: results and risk assessment. Restaurator 7, 143–168.

Bank, T.L., Kukkadapu, R.K., Madden, A.S., Ginder-Vogel, M.A., Baldwin, M.E., Jardine, P.M., 2008. Effects of gamma-sterilization on the physico-chemical properties of natural sediments. Chemical Geology 251, 1–7.

Basset, T., Draïs, M., 2011. La décongéLation d'un ouvrage inondé sans lyophiLisation. In: Bridgland, J. (Ed.), ICOM Committee for Conservation, Preprints of the 16th Triennial conference Lisbon 19–23 September 2011, CD-ROM ed. Critério – Produção Gráfica, Lda, Lisbon.

Bello-Gonzalez, T., Rosales-Pantoja, P., Acosta-Gio, A.E., de Waard, J.H., 2008. Instrument processing with lauryl dimethyl benzyl ammonium bromide: a challenge for patient safety. American Journal of Infection Control 36, 598–601.

Belloni, F., Nassisi, V., Alifano, P., Monaco, C., Panzanaro, S., 2006. The effects of UV laser radiation as sterilizer for cultural heritage. Macromolecular Symposia 238, 52–56.

Ben-Arie, R., Barkai-Golan, R., 1969. Combined heat-radiation treatments to control storage rots of Spadona pears. The International Journal of Applied Radiation and Isotopes 20, 687–690.

Bennett, J.W., Klich, M., 2009. Mycotoxins. In: Moselio, S. (Ed.), Encyclopedia of Microbiology, third ed. Academic Press, Oxford, pp. 559–565.

Blake, D.M., Maness, P.-C., Huang, Z., Wolfrum, E.J., Huang, J., Jacoby, W.A., 1999. Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. Separation & Purification Reviews 28, 1–50.

Block, S.S., 2001. Disinfection, Sterilization, and Preservation, fifth ed. Lippincott Williams & Wilkins, Philadelphia.

Brokerhof, A.W., 2002. Solarization: a cheap but effective method to disinfest museum objects. In: Vontobel, R. (Ed.), Proceedings of the ICOM-Committee for Conservation 13th Triennial Meeting, Rio de Janeiro, pp. 15–20.

Bukovsky, V., Trnkova, M., 2003a. The influence of secondary chromophores on the light induced oxidation of paper part II: the influence of light on groundwood paper. Restaurator 24, 118–132.

Bukovsky, V., Trnkova, M., 2003b. The influence of secondary chromophores on the light induced oxidation of paper Part I: the influence of light on cellulose and secondary chromophores. Restaurator 24, 18–35.

Butterfield, F.J., 1987. The potential long-term effects of gamma irradiation on paper. Studies in Conservation 32, 181–191.

Buyukleyla, M., Rencuzogullari, E., 2009. The effects of thymol on sister chromatid exchange, chromosome aberration and micronucleus in human lymphocytes. Ecotoxicology and Environmental Safety 72, 943–947.

Chawengkijwanich, C., Hayata, Y., 2008. Development of TiO2 powder-coated food packaging film and its ability to inactivate Escherichia coli in vitro and in actual tests. International Journal of Food Microbiology 123, 288–292.

Coimbra, M., Isacchi, B., van Bloois, L., Torano, J.S., Ket, A., Wu, X., Broere, F., Metselaar, J.M., Rijcken, C.J.F., Storm, G., Bilia, R., Schiffelers, R.M., 2011. Improving solubility and chemical stability of natural compounds for medicinal use by incorporation into liposomes. International Journal of Pharmaceutics 416, 433–442.

Cotton, R.T., Roark, R.C., 1928. Ethylene oxide as a fumigant. Industrial & Engineering Chemistry 20, 805.

Cox, P.J., Foote, N.M., MacManus, S.M., 2004. Solid state study of hydrogen bonding in dichlorophen crystals. International Journal of Pharmaceutics 269, 15–18.

Craig, R., 1986. Alternative approaches to the treatment of mould biodeterioration—an international problem. The Paper Conservator 10, 27–30.

Daniels, V., Boyd, B., 1986. The yellowing of thymol in the display of prints. Studies in Conservation 31, 156–158.

Dersarkissian, M., Goodberry, M., 1980. Experiments with non-toxic anti-fungal agents. Studies in Conservation 25, 28–36.

EC, 2008. Regulation (EC) No 689/2008 of the European Parliament and of the Council of 17 June 2008 concerning the export and import of dangerous chemicals. Official Journal of the European Union 51, 1–35.

EC, 2012. Consolidated List of Existing Active Substances for Which a Decision of Non-inclusion into Annex I or IA of Directive 98/8/EC Has Been Adopted, Decisions Concerning the Non-inclusion of Substances in Annex I, IA or IB to Directive 98/8/EC. European Comission, Environment Directorate-General.

ECHA, 2012. Registered Substances. European Chemicals Agency. http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances. 05/06/2012.

Escalada, J.P., Pajares, A., Gianotti, J., Biasutti, A., Criado, S., Molina, P., Massad, W., Amat-Guerri, F., García, N.A., 2011. Photosensitized degradation in water of the phenolic pesticides bromoxynil and dichlorophen in the presence of riboflavin, as a model of their natural photodecomposition in the environment. Journal of Hazardous Materials 186, 466–472.

EU-OSHA, European Agency for Safety and Health at Work, 2009. Exploratory Survey of Occupational Exposure Limits for Carcinogens, Mutagens and Reprotoxic Substances at EU Member States Level European Risk Observatory Report. Bilbao.

Fabbri, A., Ricelli, A., Brasini, S., Fanelli, C., 1997. Effect of different antifungals on the control of paper biodeterioration caused by fungi. International Biodeterioration & Biodegradation 39, 61–65.
Fernández Freire, P., Labrador, V., Pérez Martín, J.M., Hazen, M.J., 2005. Cytotoxic effects in mammalian Vero cells exposed to pentachlorophenol. Toxicology 210, 37–44.
Flieder, F., 1965. Action des différents produits fongicides et insecticides, utilisés en conservation sur la résistance physico-chimique des papiers. In: 5th Joint Meeting Of The Icom Committee for Museum Laboratories and of the Sub-Committee for the Care of Paintings. ICOM, New York, USA, pp. 1–46.
Flink, J., Hoyer, H., 1971. Conservation of water-damaged written documents by freeze-drying. Nature 234, 420.
Florian, M.-L.E., 1997. Heritage Eaters - Insects & Fungi in Heritage Collections. James & James Ltd, London, UK.
Florian, M.-L.E., 1998. "Natural products" - for insect and fungal control? Ethnographic Conservation Newsletter 18, 4–8.
Florian, M.-L.E., 2002. Fungal Facts - Solving Fungal Problems in Heritage Collections. Archetype Publications, Great Britain.
Freitas, M.B.-L.B., 1937. A arte do Livro: Manual do Encadernador, Facsimile. Lisboa: Edinova, 2000 ed. Livraria Sá da Costa, Lisboa.
Gallo, F., 1963. Aspetti della lotta preventiva e curativa contro i microrganismi dannosi al materiale bibliografico e archivistico. Bollettino dell'Istituto di patologia del libro "Alfonso Gallo" 22, 29–66.
Gallo, F., Pasquariello, G., Rocchetti, F., 1998. Biological investigation on sizings for permanent papers. Restaurator 19, 61–84.
Garside, P., Knight, B., 2011. The behaviour of books in changing environmental conditions and the implications for collection storage. In: Bridgland, J. (Ed.), ICOM Committee for Conservation, Preprints of the 16th Triennial Conference Lisbon 19–23 September 2011, CD-ROM ed. Critério – Produção Gráfica, Lda, Lisbon.
Gavriliu, S., Lungu, M., Gavriliu, L.C., Grigore, F., Groza, C., 2009. Antimicrobial colloidal suspensions of silver-titania. The Open Chemical and Biomedical Methods Journal 2, 77–85.
Giavini, E., Menegola, E., 2010. Are azole fungicides a teratogenic risk for human conceptus? Toxicology Letters 198, 106–111.
Gollapudy, R., Ajmani, S., Kulkarni, S.A., 2004. Modeling and interactions of *Aspergillus fumigatus* lanosterol 14-α demethylase `A' with azole antifungals. Bioorganic & Medicinal Chemistry 12, 2937–2950.
Guillitte, O., 1995. Bioreceptivity - a new concept for building ecology studies. Science of the Total Environment 167, 215–220.
Gunde-Cimerman, N., Sonjak, S., Zalar, P., Frisvad, J.C., Diderichsen, B., Plemenitaš, A., 2003. Extremophilic fungi in arctic ice: a relationship between adaptation to low temperature and water activity. Physics and Chemistry of the Earth 28, 1273–1278.
Gupta, P.K., Aggarwal, M., 2012. Chapter 55-Toxicity of fungicides, veterinary toxicology, second ed. Academic Press, Boston, pp. 653–670.
Gustafson, R.A., Modaresi, I.R., Hampton, G.V., Chepesiuk, R.J., Kelley, G.A., 1990. Fungicidal efficacy of selected chemicals in thymol cabinets. Journal of the American Institute for Conservation 29, 153–168.
Haines, J.H., Kohler, S.A., 1986. An evaluation of ortho-phenyl phenol as a fungicidal fumigant for archives and libraries. Journal of the American Institute for Conservation 25, 49–55.
Hanus, J., 1985. Gamma radiation for use in archives and libraries. Abbey Newsletter 9, 34.
Harding, M.W., Marques, L.L.R., Howard, R.J., Olson, M.E., 2009. Can filamentous fungi form biofilms? Trends in Microbiology 17, 475–480.
Heim, R., Flieder, F., Nicot, J., 1968. Combating the Moulds which Develop on Cultural Property in Tropical Climates, the Conservation of Cultural Property, with Special Reference to Tropical Conditions. UNESCO, Lausanne, pp. 41–52.
Hengemihle, F.H., Weberg, N., Shahani, C.J., The Library of Congress, 1995. Desorption of Residual Ethylene Oxide from Fumigated Library Materials. Preservation Research and Testing Series, Washington, D.C., 9502.
Hocking, A.D., 1989. Responses of fungi to modified atmospheres. In: Champ, B.R., Highley, E., Banks, H.J. (Eds.), Fumigation and Controlled Atmosphere Storage of Grain: Proceedings of an International Conference Held at Singapore, 14–18 February 1989. Australian Centre for International Agricultural Research, Singapore, pp. 70–82.
Hollinger, R.E., Hansen, G., 2010. Discussion: mitigation of contaminated collections. In: Charola, A.E., Koestler, R.J. (Eds.), Pesticide Mitigation in Museum Collections: Science in Conservation. Proceedings from the MCI Workshop Series. Smithsonian Institution Scholarly Press, Washington, D.C, pp. 65–70.
Huang, Z., Maness, P.-C., Blake, D.M., Wolfrum, E.J., Smolinski, S.L., Jacoby, W.A., 2000. Bactericidal mode of titanium dioxide photocatalysis. Journal of Photochemistry and Photobiology A: Chemistry 130, 163–170.
Hugo, W.B., 1995. A brief history of heat, chemical and radiation preservation and disinfection. International Biodeterioration & Biodegradation 36, 197–217.
IARC, International Agency for Research on Cancer, 1999a. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. Monographs on the Evaluation of Carcinogenic Risks to Humans 71, 769–816.
IARC, International Agency for Research on Cancer, 1999b. Ortho-phnenylphenol and its sodium salt. Monographs on the Evaluation of Carcinogenic Risks to Humans 73, 451–480.
IARC, International Agency for Research on Cancer, 2006. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol. Monographs on the Evaluation of Carcinogenic Risks to Humans 88, 39–325.
IARC, International Agency for Research on Cancer, 2008. 1,3-butadiene, ethylene oxide and vinyl halides (vinyl fluoride, vinyl chloride and vinyl bromide). Monographs on the Evaluation of Carcinogenic Risks to Humans 97, 185–309.
IARC, International Agency for Research on Cancer, 2010. Carbon black, titanium dioxide, and talc. Monographs on the Evaluation of Carcinogenic Risks to Humans 93, 193–275.
Isbell, L.H., 1997. The effects of thymol on paper, pigments, and media. Abbey Newsletter 21, 39–43.
Jenkinson, H., 1922. A Manual of Archive Administration: Including the Problems of War Archives and Archive Making. Clarendon Press, Oxford.
Karpouhtsis, I., Pardali, E., Feggou, E., Kokkini, S., Scouras, Z.G., Mavragani-Tsipidou, P., 1998. Insecticidal and genotoxic activities of oregano essential oils. Journal of Agricultural and Food Chemistry 46, 1111–1115.
Kowalik, R., 1980. Microbiodeterioration of library materials. Part 2. Microbiodecomposition of basic organic library materials. Chapter 4. Restaurator 4, 135–219.
Lamb, D.C., Kelly, D.E., Baldwin, B.C., Kelly, S.L., 2000. Differential inhibition of human CYP3A4 and Candida albicans CYP51 with azole antifungal agents. Chemico-Biological Interactions 125, 165–175.
Magaudda, G., 2004. The recovery of biodeteriorated books and archive documents through gamma radiation: some considerations on the results achieved. Journal of Cultural Heritage 5, 113–118.
Markowska-Szczupak, A., Ulfig, K., Morawski, A.W., 2011. The application of titanium dioxide for deactivation of bioparticulates: an overview. Catalysis Today 169, 249–257.
Matsunaga, T., Tomoda, R., Nakajima, T., Nakamura, N., Komine, T., 1988. Continuous-sterilization system that uses photosemiconductor powders. Applied and Environmental Microbiology 54, 1330–1333.
McBain, A.J., Gilbert, P., 2001. Biocide tolerance and the harbingers of doom. International Biodeterioration & Biodegradation 47, 55–61.
Mendes, G.C.C., Brandão, T.R.S., Silva, C.L.M., 2007. Ethylene oxide sterilization of medical devices: a review. American Journal of Infection Control 35, 574–581.
Miller, C.S., Aboul-Ela, M.M., 1969. Fate of pentachlorophenol in cotton. Journal of Agricultural and Food Chemistry 17, 1244–1246.
Napoli, E.M., Curcuruto, G., Ruberto, G., 2010. Screening of the essential oil composition of wild Sicilian thyme. Biochemical Systematics and Ecology 38, 816–822.
Neves, E., 2006. Avaliação da acção antifúngica e desacidificante de uma nova mistura: Aplicação à conservação e tratamento do papel, Master thesis. Universidade Nova de Lisboa, Lisboa, Portugal.
Neves, E., Schafer, S., Phillips, A., Canejo, J., Macedo, M., 2009. Antifungal effect of different methyl and propyl paraben mixtures on the treatment of paper biodeterioration. International Biodeterioration & Biodegradation 63, 267–272.
Nittérus, M., 2000a. Ethanol as fungal sanitizer in paper conservation. Restaurator 21, 101–115.
Nittérus, M., 2000b. Fungi in archives and libraries. Restaurator 21, 25–40.
Numpaque, M., Oviedo, L., Gil, J., García, C., Durango, D., 2011. Thymol and carvacrol: biotransformation and antifungal activity against the plant pathogenic fungi Colletotrichum acutatum and Botryodiplodia theobromae. Tropical Plant Pathology 36, 3–13.
Nyberg, S., 1987. The Invasion of the Giant Spore. SOLINET Preservation Program 5.
OSHA, Occupational Safety & Health Administration, 1984. Occupational exposure to ethylene oxide; final standard. Federal Register 49, 25734–25809. 25734.
Paulus, W., 2004. Directory of Microbicides for the Protection of Materials - A Handbook. Kluwer Academic Publishers, The Netherlands.
Pavon Flores, S.C., 1976. Gamma radiation as fungicide and its effects on paper. Bulletin of the American Institute for Conservation of Historic and Artistic Works 16, 15–44.
Pérez-Rivera, A.A., Hu, T., Aardema, M.J., Nash, J.F., 2009. Evaluation of the genotoxicity of the imidazole antifungal climbazole: comparison to published results for other azole compounds. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 672, 27–39.
Pinheiro, A.C., Macedo, M.F., Jurado, V., Saiz-Jimenez, C., Viegas, C., Brandao, J., Rosado, L., 2011. Mould and yeast identification in archival settings: preliminary results on the use of traditional methods and molecular biology options in Portuguese archives. International Biodeterioration & Biodegradation 65, 619–627.
Pinniger, D., 2003. Saving our treasures - controlling museum pests with temperature extremes. Pesticide Outlook 14, 10–11.
Pinzari, F., Pasquariello, G., De Mico, A., 2006. Biodeterioration of paper: a SEM study of fungal spoilage reproduced under controlled conditions. Macromolecular Symposia 238, 57–66.
Pinzari, F., Zotti, M., De Mico, A., Calvini, P., 2010. Biodegradation of inorganic components in paper documents: formation of calcium oxalate crystals as a consequence of *Aspergillus terreus* Thom growth. International Biodeterioration & Biodegradation 64, 499–505.
Pinzari, F., Troiano, F., Piñar, G., Sterflinger, K., Montanari, M., 2011. The contribution of microbiological research in the field of book, paper and parchment conservation. In: Engel, P., Schirò, J., Larsen, R., Moussakova, E., Kecskemét, I. (Eds.), New Approaches to Book and Paper Conservation-Restoration. Verlag Berger, Wien/Horn, pp. 575–594.
Pitt, J.I., Christia, J., 1968. Water relations of xerophilic fungi isolated from prunes. Applied Microbiology 16, 1853–1858.
Ponce-Jimenez, M., Toral, F., Fornue, E., 2002. Antifungal protection and sizing of paper with chitosan salts and cellulose ethers. Part 1, physical effects. Journal of the American Institute for Conservation 41, 243–254.

Pyrgiotakis, G., Sigmund, W., 2008. Photocatalytic particles for biocidal applications. In: Sigmund, W., El-Shall, H., Shah, D.O., Moudgil, B. (Eds.), Particulate Systems in Nano- and Bio- Technologies. RCR Press, Boca Raton, U.S.A, pp. 283–307.

Rajasekaran, A.K., Maheshwari, R., 1993. Thermophilic fungi - an assessment of their potential for growth in soil. Journal of Biosciences 18, 345–354.

Rakotonirainy, M., Lavedrine, B., 2005. Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas. International Biodeterioration & Biodegradation 55, 141–147.

Rakotonirainy, M., Fohrer, F., Flieder, F., 1999. Research on fungicides for aerial disinfection by thermal fogging in libraries and archives. International Biodeterioration & Biodegradation 44, 133–139.

Rakotonirainy, M., Heraud, C., Lavedrine, B., 2003. Detection of viable fungal spores contaminant on documents and rapid control of the effectiveness of an ethylene oxide disinfection using ATP assay. Luminescence 18, 113–121.

Rakotonirainy, M.S., Juchauld, F., Gillet, M., Othman-Choulak, M., Lavedrine, B., 2007. The effect of linalool vapour on silver-gelatine photographs and bookbinding leathers. Restaurator 28, 95–111.

Raschle, P., 2001. Microbiology for our cultural heritage. Chimia 55, 990–995.

Russell, A., 2003. Similarities and differences in the responses of microorganisms to biocides. Journal of Antimicrobial Chemotherapy 52, 750–763.

SCCP, European Commission - Health & Consumer Protection Directorate-General, 2005. Scientific Committee on Consumer Products - Extended Opinion on Parabens. Underarm Cosmetics and Breast Cancer, SCCP/0874/05.

Šegvić Klarić, M., Kosalec, I., Mastelić, J., Piecková, E., Pepeljnak, S., 2007. Antifungal activity of thyme (Thymus vulgaris L.) essential oil and thymol against moulds from damp dwellings. Letters in Applied Microbiology 44, 36–42.

Şifa, T., 2008. Evaluation of genotoxic effects of sodium propionate, calcium propionate and potassium propionate on the root meristem cells of Allium cepa. Food and Chemical Toxicology 46, 2035–2041.

Sikkema, J., Debont, J.A.M., Poolman, B., 1994. Interactions of cyclic hydrocarbons with biological-membranes. Journal of Biological Chemistry 269, 8022–8028.

Sköld, M., Börje, A., Matura, M., Karlberg, A.-T., 2002. Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. Contact Dermatitis 46, 267–272.

Soni, M., Taylor, S., Greenberg, N., Burdock, G., 2002. Evaluation of the health aspects of methyl paraben: a review of the published literature. Food and Chemical Toxicology 40, 1335–1373.

Soni, M., Carabin, I., Burdock, G., 2005. Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food and Chemical Toxicology 43, 985–1015.

Sterflinger, K., Pinzari, F., 2012. The revenge of time: fungal deterioration of cultural heritage with particular reference to books, paper and parchment. Environmental Microbiology 14, 559–566.

Strang, T., 1992. A review of published temperatures for the control of pest insects in museums. Collection Forum 8, 41–67.

Strang, T.J.K., 2001. Principles of heat disinfestation. In: Kingsley, H., Pinniger, D., Xavier-Rowe, A., Winsor, P. (Eds.), Integrated Pest Management for Collections. James & James, London, pp. 114–129.

Strassberg, R., 1978. The use of fumigants in archival repositories. The American Archivist 41, 25–36.

Strlic, M., Kolar, J., Scholten, S., 2005. Paper and durability. In: Strlic, M., Kolar, J. (Eds.), Ageing and Stabilisation of Paper. National and University Library, Ljubljana, Slovenia, pp. 3–8.

Strzelczyk, A.B., Rozanski, J., 1986. The effect of disinfection with quaternary ammonium salt solution on paper. Restaurator 7, 3–13.

Suhr, K.I., Nielsen, P.V., 2004. Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. International Journal of Food Microbiology 95, 67–78.

Takenaka, N., Ueda, A., Daimon, T., Bandow, H., Dohmaru, T., Maeda, Y., 1996. Acceleration mechanism of chemical reaction by freezing: the reaction of nitrous acid with dissolved oxygen. The Journal of Physical Chemistry 100, 13874–13884.

Tateo, F., Bononi, M., 2006. Determination of ethylene chlorohydrin as marker of spices fumigation with ethylene oxide. Journal of Food Composition and Analysis 19, 83–87.

Thevelein, J.M., Vanassche, J.A., Carlier, A.R., Heremans, K., 1979. Heat activation of phycomyces-blakesleeanus spores - thermodynamics and effect of alcohols, furfural, and high-pressure. Journal of Bacteriology 139, 478–485.

Tomazello, G.C., Wiendl, M., Maximiliano, F., 1995. The applicability of gamma radiation to the control of fungi in naturally contaminated paper. Restaurator 16, 93–99.

Triolo, L., Di Trapani, R., Santucci, L., 1968. Resistenza e stabilità della carta:VII. Trattamento con composti fungicidi. Bollettino dell'Istituto di patologia del libro "Alfonso Gallo" 27, 207–224.

Tsuen-hsuin, T., 1985. Paper and Printing. Cambridge University Press, Cambridge.

US-EPA, Environmental Protection Agency Office of Pesticide Programs, Special Review and Reregistration Division, 1991. Reregistration Eligibility Document Propionic Acid, and Salts - List D - Case 4078. Washington, D.C.

US-EPA, United States Environmental Protection Agency, Office of Preservation, Pesticides and Toxic Substances, 2002. Reregistration Eligibility Decision: Thiabendazole. EPA738-R-02, Washington, D.C.

US-EPA, United States Environmental Protection Agency, Office of Air Quality Planning and Standards, 2004. Ethylene Oxide Commercial Sterilization and Fumigation Operations NESHAP - Implementation Document. EPA-456/R-97–004, North Carolina.

US-EPA, United States Environmental Protection Agency - Prevention, Pesticides and Toxic Substances, 2005. R.E.D. Facts - Imazalil. EPA-738-F-04–011 Washington, D.C.

US-EPA, Environmental Protection Agency Office of Pesticide Programs, Office of Prevention, Pesticides and Toxic Substances, 2008b. Reregistration Eligibility Decision for Pentachlorophenol. EPA 739-R-08-008, Washington, D.C.

US-EPA, United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Subtances, 2010. Summary of Human Health Effects Data for the Thymol. Registration Review Decision Document, Washington D.C.

US-EPA, United States Environmental Protection Agency Office of Pesticide Programs, Office of Prevention, Pesticides and Toxic Substances, 2008a. Reregistration Eligibility Decision for Formaldehyde and Paraformaldehyde. EPA 739-R-08-004, Washington, D.C.

Valentin, N., 1986. Biodeterioration of library materials disinfection methods and new alternatives. The Paper Conservator 10, 40–45.

Valentin, N., 2007. Microbial contamination in archives and museums: health hazards and preventive Strategies using air ventilation Systems. In: Proceedings of the Experts' Roundtable on Sustainable Climate Management Strategies, Spain, pp. 1–26.

Valentin, N., 2010. Microorganisms in Museum Collections. COALITION, pp. 2–5.

Valentin, N., Garcia, R., 1999. El biodeterioro en el museo. Arbor: Ciencia, Pensamiento y Cultura 645, 85–108.

Valentin, N., Lidstrom, M., Preusser, F., 1990. Microbial control by low oxygen and low relative humidity environment. Studies in Conservation 35, 222–230.

Yamarik, T.A., Andersen, F.A., Cosmetic Ingredient Review, E, 2004. Safety assessment of dichlorophene and chlorophene. International Journal of Toxicology 23, 1–27.

Zappalà, M.P., 1990. Le propionate de calcium: agent désacidifiant et stabilisant des papiers anciens? In: Grimstad, K. (Ed.), ICOM-Committee for Conservation, 9th Triennial Meeting ICOM Committee for Conservation, Dresden, pp. 500–504.

Zhang, Q., Lian, M., Liu, L., Cui, H., 2005. High-performance liquid chromatographic assay of parabens in wash-off cosmetic products and foods using chemiluminescence detection. Analytica Chimica Acta 537, 31–39.

Zotti, M., Ferroni, A., Calvini, P., 2007. Inhibition properties of simple fungistatic compounds on fungi isolated from foxing spots. Restaurator 28, 201–217.