Review

Antifungals on paper conservation: An overview

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A B S T R A C T

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.

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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 bio-receptivity of paper with inappropriate storage conditions or water related emergency circumstances makes this material very susceptible to microbial deterioration, mainly fungi. Bio-receptivity, as defined by Guillaumie (1995), is the ability of a material to be colonized by living organisms. The bio-receptivity 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 bio-receptivity of paper. Native cellulose is mainly crystalline with some amorphous spots, 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 bio-receptivity. Also, the removal of lignin in papermaking processes, while contributing to the increase of the quality of paper, amplifies its bio-receptivity, 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 discolorations 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/toxigenic (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 fire hazards. 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 (1860) 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 microbialidal 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 develop-
ment (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 – fungi-
cides 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, concentra-
tion, 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 mech-
anism of action as being membrane-actives or electrophilically
actives (Paulus, 2004).

Membrane active microbicides include: alcohols, phenols, acids,
salicylanilides, carbanilides, dibenzamidines, biguanides, quater-
nary 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
outer layer.) In addition, they are able to enhance conidial activation (Florian, 2002). Lower concentrations will also act as a wetting agent on the conidial cell, and are more toxic to the fungal spores than the mycelial phase of the fungi (Paulus, 2004). Electrophilically active microbicides can also be added to the mixture by reacting through electrophilic addition or substi-
tution, electrophilically active microbicides can lead to the inacti-
vation 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 anti-
microbial efficacy is universally acknowledged. This efficacy
increases with the chain length (Paulus, 2004) and is mainly caused
by coagulation and denaturation of proteins in the microbial cell
(Baciílková, 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), depend-
ing on the kind of alcohol (Baciílková, 2006).

Alcohols can be effective against vegetative forms of bacteria,
fungi and viruses, but their effect against sporulating microorgan-
isms has not been yet proven, and so, they are only considered
disinfecting and not sterilizing agents (Baciílková, 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
Baciílková (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
colonized heritage objects, a more detailed review follows.

2.1.1.1. Ethanol. Ethanol (C2H5OH) or ethyl alcohol has been widely
used as a disinfectant. The first study concerning the effects of
ethanol on bacterial cultures dates from 1880 (Baciílková, 2006).

Ethanol acts on fungi by affecting the permeability of their cyto-
plasmic membrane, causing the leakage of cytosol constituents and
ultimately leading to the disintegration of the cell (Nittérus, 2000b;
Baciílková, 2006).

The microbicide effect of ethanol depends on its concentration
and formulation composition (Paulus, 2004; Baciílková, 2006). The
higher effect is reported for ethanol concentrations between 50% and
90% 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 inhi-
bition 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.

Baciílková (2006), reports the efficiency of vapours of ethanol
solutions on paper samples inoculated with Aspergillus niger and
Penicillium 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, Baciílková
(2006), regards that for thicker materials, like books, the treat-
ment 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 sporidical 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>
<thead>
<tr>
<th>Antifungal compound</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Effects on paper</th>
<th>First report of use</th>
<th>Use restrictions[^a]</th>
<th>LD50 for rats oral route (mg/kg bw)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azole antifungals</td>
<td></td>
<td></td>
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<tr>
<td>e.g. Thiabendazole:</td>
<td>Can be used for aerial disinfection</td>
<td>Fungistatic rather than fungicidal Can leave fatty or powdery films on treated materials</td>
<td>Thiabendazole: Slight decrease on brightness</td>
<td>1944</td>
<td>Different restrictions for each essential oil component (see references)</td>
<td>Different values for each azole compound e.g., Thiabendazole: &gt;2000</td>
<td>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., 2005; Giavini and Menegola, 2010; EC, 2012</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td>Also has deacidifying properties Low toxicity</td>
<td>Fungistatic but not fungicidal</td>
<td>Increase of pH Increase of polymerization degree Minor increase on lightness and yellowness</td>
<td>1930's (1980 on heritage materials)</td>
<td>EU: N/A USA: N/A</td>
<td>3920</td>
<td>Dersarkissian and Goodberry, 1980; Zappalà, 1990; US-EPA, 1991; Florian, 2002; Paulus, 2004; Suhr and Nielsen, 2004; Neves, 2006; Zotti et al., 2007; Šifa, 2008</td>
</tr>
<tr>
<td>Dichlorophen</td>
<td>Can be used in alkaline pH Strongly effective against fungi</td>
<td>Causes irritation on skin and eyes</td>
<td>Increase of the deterioration rate Decrease of whiteness Harmful effects on inks</td>
<td>1929</td>
<td>EU: R USA: N/A</td>
<td>1250</td>
<td>Triolo et al., 1968; Kowalsik, 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</td>
</tr>
<tr>
<td>Dimenthy-lauryl-benzyl-ammonium bromide</td>
<td>Low-level disinfectant with no sporicidal activity</td>
<td>Decrease of physical properties, pH, whiteness, and alpha cellulose content</td>
<td>1986: on heritage materials</td>
<td>EU: R USA: R</td>
<td>N/A</td>
<td>Strzelczyk and Rozanski, 1986; Bello-Gonzalez et al., 2008; EC, 2012</td>
<td></td>
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<tr>
<td>Chemical</td>
<td>Toxicity</td>
<td>Activity</td>
<td>pH Range</td>
<td>Major Effects</td>
<td>Notes</td>
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<tr>
<td>Esters of p-hydroxybenzoic acid</td>
<td>Low toxicity</td>
<td>Mainly fungistatic and bacteriostatic</td>
<td>pH range of activity</td>
<td>Minor increase on yellowing</td>
<td>€2100–&gt;8000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essential oils e.g. Linalool</td>
<td>Low toxicity</td>
<td>Fungistatic rather than fungicidal</td>
<td>Fungistatic rather than fungicidal</td>
<td>Decrease of pH</td>
<td>Different restrictions for each essential oil component (see references)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>Evaporates and does not leave toxic residues</td>
<td>Can act as a conidium activator</td>
<td>Can act as a conidium activator</td>
<td>Loss of gloss, increase in opacity and slight deformation (especially on transparent papers)</td>
<td>€10,470</td>
<td></td>
<td></td>
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<tr>
<td>Ethylene oxide</td>
<td>High antimicrobial efficacy</td>
<td>Raises the susceptibility of objects to future microbial attack</td>
<td>Raises the susceptibility of objects to future microbial attack</td>
<td>Decrease of mechanical properties and polymerization degree</td>
<td>€330</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EU: N/A USA: N/A

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

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

Valentin and Garcia, 1999; Nittérus, 2000a; Florian, 2002; Bacílková, 2006; EC, 2012; ECHA, 2012

Cotton and Roark, 1928; Flieder, 1965; Strassberg, 1978; OSHA, 1984; Ballard and Baer, 1986; Craig, 1986; Valentin, 1986; Valentin et al., 1990; Hengenihle et al., 1995; Florian, 2002; Ponce-Jime
ez 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>
<thead>
<tr>
<th>Antifungal compound</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Effects on paper</th>
<th>First report of use</th>
<th>Use restrictions</th>
<th>LD50 for rats oral route (mg/kg bw)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauryl-dimethyl-Carbethoxymethyl ammonium bromide</td>
<td>Can be used for mass treatments (nebulisation).</td>
<td>Undergoes acidification with time. Irritates the mucous membrane.</td>
<td>Minor darkening and acidification of paper. Depolymerisation of cellulose. Decrease of adhesion of inks to paper.</td>
<td>N/A</td>
<td>EU: N/A, USA: N/A</td>
<td>N/A</td>
<td>Heim et al., 1968; Triolo et al., 1968; Strassberg, 1978; Kowalik, 1980; EC, 2012</td>
</tr>
</tbody>
</table>
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 García (1999) report that in order to raise ethanol’s microbicidal 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 García, 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 inactive bacteria, fungi and viruses (Russell, 2003).

2.1.2.1. Ethylene oxide. Ethylene oxide [(CH₂)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 alkylating 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, sporidical, 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 CO₂ (10% EtO, 90% CO₂) 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 disinfection, 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 (CH₂O) 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 Valentín and García (1999), this compound, when applied by fumigation, has a low power of penetration and a limited fungicidal 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 (Valentín and García, 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; Valentín and García, 1999).

Regarding the toxicity of formaldehyde to humans, the International Agency for Research on Cancer (IARC, 2006), 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 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 anti-fungal activity was reported in 1944, but it was not until 1958 that it began to be marketed (Pérez-Rivera et al., 2009). In the last decades, intensive research has led to the establishment of azole compounds as antymycotics 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 (Gollapudi 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 synthase 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/m². 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 shown in numerous studies to possess an embryotoxic potential, including teratogenic effects in laboratory animals and therefore should be avoided for use by pregnant women (Givani 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, eucalyptus, ravensare, lavender, tea tree, thuya, wormseed and their main components linalool, linalyl acetate, eugenol, α + β 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-oI) (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 fungus 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 CD or Panacide, is a chlorinated biphenol that was first prepared in 1929 (Block, 2001). Besides being used as a fungicide for paper, cardboard, textiles, adhesives, and as a scleroprotein 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 antipporter 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/m³ ethanolic solution. They observed that this compound caused a slight increase in the
W. 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 2011 (EC, 2012).

2.1.5.2. Ortho-phenylphenol. 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 García, 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 Rakotoinarinaï 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 (Rakotoinarinaï 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 García, 1999).

OPP's sodium salt (SOPP), with 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 García, 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 García, 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 (C₆HCl₅O), 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 Valentín and García (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 García, 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 monoterpenic 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 (Segvić-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
structures of \((\text{TiO}_6\) crystallites of anatase or rutile, which are represented as octahedral occurring compound that exists more commonly as tetragonal \(\text{TiO}_2\). Irradiated with UV can only act as a fungistatic and do not affect the conidia (Gavriliu et al., 2009; Markowska-Szczupak et al., 2011). According to Markowska-Szczupak et al., 2011), the microbicidal activity of titanium dioxide is due to its photocatalytic properties. When illuminated with UV light of less than 385 nm, \(\text{TiO}_2\) generates strong oxidizing power, producing reactive oxygen species.

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. 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 (\(\text{TiO}_2\)) is a natural occurring compound that exists more commonly as tetragonal crystallites of anatase or rutile, which are represented as octahedral structures of \((\text{TiO}_6\) (Gavriliu et al., 2009). Crude \(\text{TiO}_2\) 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). \(\text{TiO}_2\) 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 \(\text{TiO}_2\) 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, \(\text{TiO}_2\) 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 \(\text{TiO}_2\) 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, \(\text{TiO}_2\) 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, \(\text{TiO}_2\) 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 \(\text{TiO}_2\) were tested on \(A. \text{niger}\) and \(P. \text{penicillium}\) 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 \(\text{TiO}_2\) 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 \(\text{TiO}_2\) 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 \(\text{TiO}_2\) (Afsharpour et al., 2011). According to Chawengkijwanich and Hayata (2008), a \(\text{TiO}_2\) surface when illuminated, forms hydroxyl radicals and reactive oxygen species. These unstable chemical species can react with cellulose, which can justify the results observed. 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 \(\text{TiO}_2\) 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 sporidical 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°C 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 Kowalk (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 (benzylalkyl dimethyl (alkyl from C8-C22, saturated or unsaturated, tallow alkyl, cocoa 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(C2H5COO)]2 is a salt of propionic acid, that has been commonly used as a food preservative since the late 1930s (Paulus, 2004). Its ability to inhibit moulds but not yeasts to the same extent, have made it a popular 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 decadify 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 (ΔL* = 0.43 and ΔE* = 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 Sifa (2008) indicates that this compound has chromotoxic effects, and advises caution on the use of this compound as a food preservative.

2.1.2. 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 fungistic 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 fungistic 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 tumor 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
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. 

<table>
<thead>
<tr>
<th>Antifungal method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Effects on paper</th>
<th>First report of use</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration</td>
<td>Does not leave toxic residues</td>
<td>High volumes of wet paper respond slowly to a dry environment and meanwhile fungi development can occur</td>
<td>Physical deformations</td>
<td>General: Since early civilizations</td>
<td>Hugo, 1995; Florian, 1997, 2002; Garside and Knight, 2011</td>
</tr>
<tr>
<td>Freezing</td>
<td>Does not leave toxic residues</td>
<td>Can prevent soluble compounds in the materials from bleeding and migrating</td>
<td>Ice crystals increase the porosity and thickness of paper materials, which can alter its absorbance and mechanical properties</td>
<td>1000 BC</td>
<td>Flink and Hoyer, 1971; Takenaka et al., 1996; Florian, 1997, 2002; Gunde-Cimerman et al., 2003; Archer, 2004; Basset and Drais, 2011</td>
</tr>
<tr>
<td>Gamma irradiation</td>
<td>Does not leave toxic residues</td>
<td>Can be used for mass treatments</td>
<td>Can induce fungi to produce more coloured metabolic products</td>
<td>Diminishment of mechanical resistance, pH and polymerization degree, raise of copper number and yellowing</td>
<td>1896 (1960’s on heritage materials)</td>
</tr>
<tr>
<td>High frequency current</td>
<td>Also dries the materials, which is an advantage in a case of wet documents.</td>
<td>Can turn cellulose more susceptible to further infestations</td>
<td>Increasing of carbonyl groups on cellulose Diminishment of tear resistance.</td>
<td>1947</td>
<td>Flieder, 1965</td>
</tr>
<tr>
<td>Low-oxygen environments</td>
<td>Can lower the moisture content on materials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigeration, 4 °C</td>
<td>Does not leave toxic residues</td>
<td>Only causes a decrease on fungal growth, does not stop it</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultraviolet radiation</td>
<td>Does not leave toxic residues</td>
<td>Can act as a fungicidal, depending on the radiation dosage</td>
<td>Causes oxidation of cellulose, leading to yellowing, bleaching and brittleness of paper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990: on heritage materials</td>
<td></td>
<td></td>
<td>Atmospheres high on CO2 can change the acid/base balance on the materials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19903</td>
<td></td>
<td></td>
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</tbody>
</table>

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).
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-ray radiation 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 of γ-radiation 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-Arié 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 catalyster 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 use 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 CO₂ are more effective than reduced O₂ 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. funigus or exposed to environmental contamination. The low O₂ environments (5%, 1% and 0.1%) were achieved by addition of nitrogen. The results showed that even though the reduction of O₂ 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 grow, 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 CO₂ 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 actinomycente 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 conducive 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 efficient 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 loss 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 Drais (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 polys, 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, dehydroxylation 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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