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# The initial disinfection of paper-based historic items – Observations on some simple suggested methods

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## 1. Introduction

Fungi and bacteria develop on historic paper-based items containing more than 8–10% water. The collections undergo attacks of microorganisms as a result of water system failures, air-conditioning failures, floods and storage the collections in rooms which are humid, poorly ventilated or have a microclimate characterised by large temperature fluctuations. Organic materials constituting books and archival documents quickly and easily absorb water, and can soon become subject to microbial attack. The most frequently encountered fungi are common mould species, such as *Chaetomium globosum*, *Trichoderma viride*, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Aspergillus niger*, *Stachybotrys atra*, *Trichoderma koningii* and *Chaetomium elatum* (Nyuksha, 1994; Zyska, 1997). Microorganisms cause biodeterioration of historic items and mould fungi, in particular, may have negative effects on human health.

Thanks to chemical biocides biodeterioration of paper-based items could be inhibited within a few minutes or hours. In total, dozens of different chemical compounds have been used or attempted to be used for the disinfection of paper-based collections, but as early as the 1970s and 1980s, the threat to historic materials connected with the use of these chemically reactive compounds was noticed as well as their harmfulness to the environment and human health (see Sequeira et al., 2012).

What main requirements should the biocides for the conservation of moulded cultural property meet? Generally, they should be effective against microorganisms deteriorating paper items, especially microscopic fungi and they cannot reveal any harmful effects to paper and media on paper, also in a long period of time. Moreover, they should be safe for humans and environment.

Nowadays, many authors are considering disinfection to be unnecessary and recommend mainly dry mechanical removal over suction, more rarely freeze drying, gamma irradiation or 70% ethanol (Florian, 2002; Havermanns, 2011). However, all these methods are not completely satisfactory for the conservators of art. In the case of significant amounts of viable microorganisms developing in a paper-based object dry cleaning cannot replace a disinfection due to several reasons. The dry cleaning allows us to remove spores and hyphae only from the surface of the object but the biodeterioration processes may continue inside paper for some time. During conservatory treatments requiring water solutions the viable microorganisms can activate and cause further destruction. As a matter of fact analogous destruction may be caused also by microorganisms from bioaerosol or settled dust but the probability of the development of the viable microorganisms which have remained in paper is much higher. Moreover, some fungal or bacterial spores may remain viable for several or even some dozens of years and in case of any movement they may contaminate air and consequently surfaces of other items. The inhaling of the air with significant amounts of spores of microorganisms included in Biosafety level 2, i.e. *Aspergillus niger, A. flavus, A. fumigatus, A. terreus, Acremonium kiliense, A. falciforme, Paecilomyces variotii, Scopulariopsis brevicaulis, Sporothrix schenckii, Fusarium oxysporum, F. solani*, can result in a risk of fungal diseases to humans (de Hoog and Guarro, 1995). Gilot et al. (2012) state that mechanical dry cleaning of moulded surfaces did not bring about a statistically significant limitation of microorganisms because despite the reduction of fungal spores in some places, the increase in number of spores occurred somewhere else due to their dispersion.

Gamma irradiation is efficient against microorganisms deteriorating paper, but causes a decline in the degree of polymerization (DP) of cellulose followed by a decline in mechanical properties of paper as well as leads to a decrease of paper pH (Hofenk de Graaf and Roelofs, 1994). Freeze drying is the best method for drying the wet objects affected by flooding or other disasters (Thiel and

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Weiler, 2013) but fungal spores may survive it (Strzelczyk et al., 2001). Some attempts have been made using alcohols, mainly ethanol as a biocide, to kill the microorganisms growing on paper-based objects (Nittérus, 2000; Bacílková, 2006; Meier, 2006; Weiβ, 2006; Castaneda et al., 2010; Gilot et al., 2012), but the effects of this treatment have been still poorly recognised. Also, some trials with essential oils (Rakotonirainy and Lavédrine, 2005; Rakotonirainy et al., 2007) or low temperature plasma (Laguardia et al., 2005) have been performed but they have not been applied in practice yet.

In this paper, the results concerning three biocides (hydrogen peroxide, tea tree oil and ethanol) used as vapours are presented. Disinfection in vapours seems to be more adequate because of the lack of direct contact of paper with the biocide in solution. The aim of this research is to investigate the effectiveness of vapours against fungi and their influence on paper and some media on paper.

## 2. Materials and methods

### 2.1. Preparation of samples for disinfection

Samples were cut out from the 19th century handmade paper containing fibres of linen, hemp, jute and cotton. This kind of paper was susceptible to fungal growth, easily attacked by different species of fungi and therefore useful in this investigation. Samples were autoclaved (121 °C, 1 atm) and then infected by immersion with suspensions of fungal spores ($10^5$ cfu/cm$^3$) of *Trichoderma pseudokoningii* Rifai (ŁOCK, 1120), *Chaetomidium subfimeti* Seth. (ŁOCK, 1122), *Cladosporium cladosporioides* (Fresen.) de Vries (ŁOCK, 1121) and *Penicillium spinulosum* Thom (ŁOCK, 1123). These strains were previously isolated from archives and their cellulolytic properties have been determined in the previous studies (Karbowska-Berent, 2014). The infected samples were incubated in humid chambers at 28 °C for 3 weeks and then disinfected.

### 2.2. Disinfection

Samples prepared for disinfection in the vapourised hydrogen peroxide were placed in a 1 m$^3$ chamber. The vapourised peroxide was produced by flash heating (vapourisation) of 35% liquid hydrogen peroxide in a VHP 1000ARD generator (Steris Life Sciences) in "Impuls", Pruszcz Gdański, Poland. In the course of treatment hydrogen peroxide was maintained in a dry vapour form to avoid the condensation effect. Two different processes were carried out—the decontamination in the concentration 250 ppm within 90 min and the decontamination in the concentration 400 ppm within 30 min. Each sample was placed in an envelope together with three layers of filter paper from each side to examine the permeability of the vapours through paper.

The samples intended for disinfection in vapours produced by tea tree oil (Avicenna Oil, Poland) were hung in 1-L jars over 1 or 3 ml of tea tree oil (for fungi) and 0.1 or 1.0 ml (for paper and media). The incubation lasted for three weeks. This treatment was performed for *P. spinulosum* only. The disinfection in vapours produced by 45% water solution of ethanol was performed in a chamber of 0.125 m$^3$ and the area of evaporation was 600 cm$^2$. The times of exposition were 3 h, 6 h, 18 h or 24 h. This treatment was performed for all the strains mentioned above.

All decontamination treatments were carried out in parallel on two paper samples because of the scantiness of the paper material. The controls were inoculated and incubated as above but not disinfected.

### 2.3. Control of effectiveness of the disinfection

After disinfection the samples were intensively shaken off in 100 ml of sterile water and created suspension was inoculated with the poured plates method in the amount of 0.5 ml per plate on malt extract agar in duplicates. When taking into consideration that every treatment was done in parallel on two paper samples, we obtained four replications from one disinfected paper sample. After 5 days of incubation, the colonies were counted and the amount of the microorganisms in cfu/cm$^2$ was calculated. The results were given as the logarithmic degree of the reduction of the number of fungi (R) according to the formula:

$$R = \log_{10}F_c - \log_{10}F_t$$

Where:

$F_c$—the arithmetic mean of the number of microorganisms in control sample [cfu/cm$^2$].

$F_t$—the arithmetic mean of the number of microorganisms in treated sample [cfu/cm$^2$].

The disinfection was assumed as successful when $R \geq 4{,}00$ according to PN-EN 1650+A1:2013-08E (2013).

### 2.4. Research on the influence of disinfection on paper

Two kinds of other test paper called here A and B were used in this research (Table 1). These kinds of paper were produced as standard test papers for investigation of papers used for conservatory purposes (Havermanns, 1995). The disinfection of samples of paper A and B was carried out as described in 2.2 section.

After disinfection, the influence on the paper properties, i.e. the surface pH, the total colour difference $\Delta E^*$, the difference in the yellowing $\Delta R_z$ and the tear resistance, was examined. In order to estimate the influence of disinfection on test papers after a long period of time, artificial ageing of the samples in a Vösch VC 403 climate chamber was carried out (21 days, 80 °C, RH 65%). The samples were investigated before disinfection, after disinfection and after disinfection and artificial ageing.

The surface pH was measured on the wire side of the test paper samples using a Mettler Toledo EL 20/EL2 pH-meter, electrode for flat surfaces Hydromet ERM-115 and de-ionized water with conductivity lower than 0.1 mS/m at a temperature of 21 °C. The paper samples, sized 2 cm × 2 cm, were put on the HDPE (high density polyethylene) support; 2–3 droplets of de-ionized water were put on each paper sample and spread by a glass rod. The final result was taken as the average of 5 measurements.

The optical properties were measured using a Colour Pen Dr. Lange colorimeter. The measurements were taken in 7 points. The total colour difference $\Delta E^*$ was calculated using the following formula:

$$\Delta E^* = [(L_1^* - L_c^*)]^2 + (a_1^* - a_c^*)^2 + (b_1^* - b_c^*)^2]^{1/2},$$

where:

$L^*_1, a^*_1, b^*_1$—results of the treated samples, $L^*_c, a^*_c, bc_2$—results of the untreated control samples.

The $\Delta E^*$ results were interpreted according to the scale (Drzewińska, 2002):

- $\Delta E^* < 1{,}00$ - the difference unnoticeable
- $1{,}00 < \Delta E^* < 2{,}00$ - very small difference, noticeable to an experienced observer only
- $2{,}00 < \Delta E^* < 3{,}50$ - medium difference, noticeable to an experienced observer only
- $3{,}50 < \Delta E^* < 5{,}00$—significant difference

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