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Microbial contamination of libraries and archives: risk assessment and contamination control

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Abstract. Freezing of the paper material is a way to control the growth of microorganisms and the possibility of "acute" biodeterioration and illness, after accidental events, e.g. flooding. But as the paper-born microorganisms can survive after lyophilisation, they must be controlled also after the drying process. The decision-making about the adoption of prevention and protection measures for the exposed operators' safety, among which the possibility to control the biodeteriogenic and toxigenic microflora growth by the application of *Thymus vulgaris* oil, was supported not only by the experimental results but also through a dedicated risk assessment procedure.

1. Introduction

Paper documents are, in general, damaged by contact with water, which can occur as a result of accidental events such as rainstorm, flooding or fire extinction. The damage can be of different types: chemical, physical or biological (i.e. biodeterioration). In the last case, the presence of water could favour the growth of indigenous biodeteriogenic microorganisms, causing inestimable damages, in particular when the paper material is characterized by a high historical or archival value (e.g. flooding in Florence, 1966, and, more recently, in Venice, 2019). When dealing with biological damages, it is necessary to introduce the concept of "bioreceptivity", that is the ability of a material to be colonized by living organisms. In particular, paper bioreceptivity is high because cellulose, one of the main constituents of paper materials, represents a primary carbon source for several microbial classes (i.e. bacteria and filamentous fungi).

Microbial growth may be also responsible for health effects: several respiratory diseases have been correlated to the presence of microorganisms and their metabolites or components (allergens, mycotoxins, beta-glucans, volatile organic compounds - VOCs), as may have irritating effects on the skin, mucous membranes and respiratory tract [1]. Among the paper-borne microorganisms, moulds and, in particular, mycotoxin producers represent a serious risk, quite often underestimated. They can grow on paper, in the presence of an adequate combination of humidity and temperature conditions [2]. When an accidental event occurs, the fungal growth rate is enhanced, and the health effects are consequently more serious. In both cases, i.e. biodeterioration on paper documents and health effects on humans, the control of microbial growth on paper documents is mandatory.

As far as the accidental events, e.g. flooding, are concerned, freezing of the paper material is a way to stop the growth of microorganisms and, consequently, the possibility of "acute" biodeterioration and illness. The frozen paper material can be stored for a very long time without negative consequences. In these cases, freeze-drying represents an interesting possibility of recovery the documents, since the first phase of the process, i.e. the freezing of the material, has already been carried out. Drying of water-

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damaged archival documents and books by means of freeze-drying is a well-known technology, which can give excellent results avoiding further damages to paper material because the operating temperatures are very low and there is no presence of liquid water (during the process) that can displace soluble components present in the material, such as dyes and glues [3-4]. Moreover, as said before, freezing is a fast method to control microbial growth and consequently health risks. In the scientific literature, results of freeze-drying treatment on the viability of paper-born microorganisms are not univocal and sometimes contradictory. As an example, according to the work by Troiano *et al.* [5], lyophilisation in general reduces significantly the microbial load present. Fissore *et al.* [6] investigated the influence of different operational variables of the lyophilisation process (in particular, freezing temperature, freezing speed and sublimation temperature) on flooded paper contaminated with biodeteriogenic microorganisms (i.e. a bacterium *Staphylococcus epidermidis*, a yeast *Rhodotorula mucilaginosa* and a filamentous fungus *Alternaria alternata*). The effect of freezing and drying on microorganism survival and growth was separately evaluated: even if all the strains were able to survive after lyophilisation, the growth of *S. epidermidis* was the same as that of the control, *R. mucilaginosa* was stimulated, while *A. alternata* was inhibited, in particular when a non-sporulated mycelium was tested. When evaluating results presented in the Literature it has to be taken into account both the type of microorganism considered, and that the sensitivity of a specific strain varies in relation to the phase of the growth curve; this last aspect is particular relevant for filamentous fungi. In fact, it is well known that, given the same species of filamentous fungi, the spores are more resistant than the vegetative mycelium to the lyophilisation process [5-6]. There is little scientific work on the effects of lyophilisation on biodeteriogenic microorganisms, probably in relation to the fact that the different microbial classes respond to the treatment in a species-dependent manner (and, as said, differently depending on the growth phase).

Based on the obtained results, the necessity to control the paper-born microorganisms, after the lyophilisation process, appears inevitable. Moreover, the control of toxigenic microflora is an important issue in libraries or archives when the temperature and humidity conditions could support the microbial growth. In this framework, the possibility to control the biodeteriogenic and toxigenic microflora growth by using essential oils [2] appears particularly interesting as they can be effective and, with respect to other treatments, they do not appear to further deteriorate the archival materials. The use of *Thymus vulgaris* oil was investigated in this study, considering both a book where the contamination came from the environmental conditions, and a system where we reproduced the conditions of flooding and successive contamination, followed by lyophilisation process. The decision-making about the adoption of the measure above described was supported not only by the experimental results but also from a risk assessment point of view. The selection of the possible measures to be implemented to control the growth of microorganisms can in fact be based on the level of risk, according to the general risk assessment procedure summarised in Figure 1 [7].

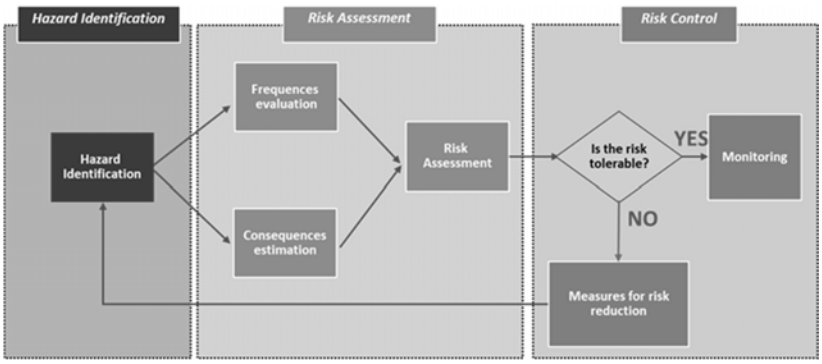

Figure 1. Risk assessment general procedure (reproduced from [7]).

In general terms, after the identification of hazards, both for the operators and the document collection, a risk estimation can be carried out, given the probability of occurrence of an unwanted event and the severity of the possible consequences. The risk can be thus compared to a threshold value and:

- in case of a risk lower than the threshold, the risk can be considered tolerable and no need for preventive or protective measures is highlighted, but only monitoring and awareness measures are required;
- in case the risk should be higher, then some preventive or protective measures should be implemented, and the risk assessed again to verify their effectiveness in risk reduction.

2. Material and methods

In order to investigate the drying of books flooded and contaminated, an experimental protocol was set-up aiming to carry out the study in controlled and reproducible conditions, obviously close to what occurs in a real case study. Modern 80 g/cm^2 white paper blocks (size 6 x 6 cm, 10 ± 1 mm height) were prepared, immersed in distilled water for 18 hours and then drained for 1 minute. The tested microorganisms (*S. epidermidis, R. mucilaginosa* and *A. alternata*) were inoculated separately on a sterile paper sheet (6 x 6 cm) and cultivated as described in [6]. Contaminated samples were obtained positioning inoculated sheets in the middle of the soaked paper blocks which were then frozen inside plastic bags, in a domestic freezer at about -20°C and lyophilised in a Millrock REVO freeze-dryer at 200 μbar and 0°C for about 72 hours, till the end of the ice sublimation step.

At the end of the drying phase, the contaminated sheets were recovered and placed on Malt Extract Agar (MEA), Nutrient Agar (NA) or Czapeck Dox Agar (CZ) plates, as described in our previous work [6], and treated with *Thymus vulgaris* oil solution (0.75% v/v in 1.5% TEGO 20). The essential oil (EO) solution was sprayed (10 sprays for each sheet) at a sheet distance of 12 cm.

The treatment was carried out immediately after the drying phase (i.e. time 0) or after 24 hours of incubation. The treated samples were closed and wrapped in a polyethylene film (*see* Figure 2) to avoid the evaporation of the essential oil, and incubated at the optimal growth temperature; further details can be found elsewhere [8]. For each microorganism, an inoculated sheet, untreated with essential oil and maintained under the same conditions of the treated samples, was used as control. Each test was carried out in triplicate.

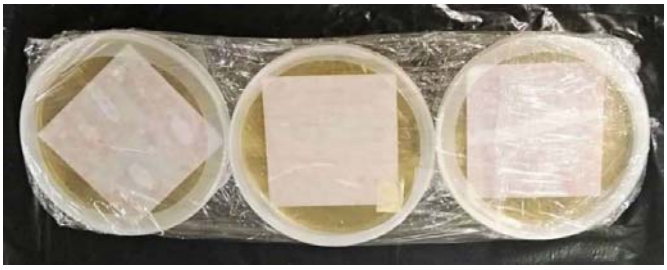

Figure 2. *R. mucilaginosa* contaminated sheets treated with *Thymus vulgaris* oil at time 0 and wrapped in polyethylene film.

The microbial growth was visually monitored every 24 hours and for 5 days from the deposition of the contaminated paper sheets on the surface of the agar. Moreover, for *A. alternata* colonies, at the same time, two perpendicular diameters of the colony were measured until the stop of the mycelium growth, or when the colony reached the edge of the Petri dishes. The mean diameter values were used to calculate the inhibition percentage of mycelial growth (*MGI*%):

$$MGI\ (\%) = \frac{d_c - d_t}{d_c} \cdot 100$$

where d_c is the colony diameter in the control sheet and d_t is the colony diameter in the treated sheet.

The treatment with *Thymus vulgaris* oil was also applied to a contaminated book, taken from a library where the environmental conditions were favourable to microbial growth. The microorganisms present on the book surface were transferred on Malt Extract Agar (MEA) by means of the Replica Plating technique and incubated at 30°C for three days. The efficacy of *Thymus vulgaris* oil (0.75% v/v) on the isolated microorganisms was tested as previously described for the contaminated paper sheets. Then, the contaminated book was placed inside a box covered with polyethylene film and was treated with essential oil in three different ways (*see* Figure 3): in area 1 the oil was sprayed and, to limit its dispersion, a mask (6 cm x 3 cm) was used. In the two other areas, sterile paper sheets (6 cm x 3 cm), previously soaked in the essential oil solution, were deposited for 2 and 24 hours (area 2 and area 3, respectively).

Figure 3. Contaminated book treated with essential oil solution and placed inside a box covered with polyethylene film.

3. Risk assessment

According to the general procedure described in the introduction, a risk assessment approach to the specific problem has been drawn, mainly built on expert judgment.

The risk, R, is here intended as $R = P \times E \times M$, where P is the probability of occurrence of an unwanted event that could trigger the mould formation; E is the exposure factor to the hazard; M is the magnitude or severity of the consequences.

For our purpose, in this case the P factor is intended to be 1, since the unwanted event, a flood or the exposure to humidity, is taken for granted. The exposure and magnitude indexes are defined as follows, limited for the moment to the risk for the operators (Table 1 and 2):

Table 1. Exposure Factor values for different operators.

Exposure Factor, *E*	Librarian (h/week)	Reader (contacts/week)	Restorer (contacts/week)
4	30÷40	>10	>5
3	20÷30	7÷10	3÷5
2	10÷20	4÷7	2÷3
1	<10	<4	<2

The difference between librarian and reader and/or restorer is that not necessarily the librarian has to manipulate and open the contaminated books, thus his exposure is a continuous one to a potentially contaminated air. Both the reader and the restorer have a discontinuous exposure to the potentially contaminated book, but this is closer for the restorer.

Table 2. Magnitude Factor values.

Magnitude Factor, *M*	Description
4	High contamination with toxins production
3	Low contamination with toxins production OR high contamination with allergens production
2	Low contamination with allergens production
1	Negligible contamination

The above described parameters have been used to develop the risk matrix shown in Figure 4, that will allow the risk-based decision making to be completed. It has also to be considered that the level of contamination can change with time, depending of the storage conditions, factor that has not be introduced in this first model.

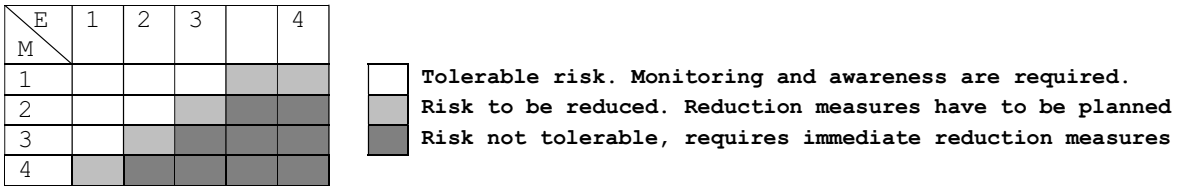

Figure 4. Proposed risk matrix (with E, exposure factor and M, Magnitude factor).

In case the risk should result to be not tolerable, the measures discussed in this paper could be implemented reducing the risk. The effectiveness of protective and preventive measures has been assigned through expert judgement, as summarised below.

The list of measures is partial and can be extended based on experience. For the exposure reduction, with an assigned effectiveness of 1, a local ventilation system could be implemented and, if not possible, personal protective equipment (PPE) as filtering mask could be supplied to operators, with a similar effectiveness. For the magnitude control, instead, it must be considered that the freeze-drying process allows a reduction of the severity index of 1 point, as the adoption of essential oils. Their combination will have an additive effect, as testified by experiments.

4. Results and discussion

4.1. Experimental results

At the end of the drying process, sheets contaminated with *R. mucilaginosa* were extracted from the blocks, deposited in Petri dishes containing MEA and treated with *Thymus vulgaris* oil (0.75% v/v) immediately after the drying phase or at 24 hours of incubation (*see* Materials and Methods section).

In general, all the treated sheets exhibit an inhibition of microbial growth; in fact, the colony diameter did not increase and the pink pigmentation due to carotenoid production was less evident. When the contaminated sheets were treated after 24 hours of incubation, the microbial growth was higher than that observed for the sheets treated earlier (time 0). The colonies actually grew during the 24 hours of incubation before the treatment with essential oil; after that a growth inhibition was observed. The results obtained in these tests clearly showed that *Thymus vulgaris* oil (0.75% v/v) was able to inhibit the growth of *R. mucilaginosa*, after the freeze-drying process; the efficacy was higher when the oil was applied earlier. Sheets contaminated with *S. epidermidis* at the end of the drying process were recovered from the blocks, deposited on NA plates, treated with *Thymus vulgaris* oil (0.75% v/v) as reported for *R.*

mucilaginosa, and incubated for 72 hours at 37°C. In order to observe the *S. epidermidis* growth on the sheets, after the treatment with the essential oil, the replica-plating technique was applied, since the colour of the colonies was very similar to that of the paper. As for *R. mucilaginosa*, the major inhibition effect on microbial growth was observed when the contaminated sheets were treated with essential oil immediately after the freeze-drying process.

After freeze-drying, the sheets contaminated with *A. alternata* were extracted from the blocks, deposited on CZ agar plates, treated with *Thymus vulgaris* oil (0.75% v/v) and incubated for two weeks at 30°C. The mycelium, with or without sporification, treated immediately after the drying, showed a total inhibition of growth during the evaluation period; these results confirm the efficacy of *T. vulgaris* oil on *A. alternata*, previously reported in different works [9-11]. In both cases, the essential oil treatment, carried out after 24 hours of incubation, inhibited the *A. alternata* growth for a week, after which the growth rate of the treated fungus was always lower than that of the control, but in the case of the non-sporulated mycelium a higher value of *MGI*% was observed. In particular, the maximum value was obtained when the essential oil was sprayed immediately after drying: 51.4% and 81.4% for *A. alternata* with mycelium sporulated or not-sporulated, respectively.

In the case of the contaminated book, at first the inhibition effect of *Thymus vulgaris* oil (0.75% v/v) was verified on the microorganisms previously isolated from the book surface by means of the replica plating technique. On the contaminated book, two different application methods of the essential oil were tested: spraying and contact sheets (*see* Material and Methods section). In Figure 5, the images before and after treatment, at 72 hours of incubation, are showed and compared.

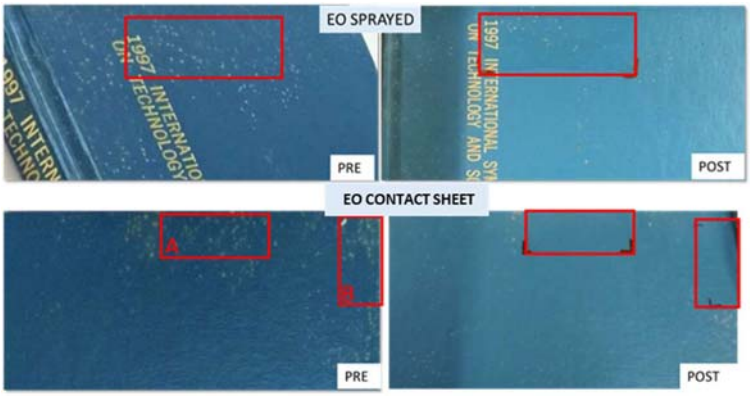

Figure 5. Contaminated book treated with *Thymus vulgaris* oil: pre-treatment with EO (on the left), post-treatment with EO (on the right). EO contact time: A) 2 hours; B) 24 hours.

The inhibition effect of the essential oil was higher in the areas where it was applied through the contact sheets. This could be due to the presence of the paper which reduced the evaporation rate of the volatile components of the oil. For this reason, in the areas where the oil was sprayed, the microorganisms grew, even if an inhibition was also observed in this case. The obtained results suggested a higher toxicity of the volatile component of the *Thymus vulgaris* oil; specific tests that allow to only evaluate the effect of the volatile components have to be applied.

4.2. Risk based decision-making

Considering, as an example, a case in which a flooding due to the spurious intervention of the firefighting system sprinklers has damaged a shelf containing books, with the possible formation of filamentous fungi that could spread in the air toxins in case of manipulation. The librarian, who should not manipulate the books after the contamination, will not be exposed, while the restorer will be potentially exposed. Considering a restoring activity on the contaminated books requiring 5 contacts during the week, the exposure factor should be 3 and the magnitude 4, thus the risk index will be 12

and, according to the risk matrix, this is a not tolerable risk, that should require immediate risk reduction interventions. With the decision of equipping the area in which the restorer has to work with a local ventilation system, the exposure index will move from 3 to 2 and the risk from 12 to 9, being still to be reduced. In case the books have been freeze-dried, this has reduced the severity to 3 and the risk to 6, bringing the risk to a medium level. This residual risk can be treated with the PPE, moving the exposure index to 1 and thus the risk to 3, a low and tolerable one. If the risk for a user, a reader, after the restoration has been carried out is taken into account: after the freeze-drying the severity is reduced to 3, corresponding to a lower contamination, but with the possibility of having some toxins spread during the consultation of the book. Considering that the user could consult the book within 7 to 10 times in a week: in this case the risk for the reader will be 9, a high risk that requires reduction. Making the hypothesis the reader cannot use the book for less time, it should be required to have a better control of the microorganisms, thus adding treatment with essential oils to the freeze-dried book. In this way the magnitude index will be reduced to 2 and the risk to 6: a medium risk that could be further managed using PPE. With the combined used of both treatments also the risk for the restorer will be better managed, having an initial medium risk of 6, that can be managed through PPE, without local ventilation. This is clearly an initial risk modelling work that should be enriched as far as the knowledge of the effects of microorganisms on the human health and their growth and diffusion will increase. It should and will also be considered in better detail the influence of the storage time and storage conditions, that can significantly influence the level of contamination, and thus the Magnitude factor.

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