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Mass Treatment of Flooded Archival Materials by Gamma Radiation
Massenbehandlung durch Gammastrahlung von flutbeschädigten Archivalien

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Abstract: This work describes mass recovery processes of flooded archival materials at industrial scale. The presence of fungi on paper represents a threat to the integrity of the document because they degrade cellulose, one of the main components of paper. Gamma radiation treatments are investigated as mass disinfection agents for their high penetrating power, speed of treatment, and absence of risk due to chemical residuals. We compared two different recovery processes: thermal drying followed by gamma irradiation and gamma irradiation followed by thermal drying. Both these processes were conducted simultaneously on naturally contaminated archival items and on paper specimens artificially contaminated with test species. Efficacy was assessed by culture method and ATP assay, right after the treatments and after four years of storage at room temperature. Coupling gamma irradiation with a drying step with dry heat at 55–60 °C reduces the fungal loads on natural items up to levels close to the detection limits, and the reduction is maintained after four years. On artificial specimens, spore germination is completely inhibited, mycelia growth is also highly affected, but the melanised test species appear to be more resistant. A synergistic effect between gamma irradiation, water content, and thermal drying is highlighted in this paper.

Keywords: flood damage, gamma radiation, mass treatment, microbial infestation

Zusammenfassung: Dieser Beitrag beschreibt die Behandlung flutbeschädigter Archivmaterialien in industriellem Maßstab. Schimmelpilze auf Papier stellen ein

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potentielles Risiko für Archivgut dar, weil sie die Zellulose, einen der Hauptbestandteile von Papier, abbauen. In der vorliegenden Studie wurden Möglichkeiten einer Behandlung von gefährdetem Archivmaterial mittels Gammastrahlung untersucht. Gammastrahlen haben eine hohe Durchdringungskraft, die Behandlung ist in kurzer Zeit durchführbar und hinterlässt keine chemischen Rückstände. Zwei verschiedene Verfahren wurden dabei verglichen: thermische Trocknung gefolgt von Gammabestrahlung und Gammabestrahlung gefolgt von thermischer Trocknung. Beide Prozesse wurden gleichzeitig an natürlich kontaminierten Archivalien und an künstlich mit Schimmelpilzen kontaminierten Papierproben durchgeführt. Die Wirksamkeit wurde durch Anlegen und Auswerten von Schimmelkulturen und ATP-Assay direkt nach den Behandlungen und nach vierjähriger Lagerung bei Raumtemperatur beurteilt. Durch die Kopplung der Gammabestrahlung mit einem Trocknungsschritt bei trockener Hitze (55–60 °C) konnte die Schimmelpilzbelastung bis nahe an die Nachweisgrenze reduziert werden; auch nach vier Jahren konnten kaum Schimmelpilze nachgewiesen werden. An künstlich kontaminierten Proben wurde die Sporenkeimung vollständig gehemmt und auch das Myzelwachstum stark beeinträchtigt, die melanisierte Spezies scheint jedoch resistenter zu sein. Besonders zeigte sich in dieser Studie ein synergetischer Effekt zwischen Gammabestrahlung, Wassergehalt und thermischer Trocknung.

Keywords: Gammabestrahlung, mikrobieller Befall, Flutschäden, Massenbehandlung

1 Introduction

Fungi can degrade cellulose in paper, affecting its integrity. These damages are due to chemicals produced by fungi in metabolic processes, using cellulose as a nutrient source, and also due to the pigmented mycelium and spores (Otero D'Almeida et al. 2009). Cellulose degradation is caused by hydrolysis, which is catalyzed by enzymes produced by fungi (Nevell and Zeronian 1985). The presence of fungi in paper represents a risk to the integrity of paper-based documents and to human health (Gambale et al. 1993), especially on wet or very humid substrates on which they can spread within few hours. For this reason, after flooding or plumbing accidents involving library depots and archives, immediate drying or freezing of paper is of outmost importance. Unfortunately, in most cases days or even weeks pass after the flood and microorganisms, in particular fast-growing species flourish on the organic substrates. Toxic moulds such as *Stachybotrys chartarum* or *Aspergillus fumigatus* or even water borne pathogenic bacteria could spread on wet materials. Massive microbial contamination of organic materials

intended for conservation and public use requires rapid and thorough disinfection, not only for preservation, but also for safety reasons. The most common treatment methods for objects infested by microorganisms and insects are the application of ethylene oxide gas and radiation processes. Although ethylene oxide does not affect the ageing of the paper, it is a harmful product classified as carcinogenic. For this reason, its utilization is severely limited by regulations in many countries, and its use might be banned in the future (Havermans 2017). Therefore, ionizing gamma radiation may become the only alternative for mass disinfection in case of heavily contaminated items because of its higher penetrating power and absence of chemical residues (Rafalski et al. 2017).

Since the early 1960s, gamma radiation has been applied and tested in the field of cultural heritage (Głuszewski 2011). Secured results were achieved for wood disinfection, in particular for the protection of waterlogged archaeological wood (Poiting et al. 1998) and also for polychrome wood (Cutrubinis et al. 2008). In Poland, gamma radiation at 25 kGy was applied for the sterilisation of artefacts from the Holocaust Museum, mainly made from different types of leather and cloth. The effect of gamma radiation on paper-based objects has been investigated several times during the last decade by many researchers but up to now no shared conclusions have been reached.

The Italian *Istituto Centrale del Restauro* recently banned the use of gamma radiation for library and archival material decontamination even at doses lower than 2 kGy (Bicchieri et al. 2016). On the other side, in the last 20 years, many researchers in Italy (Adamo et al. 2001; Magaudda 2004) and in many other countries (Area et al. 2014; Bratu et al. 2009; Da Silva et al. 2006) stated minimal side effects by gamma rays, at least regarding the mechanical properties of the paper, at a dose range between 3 and 10 kGy.

Different outcomes depend largely on different experimental conditions, different evaluation methods, and different objectives. Devices generating gamma radiation change from one experiment to another by size, technology, and dose rate, as well as the paper specimens studied that can be different by composition and water content. According to many authors, dose rate and water content affect both disinfection efficacy and side effects.

According to Bicchieri et al. (2016) gamma radiation on paper is inappropriate since a large decrease of the degree of polymerization (DP) of cellulose occurs at doses higher than 2–3 kGy, thus provoking a loss in the physical properties of the substrate, particularly at a long-term perspective. This finding is in agreement with Henniges et al. (2012) where a decrease in DP and an increase in carbonyl groups due to oxidative modification of cellulose molecules after gamma radiation at 5–10 kGy was demonstrated.

On the other side, if we consider many other mechanical and chemical parameters, such as traction, folding, macrostructure morphology, colour changes, and spectroscopy responses (FTIR and Raman techniques), the majority of the studies report no or low side effect at dosage <10 kGy even after ageing (Adamo et al. 2001; Coppola et al. 2018).

The minimal gamma radiation dosage for disinfection or sterilization is also a matter of debate. On the one hand, a dosage of 0.2–0.5 kGy up to 3 kGy for insects' eradication is quite well established (Magaudda et al. 2000; Unger et al. 2001), on the other hand there are divergent statements for microbial disinfection. Once more, different experimental conditions – as paper water content and target species susceptibility – account for different conclusions. Da Silva et al. (2006) report 15 kGy for inactivation of fungal strains in pure culture isolated from paper, while Adamo, Baccaro, and Cemmi (2015) recommend 3–8 kGy for an efficient eradication of moulds growing on paper specimens.

The dose of 25 kGy is the value accepted as sterilizing dose and is usually related to bacteria eradication, since some spore-forming bacteria can resist up to 22.4 kGy (Dang et al. 2001). Nonetheless, even if immediately after flooding bacteria can rapidly grow on paper, it is quite improbable that at standard storage condition after recovery, bacteria pose a real threat for the items.

Regarding fungi, resistance to gamma radiation varies from species to species. Species with melanised hyphae generally are more resistant than species with colourless mycelia: *Curvalaria geniculate* (a melanised dematiaceous species) and *A. fumigatus* (mostly hyaline) survive at 20 and 2.5 kGy respectively (Saleh et al. 1988). On the other hand, 1 Log reduction (D_{10}: 90% of reduction) usually is achieved with a much lower dosage. Typical D_{10} values are reached in the interval 0.1–2.0 kGy even for the more resistant species. According to many authors, a drastic bioburden reduction to acceptable limits instead of complete eradication is a more realistic objective (Bratu et al. 2009).

Finally, we should consider gamma radiation on contaminated archival material as part of a wider safety process following a disaster, such as a massive flood, to stop rapid microbial spreading and to reduce the infective load below an acceptable threshold for a safe handling during the subsequent conservation steps such as drying, dry cleaning, and substitution of damaged components. This can only be achieved at an industrial level, using a simple and convenient logistic and a fast mass treatment. Industrial mass recovery by gamma radiation of archival materials after disasters has been already employed with success in several countries. One of the first reported cases is the irradiation of a large quantity of heavily contaminated paper documents of the Dr. Gantt Collection (medical files, manuscripts, and photos, dated from beginning of XX century). The collection was irradiated at 4.5 kGy and was in good condition after more than 20 years of storage (Sinco 2000). In 1997, thousands of flooded and contaminated documents

belonging to the Morgan Library in Fort Collins, Colorado (USA) were successfully irradiated at 15 kGy, then immediately stored after drying and dry cleaning.

Unfortunately, most of the past mass treatments for paper item recovery by gamma radiation were performed in an emergency situation with poor or no evaluations on the radiation side effects, and never considering the whole recovery process. In fact, after flooding, a dehydration step of paper items is often required since the substrates are not only contaminated but also saturated or even over-saturated by water. Furthermore, drying is a preparatory step for spores and debris removal. Mass dehydration using industrial plants could be introduced in the process, upstream or downstream the gamma irradiation.

Some companies employ the thermal drying technique in specific climate chambers at about 60 °C. Through this procedure, wet archival materials with more than 14% of water content could be recovered at 8% after only 24–48 h, by ensuring a good air circulation among the folders. According to several authors, there might be a synergetic effect between gamma irradiation and thermal treatment (Ben-Arie and Barkai-Golan 1969; Justa and Stifter 1992). This effect has also been observed after artificial ageing at high and dry temperature (105 °C) (Hanus 1985).

To assess the disinfection effect of a treatment, different methods have been proposed (Pinzari et al. 2011). Besides classical culturing methods by semi-selective media, biochemical analysis of adenosine tri-phosphate (ATP) concentration is usually proposed for its sensitivity and capacity to detect cellular vitality of unculturable microorganisms or in a Viable But Non Culturable (VBNC) state (Rakotonirainy et al. 2003; Robben et al. 2019).

Sub-lethal ionizing irradiation at relatively low dosage level as well as other disinfection treatments (Robben et al. 2018) might stress normal biochemical processes, leading the microbial cells to a temporary VBNC state but retaining their activity. For this reason, in the present research we have studied the effectiveness of recovery processes in the short and long-term, both through standard cultivation and ATP assay. Related data on the physico-chemical side effects of the same processes on model paper are reported in a correlated study (Coppola et al. 2018).

2 Materials and Methods

2.1 Treated Items

2.1.1 Disposable Archival Samples

A heavily contaminated folder belonging to the *Archivio di Stato* in Aulla (Massa-Carrara, Italy) was discarded after the flooding in 2011 (Figure 1). The folder is

Figure 1: Disposable archival sample before sub-samples dissection.

composed of documents of different age (not older than 30 years) and type, including printing paper and different sizes of cardboard. Before recovery, the water content measured by a surface conductivity hygrometer (Aqua-boy PMII) varied between 14% on the surface and 17% in the middle of the folder.

2.1.2 Artificial Specimens

Chromatography paper MN 261 (Macherey-Nagel, Germany) made of pure cellulose (ash and lignin free) was artificially contaminated with fungal strains *Penicillium rugulosum* and *Cladosporium cladosporioides*, previously isolated from bio-deteriorated cellulosic materials. These strains were identified by ITS sequencing and characterized for their cellulolytic ability in a previous work (Montanari et al. 2009). The choice of these two fungal genera was made because, according to the literature, they show different resistance to gamma irradiation, the melanised *Cladosporium* being more resistant than the *Penicillium* species (Saleh et al. 1988). Two different types of specimens were prepared according to Bonetti et al. (1979):

i) *Specimens with active mycelia* – for each tested fungus, sterile strips of test paper (1 × 5 cm) were placed vertically (two strips per tube) in a 50 mL plastic tube containing 5 mL of a conidial suspension at 10^5 conidia/mL in Czapeck broth. The tubes were incubated for two weeks at 24 °C until an evident mycelial growth all over the paper surface appeared. Five replicates (strips) were considered.
ii) *Specimens with quiescent spores at three different concentrations* – for each tested fungus, a conidial suspension in Czapeck broth was prepared. The initial suspension was diluted 1/10 and 1/100. For each solution (concentrated and

diluted) eight drops (10 μL) of the conidial suspension were separately placed 1 cm apart along a sterile strip of test paper (1×8 cm). Three replicates (strips) were considered.

The highest amount of conidia per drop was about 6×10^4 and 2×10^5 for *P. rugulosum* and *C. cladosporioides,* respectively. Strips were dried for 1 h in a sterile cabinet before the recovery treatments. Artificial specimens were separately placed in sterile paper envelopes before any treatment step.

2.2 Recovery Processes

Two different types of processes were compared:
- Recovery process I (RI): thermal drying (step 1) – gamma irradiation (step 2)
- Recovery process II (RII): gamma irradiation (step 1) – thermal drying (step 2).

2.3 Recovery Technologies

2.3.1 Gamma Irradiation

Gamma irradiation was conducted in an industrial plant (Sterigenics, Minerbio-BO, Italy). It employs ExCell high-precision gamma irradiator powered by a Cobalt-60 source able to treat sequentially pallets of raw materials as large as $80 \times 120 \times 180$ cm at a high dose rate. Each pallet contains up to 24 linear meters of archival materials, packaged in plastic film placed in 16 (dimension) plastic bins (Figure 2). The total gamma dosage received by the treated material is monitored at the end of the irradiation process with dosimeters placed in different positions inside the volume of the pallet (Table 1). In the present study Red 4034 Perspex Dosimeters (nominal accuracy 4%) were applied attached to the specimen envelopes.

2.3.2 Thermal Drying

Thermal drying was performed in a restoration company using a climatic cell specific for paper items (Figure 3) (Book's Wind 2, Frati & Livi Srl, Castelmaggiore, Italy). The cell loads up to 24 linear meters of archival items previously prepared to ensure maximum penetration of hot air into the inner parts of the folders: all folders are manually divided with rigid plastic nets in reams of documents 10–15 cm large and placed on metal carts. The entire thermal process takes about

Figure 2: Eight bins containing 24 linear meter of archival material ready to be irradiated.

48 h (the duration depends on the amount of material inside the cell and on the initial moisture content) and it is controlled by thermo-hygrometric sensors placed inside the items. When the temperature reaches 55–60 °C, the items stand in the cell for about 20 h until paper substrates reach 8% water content. In our case the archival materials remained for 23 h in both the processes.

2.4 Experimental Design

Both the disposable archival samples and the artificial specimens were introduced in each recovery process. By comparing the two processes, the disposable archival materials, consisting of a contaminated folder, were divided in three portions of the same dimension (Figure 4). One portion (*r*1) followed recovery process I, one portion (*r*2) process II, the third being the control (not treated). Both *r*1 and *r*2 sub-specimens were in turn split in three reams of the same thickness and located in three different positions of the pallet before the irradiation step: external (*E*), middle (*M*) and inner position of the pallet (*I*) (Figure 5). The artificial specimens were coupled with each ream in the three different positions inside the irradiated pallet.

It is worth pointing out that the position of the sub-specimen samples during the drying step was irrelevant since hot air circulates uniformly inside the mass of the archival items.

Not treated disposable archival samples were stored indoor at room temperature inside a clean paper bag for the duration of the experimental tests.

Figure 3: Thermal drying step in the climatic chamber.

At the end of the recovery processes and after the microbial analysis at short-term, treated and not treated disposable archival samples were stored in closed and clean paper bags and in a cardboard box for four years in a repository at room temperature in a non-air-conditioned environment for subsequent long-term analysis. The temperature inside the repository was between 15 °C in winter and 30 °C in summer, with relative humidity ranging from 35 to 70%.

Figure 4: Dividing of the archival samples in three sub-samples.

Figure 5: Position of archival sub-samples (coupled with artificial specimens) in the pallet, before irradiation.

2.5 Short-Term Analysis

Analyses were performed at the beginning of the trails and immediately after each step of the two recovery processes.

2.5.1 Analysis of Disposable Archival Samples

After each step (irradiation or thermal drying), three sub samples (replicates) of paper material (about 2 cm^2 consisting of a parcel composed by several layers of slips) were cut randomly from each ream of the treated sub-specimen. Any square

was in turn shared in three sub-parcels of about the same thickness. One sub-parcel was used for culture analysis, one for biochemical analysis and one for water content determination for dry weight determination. For not treated sub-specimen the same procedure was performed only at the beginning of the experiment.

Culture analysis: Any fresh sub-parcel was weighted and placed in a 50 mL sterile tube containing 9 mL of extraction solution (0.1% of sodium pyrophosphate) and 1 g of glass beads (5 mm diameters), shaken for 10 min at 460 rpm and filtered through two layers of sterile cheese cloth. The homogenate was diluted in Ringers' solution and plated on semi selective agar for fungal counting, specifically Dichloran Rose Bengale Chloramphenicol Agar (DRBC) and Dichloran-Glycerol (DG18). After seven days at 20 °C, fungal colonies growing on the plates were counted as colony forming units (CFU) and referred to gram paper dry weight (gdw) assessed after water content determination.

Biochemical analysis: ATP assay was performed by using the detection kit ENLITEN ATP Assay System (Promega, USA, Madison) and a Luminometer (PBI, Italy). Any fresh sub-parcel was weighted, chopped in small pieces, and placed inside a 2 mL Eppendorf tube with 1 mL of Glo Lysis Buffer (Promega). After 1 min vortexing the tube was shaken with an orbital shaker at 150 rpm for 40 min. The homogenate was filtered with one layer of sterile cotton gauze and centrifuged at 4000 rpm for 4 min. The supernatant (100 μL) was placed with 100 μL of reconstituted luciferin-luciferase reagent and the chemiluminescence was immediately measured in Relative Lightness Unit (RLU) within the luminometer. In each sample the ATP amount, expressed in picomoles, was determined by comparing the RLU of samples with those of standards in the range of 10^{-8}–10^{-12} M and referred to grams of paper dry weight (gdw) assessed after water content determination.

Water content determination: The amount of water in the samples was measured by the gravimetric method. Any fresh sub-parcel was weighted and then dried at 103 °C up to constant weight. Water content was calculated on a dry basis (weight of water divided by the weight of the dry solid) following conventional standards for food, soil, and wood (for example ASTM D4442 – 20: Standard Test Methods for Direct Moisture Content Measurement of Wood and Wood-Based Materials).

2.5.2 Analysis of Artificial Specimens

Artificial specimens with active mycelia: Independent from the recovery process, after the first step each paper strip artificially contaminated (i.e., replicate) was cut in two halves longitudinally, one half was cut into five equal rectangles (1 × 0.5 cm) and plated on ¼ strength potato dextrose agar (¼ PDA; 10 g PDA, 16 g agar, 100 mg streptomycin sulphate, 10 mg tetracycline hydrochloride and 2 mL Triton X–100 in 1 L deionised water) and the other was placed back in the envelope for the

subsequent step. After the second step, the remaining half was cut as the first one and plated on ¼ PDA as well. For each strip, the percentage of rectangles showing mycelial regrowth on agar after one week of incubation at 24 °C was calculated.

Artificial specimens with quiescent spores at three different concentrations: Independent from the recovery process, after the first step from any paper strip artificially contaminated (i.e., replicate), four of the eight drops laid on any paper strip (at any different concentration) were cut in squares of about 1 × 1 cm and plated on ¼ PDA. Remaining strips were returned to the envelope for the subsequent step. After the second step the last four 1 × 1 cm drops were cut from the remaining strip and plated as reported before. For each strip the percentage of squares showing germination on agar after one week of incubation at 24 °C was calculated.

2.6 Long-Term Analysis

The analysis on the *disposable archival samples* were repeated after four years, using the same methodologies and instrumentations, in order to verify the long-term efficacy of the treatment with gamma irradiation.

2.7 Statistical Analysis

2.7.1 Disposable Archival Samples

CFU/gdw counting from disposable archival samples culture analyses were log transformed (log10(CFU + 1)). Quantitative data were checked for normal distribution.

Log CFU/gdw and picomole ATP/gdw data obtained from disposable archival samples were statistically elaborated with a multi-factor analysis of variance (ANOVA) performed with Statgraphics plus 2.1 (1996), accounting for the type of recovery process, sub-specimens' position in the pallet, and sampling time (short and long-term). Data from short-term were those obtained at the end of both the recovery processes (after the second step).

Differences between the means were evaluated by the Least Significant Difference (LSD) test at a significance level of $P < 0.05$.

The position factor was achieved only during the irradiation step, and it was not possible to introduce in this analysis the not treated control, since it was not subjected to this factor. Statistical comparison with not treated sample at any single step could be performed only through a one-way ANOVA, comparing all the thesis with each other.

2.7.2 Artificial Specimens

Percentage data were checked for normal distribution. Data obtained at the end of both the recovery processes (after step 2) were statistically elaborated with a multi-factor analysis of variance (ANOVA) performed with Statgraphics plus 2.1 (1996), accounting for type of recovery process and sub-specimens' position in the pallet.

As above it was not possible to introduce in this analysis the not treated control, since it was not subjected to the position factor during the thermal step. Statistical comparison with not treated sample at any single step could be performed only through a one-way ANOVA, comparing all the thesis with each other.

3 Results

Table 1 reports the final dosages achieved after irradiation during the recovery processes inside the pallet at the three different positions.

Table 1: Final irradiation dose (kGy) at each position.

Position	*Irradiation dose (kGy)*	
	Recovery process	
	RI	**RII**
External (E)	6.11	5.64
Middle (M)	5.14	4.88
Internal (I)	5.03	3.88

3.1 Disposable Archival Samples

3.1.1 Culture Analysis

In Figure 6, Log CFU/gdw on DRBC agar media from the samples before and after each step of the recovery processes and after long-term storage are reported. One-way ANOVA shows significant differences among the thesis: In the RI process, fungal loads after the first step are not different compared to the control (NT); fungal loads are significantly reduced only after step 2. The RII process shows significant loads reduction already after step 1. In treated samples, the low fungal loads (close to the detection limit) are maintained after long-term storage. On the

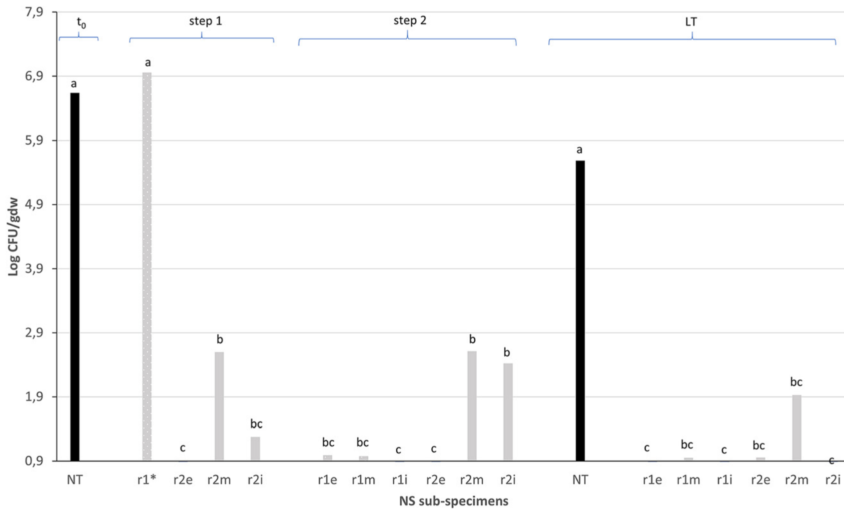

Figure 6: Average fungal loads (Log CFU/gdw) on disposable archival samples. * This value represents the average of Colony Forming Units (CFU) load obtained from all the sub-samples treated with the thermal drying process in step 1, where position in the cell is irrelevant. Different letters over column bar indicate significant differences among the averages per $P \le 0.05$ (LSD = 2.0) after One-Way ANOVA analysis. Detection limit: 0.93 (Log CFU/gdw). Legend: (t_0) analysis of not-treated samples before the recovery processes; (LT) analysis of treated and non-treated samples after 4 years of storage; (NT) non treated sub-samples; (r_1) sub-samples treated following process RI; (r_2) sub-samples treated following process RII; (*e*) sub-samples positioned in the external part of the pallet; (*m*) sub-samples positioned in the middle part of the pallet; (*i*) sub-samples positioned in the internal part of the pallet.

other side, in the not treated sample, fungal loads still remain at high level. Data obtained on DG18 agar media were always slightly lower than those on DRBC media and so they are not reported.

Multifactor ANOVA analysis on treated samples shows that the three factors, i.e., type of recovery process, position in the pallet, and analysis time (short and long-term), are not significant.

3.1.2 Biochemical Analysis

In Figure 7, picomole ATP/gdw data from disposable archival samples before and after each step of the recovery processes and after long-term storage are reported. One-way analysis shows no significant differences among the thesis, due to high variability between the replicates. Therefore, in Figure 7 only the standard deviations that highlight the intra-thesis variability are reported.

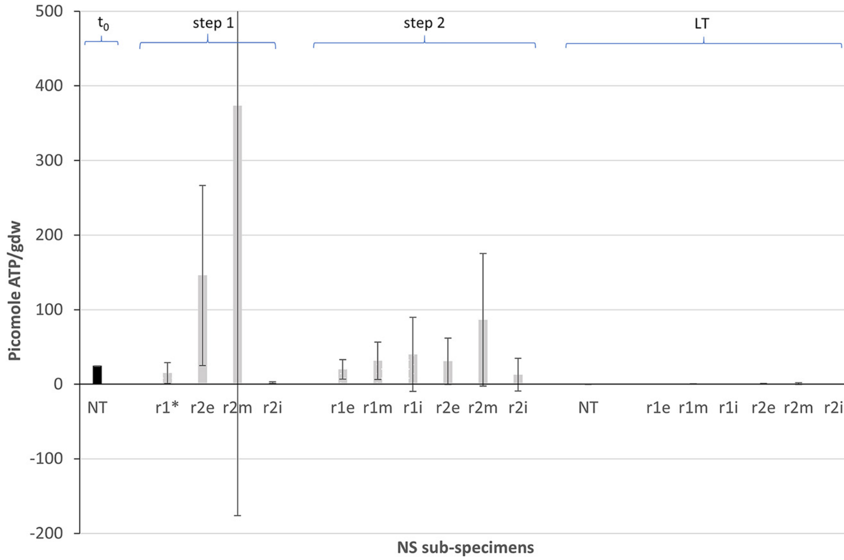

Figure 7: Average ATP amount (picomole/gdw) on disposable archival samples. * This value represents the average of picomole of Adenosin Tri-Phosphate (ATP) from all the sub-samples treated with the thermal drying process in step 1, where position in the cell is irrelevant. Bars represent standard deviation. Detection limit: 5.3×10^{-4} (picomole/gdw). Legend: (t_0) analysis on not-treated samples before the recovery processes; (LT) analysis on treated and non-treated samples after 4 years of storage; NT non treated sub-samples; (r_1) sub-samples treated following process RI; (r_2) sub-samples treated following process RII; (*e*) sub-samples positioned in the external part of the pallet; (*m*) sub-samples positioned in the middle part of the pallet; (*i*) sub-samples positioned in the internal part of the pallet.

Multifactor ANOVA on treated samples shows that there is a statistical difference between average ATP amount after short-term analysis (36.91 pmol/gdw) and after long-term analysis (0.32 pmol/gdw) for $P < 0.05$ (LSD = 22.26), independent from the type of recovery process and samples' position in the irradiated pallet. After four years of storage ATP could only be detected in very few samples, control included.

3.2 Artificial Specimens

3.2.1 P. rugulosum

Specimens with active mycelia and with quiescent spores at three different concentrations: Mycelial regrowth and spores' germination are completely inhibited after

gamma irradiation, in both the recovery processes (RI and RII), the percentage of regrowth and germination being at 0%. In RI, thermal drying before gamma radiation (step 1) does not affect *P. rugulosum* germination and mycelia neither in appearance nor in percentage of regrowth, the percentage of regrowth and germination being at 100%, equaling the not treated control. Since no variability is observed among the replicas statistical output is not reported.

3.2.2 C. cladosporioides

Specimens with active mycelia: One-way ANOVA shows significant differences among the thesis. In Figure 8 percentages of paper specimens showing mycelial regrowth after any step of the recovery processes are reported, NT being the not-treated control. Multifactor ANOVA shows significant differences between RI and RII process, independent from sample position, mycelial regrowth being higher in

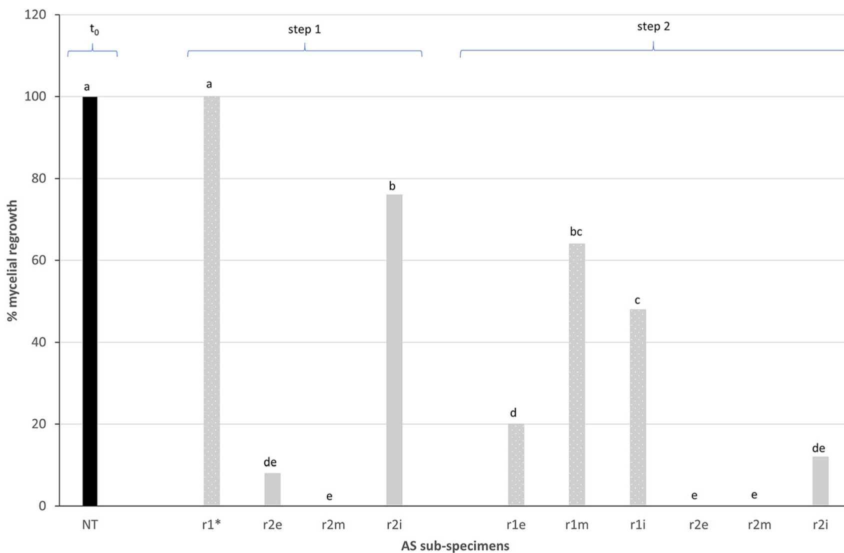

Figure 8: Average percentage of mycelial regrowth of *C. cladosporioides* from artificial specimens. * This value represents the average of % mycelial regrowth from all the sub-specimens treated with the thermal drying process in step 1, where position in the cell is irrelevant. Different letters over column bar indicate significant differences among the averages per $P \leq 0.05$ (LSD = 15.74) after One-Way ANOVA analysis. Legend: (t_0) analysis on not-treated samples before the recovery processes; (LT) analysis on treated and non-treated samples after 4 years of storage; (NT) non treated sub-samples; (r_1) sub-samples treated following process RI; (r_2) sub-samples treated following process RII; (*e*) sub-samples positioned in the external part of the pallet; (*m*) sub-samples positioned in the middle part of the pallet; (*i*) sub-samples positioned in the internal part of the pallet.

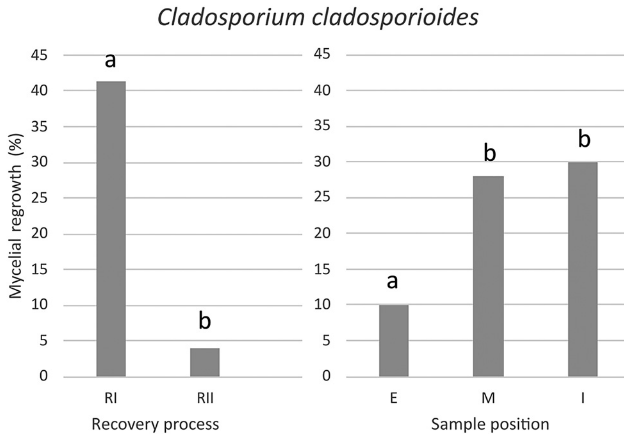

Figure 9: Average percentage of mycelial regrowth *C. cladosporioides* from artificial samples for each recovery process (RI and RII) and for each position in the pallet (*E*, *M* and *I*). Different letters over column bar indicate significant differences among the averages per $P \leq 0.05$ (LSD = 16.05 and 19.65, for recovery factor and position factor respectively), after Multifactor ANOVA.

RI than in RII. Independent from the recovery process, mycelial regrowth is lower for samples positioned in the external part of the pallet than in the other two sample positions. There are no differences among inner and middle position (Figure 9).

Mycelial regrowth is completely inhibited only at the end of process RII on specimens positioned at the external and middle part of the pallet receiving the higher amount of gamma radiation. It is worth mentioning that mycelial regrowth after radiation process both in RI and RII process at a visual inspection turns out to be partially affected, being more restricted and less pigmented.

In RII, drying after gamma radiation (step 2) further reduces mycelial regrowth. In RI, gamma radiation after drying (step 2) clearly affects mycelial survival, specimens positioned in the inner and the middle area of the pallet show a reduction of the growth of about 50%, while the specimens positioned in the external area show a reduction of 80%. In RI drying before gamma radiation (step 1) does not affect *Cladosporium mycelia* neither in appearance nor in percentage of regrowth.

Specimens with quiescent spores at three different concentrations: Spores' germination on paper is completely inhibited after gamma irradiation at any concentration and in both recovery processes. In RI, drying alone does not affect the germination at any concentration, comparable to the not treated control. Since no variability is observed among the replicas, statistical output is not reported.

4 Discussion

Under the described experimental conditions, recovery processes that include gamma radiation at about 5 kGy dosage show high effectiveness on heavily contaminated archival materials and selected fungal strains, reducing microbial load to levels close to the detection limits, up to six log units. The reduction of fungal load on archival samples is maintained after four years. This means that fungal propagules left inside the archival items after treatment were completely devitalized.

Thermal drying alone does not affect the viability of fungi on both natural and artificial specimens. Nevertheless, thermal dehydration seems to have a synergetic effect with gamma irradiation when the latter precedes the former, as shown by the results obtained on samples artificially contaminated with the test fungus *C. cladosporioides*. Gamma radiation is more effective against *C. cladosporioides* on wet substrates than on dehydrated ones, probably because the higher content of water-derived free radicals enhances the biocidal effect, as already observed by Adamo et al. (2001). *C. cladosporioides* on artificial specimens irradiated at a higher dosage is completely eradicated only after the subsequent thermal drying treatment.

As expected, the melanized test fungus *C. cladosporioides* showed more resistance to gamma radiation compared to the *Penicillium* strain. Radiation at a dose of 4–6 kGy alone does not eradicate it completely for the entire thickness of the irradiated pallet, but is worth mentioning that even at the lower dosage (inner part of the pallet) mycelium consistence and pigmentation is visibly reduced.

Conidial germination of both fungal strains is completely inhibited even at the lower dosages, ensuring a reduction of the risk of recontamination by residual fungal spores.

The ATP data obtained on natural samples are difficult to compare. In the short-term, gamma irradiation seems to increase the ATP levels (although there is a high variability among the replicates) despite a drastic devitalizing effect on the microbial load. This unexpected data could be explained by the study conducted by Tsukimoto et al. (2010) on human cell lines which show extracellular ATP release following low doses of gamma irradiation. Then, according to Tsukimoto, the ATP present in the microbial cells could be released randomly right after the ionizing treatment, remaining quantifiable in the substrate for a few hours or days. In addition, Alfa et al. (2015) has estimated that, in absence of a chemical disinfection, the ATP produced by devitalized bacterial cultures can remain on a surface for several days, up to over one month. In light of these statements, we must conclude that the ATP test is not a suitable method to verify the effectiveness of a gamma treatment on short-term. These conclusions are confirmed by the long-term

ATP test (after four years), in which very low ATP values are observed on treated samples in accordance with very low CFU values.

5 Conclusions

In case of a severe microbial contamination on archival or library materials due to slow emergency responses after a flood or an accident, a highly effective recovery process is needed to save documents and reduce biological risk for operators and users. A proper operating protocol should follow several milestones including recovery and packaging, disinfection, dehydration, and residual cleaning. The last two steps are of outmost importance to reduce the allergenic potential of residual spores and the risk of subsequent microbial regrowth.

In our study we demonstrate that, at an industrial scale, thermal drying following gamma radiation eliminates almost completely the microbial load on the substrate in the long-term perspective. Furthermore, the use of radiation instead of ethylene oxide during the disinfection step appears to be safer for the recovery of wet contaminated material. In fact, fumigation with ethylene oxide on moist materials produces the by-product ethylene glycol, leading to a reduction of its effectiveness (Moerman and Mager 2016). Therefore, the dehydration step must necessarily be carried out upstream of the fumigation process. This means that the final step of dry cleaning of dead microbial residues with brushes and compressed air nozzles would take place in the presence of potentially toxic compounds. Even if zero ethylene oxide concentration can be achieved with a proper ventilation technology, a mass treatment conducted on mixed materials composed of many different substrates (paper, parchment, synthetic materials, and films) with different rates of ethylene release (Hengemihle et al. 1995) could be considered not completely safe.

As stated by Coppola et al. (2018), gamma radiation does affect the integrity of cellulose, probably reducing paper life but, when dealing with large amounts of materials heavily contaminated, it may represent a safe, fast, and effective solution to put in place, especially if saving the content of a document is more important than its materiality itself. Institutions should improve their emergency response plans by developing an *ad hoc* rescue path that includes mass decontamination through gamma irradiation, identifying on a national scale the professionals and facilities capable of carrying out these services.

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