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# Bioburden assessment and gamma radiation inactivation patterns in parchment documents

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## HIGHLIGHTS

- Characterization of the microbial population of parchment documents.
- Study the inactivation pattern of parchment microbiota by gamma radiation.
- Assessment of the minimal gamma radiation dose for the parchment decontamination.
- The microbial population in tested parchments was mainly constituted by bacteria.
- *Cladosporium cucumerinum* was the most frequent fungal species.

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## ABSTRACT

Parchment documents are part of our cultural heritage and, as historical artifacts that they are, should be preserved. The aim of this study was to validate an appropriate methodology to characterize the bioburden of parchment documents, and to assess the growth and gamma radiation inactivation patterns of the microbiota present in that material. Another goal was to estimate the minimum gamma radiation dose ($D_{min}$) to be applied for the decontamination of parchment as an alternative treatment to the current toxic chemical and non-chemical decontamination methods. Two bioburden assessment methodologies were evaluated: the Swab Method (SM) and the Destructive Method (DM). The recovery efficiency of each method was estimated by artificial contamination, using a *Cladosporium cladosporioides* spore suspension. The parchment samples' microbiota was typified using morphological methods and the fungal isolates were identified by ITS-DNA sequencing. The inactivation pattern was assessed using the DM after exposure to different gamma radiation doses, and using *C. cladosporioides* as reference. Based on the applied methodology, parchment samples presented bioburden values lower than $5 \times 10^3$ CFU/cm$^2$ for total microbiota, and lower than 10 CFU/cm$^2$ for fungal propagules. The results suggest no evident inactivation trend for the natural parchment microbiota, especially regarding the fungal community. A minimum gamma radiation dose ($D_{min}$) of 5 kGy is proposed for the decontamination treatment of parchment. Determining the minimal decontamination dose in parchment is essential for a correct application of gamma radiation as an alternative decontamination treatment for this type of documents avoiding the toxicity and the degradation promoted by the traditional chemical and non-chemical treatments.



## 1. Introduction

Art in its different forms such as paintings, sculptures or written documents, is exposed to physical, chemical and biological deterioration agents. Mainly made of degradable raw materials, art objects can be altered and destroyed according to their intrinsic characteristics and structure, and due to the effects of environmental conditions, such as humidity and temperature. Organic raw materials are usually good substrates for insects, bacteria and fungi, which makes biodeterioration a central issue for scholars and art curators (Katusin-Razem et al., 2009; Guiamet et al., 2011). This fact leads to the necessity of developing new approaches in restoration, preservation, conservation and decontamination procedures.

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Microorganisms such as bacteria and fungi have been described as important agents in the degradation of documents from archives and libraries (Mesquita et al., 2009; Guiamet et al., 2011). The small size of bacteria allows them to penetrate between polymer fibers, leading to strong substrate aggregation and consequent colonization. Some bacteria can even accelerate the deterioration process due to the excretion of specific substances, such as cellulolytic enzymes or organic acids, which reduce the pH, promoting the attack of new microorganisms (Tiano, 2002). Fungi can also play an important role in the biodeterioration of cultural heritage (Sterflinger, 2010). Apart from the problems caused by the production of some toxic (and sometimes pigmented) secondary metabolites, fungal biocontaminants are especially problematic due to their morphological characteristics (e.g. hyphae penetration capacity), the fact that they can feed on of almost every substrate, and their nutrition pathway, that eventually leads to the secretion of enzymes (Szczepanowska and Cavaliere, 2000).

Archive materials present different susceptibility to microorganisms according to their structure and specific components: paper is mainly constituted by lignin, hemicelluloses and cellulose, and is frequently contaminated by fungi (Tiano, 2002). Parchment, along with papyrus, was one of the most important precursors of paper. It is a natural and organic material consisting of animal skin (from goat, cow, sheep, etc.) that undergoes a special treatment. Because of its animal origin, parchment is rich in compounds such as collagen, keratin, elastin, albumin and globulin making it an excellent biological substrate that is preferably contaminated by bacteria under aerobic conditions, but can also be contaminated by certain fungal species (Tiano, 2002).

Research on the biodeterioration of old historical documents is important for the development and optimization of methodologies that help to prevent their degradation. Many chemical and non-chemical methodologies have been applied in order to decontaminate and protect different art forms, especially archive documents. Between the different chemical methods, we can find fumigants agents such as Ethylene Oxide (ETO), Methyl Bromide or Sulphuryl Fluoride (Hengemihle et al., 1995; Bond, 1998). However, fumigants and pesticides are in disuse due to their toxicity to humans. Non-chemical treatments such as modified indoor atmospheres could help in preventing contamination, but the toxicity to the staff of archives and libraries is still high (Kaplan and Schulte, 1996). However, other non-chemical treatments outweigh the toxicity problems.

Gamma radiation has been applied and tested in cultural heritage since the early 60's (Sinco, 2000; Magaudda, 2004). Low doses seem to be effective on the decontamination of archive documents, with little evidence of material alterations (Adamo et al., 1998). However, the most studied documents are mainly constituted by paper (Adamo et al., 2001; Gonzalez et al., 2002) and rare studies were made regarding the radiation effects on parchment (Nunes et al., 2012).

Considering all that was stated above, this study aimed to: (i) The validation of a methodology to estimate the bioburden of parchment (using documents from the Archive of the University of Coimbra (AUC)). (ii) Characterize the microbiota to identify parchment contamination patterns. (iii) Evaluate the inactivation patterns of parchment microbiota. (iv) Propose a minimal radiation dose ($D_{min}$) to be applied in parchment decontamination treatments using gamma radiation as an alternative decontamination method.

## 2. Material and methods

### 2.1. Sample identification

Four different parchment samples ($n=4$) from the Archive of the University of Coimbra (AUC) were used: 1CSC, 2LBA, 5CFF and 6SXVI, respectively, "1-Cabido da Sé de Coimbra (1472)"; "2 – Livro de Baptismos de Alvarenga (1719–1751)"; "5 – Colações – Figueira da Foz" and "6 – An unclassified parchment document from the 16th century". When acquired, the samples presented some small tears and folds (especially in the edges) as well as symptoms of humidity and handling damage. In general, four samples were soiled and the parchments 2LBA, 5CFF and 6SXVI showed visible signs of contamination by molds. Only the parchment 6SXVI presented visible biodegradation. During the period between acquisition and the tests, all samples were individually kept in paper bags at room temperature. Storage conditions before the acquisition were unknown.

The thickness of parchment samples was measured using a micrometer (Mitutoyo 7360, precision=0.01 mm), as described by Nunes et al. (2012) ($n=96$, 24 per parchment sample). Measurements showed a thickness variation between 0.179 and 0.384 mm (mean value=0.288 mm $\pm$ 0.009).

### 2.2. Sampling procedure

Each parchment sample (1CSC, 2LBA, 5CFF and 6SXVII) was divided in two halves. The first half was subdivided into twelve similar subsamples ($\approx$11.1 cm $\times$ 4.8 cm) from which, six were used for the determination and characterization of the parchment bioburden (Section 2.3.2), using both the Swab Method (SM) and the Destructive Method (DM) ($n=3$ subsamples per method), while the remaining six were used for the evaluation of the recovery efficiency of these methods (Section 2.3.1) using an artificial contamination.

The second half of each parchment sample was subdivided in 24 similar subsamples ($\approx$7.2 cm $\times$ 4.7 cm) and used in the inactivation studies (Sections 2.3.3 and 2.3.4).

To avoid cross-contamination between subsamples, replicas and environment, the parchment division, artificial contamination and packing procedures were performed in a Biohazard safety cabinet class II (Biochem Grad; the Baker Company, inc.). Furthermore, the dissection material was sterilized between samples and the artificial contamination was performed sample by sample.

Environmental controls, inside the Biohazard safety cabinet, were applied by settle plate method using Tryptic Soy Agar (TSA) Petri dishes ($n=3$). No colonies were detected in the environmental control plates after incubation (5 days at 25–28 °C and 30 $\pm$ 2 °C until the seventh day).

### 2.3. Experimental procedure

#### 2.3.1. Validation of the bioburden determination method

A Swab Method (SM) was compared with a washing Destructive Method (DM).

The choice of the described methods was based on the natural characteristics of parchment (e.g. non-liquidity, flexibility, fibrousness) and the guidelines provided in the ISO, 2006 11737-1.

The validation of the parchment bioburden determination method was performed using an artificial contamination of subsamples with a spore suspension of *Cladosporium cladosporioides* (AUC indoor air's isolate – 2008 sampling). The spore suspension was prepared from axenic cultures of *C. cladosporioides* (seven days in Malt Extract Agar medium), using a saline solution (0.9% NaCl with 0.1% of Tween 80) as suspension medium. Concentration was assessed using a Neubauer chamber (~$10^7$ spores/mL). Six subsamples of each parchment ($n=6$) were artificially contaminated with $5 \times 50$ μL of the spore suspension, corresponding to approximately $8 \times 10^7$ spores/mL. The subsamples were dried overnight in the laminar flow chamber.

In the SM, three spiked subsamples per parchment were sampled by scraping using a swab. Each swab was then individually placed in a