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Original article

Decontamination of archival cultural heritage materials with gamma radiation: a non-destructive investigation of paper and parchment documents from Montecassino Abbey (Cassino, Italy)

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ABSTRACT

The conservation of Cultural Heritage (CH) materials, particularly archival documents and manuscripts, faces ongoing challenges due to biological deterioration caused by mold, bacteria, and insects. Traditional conservation and restoration methods are often invasive, time-consuming, and may involve the use of hazardous chemicals. In this context, ionizing radiation emerged as an effective, non-invasive alternative for the stabilization of fragile historical materials. This study presents the results of an experimental treatment using gamma irradiation applied to selected parchment fragments and paper documents from the historic collection of the Archive of Montecassino Abbey (Italy). The treatment aimed to assess the compatibility of gamma radiation for the conservation of both paper and parchment-based heritage objects. Pre- and post-irradiation analyses were conducted by coupling microbiological analyses with Raman, Attenuated Total Reflectance Fourier Transform Infrared (FTIR/ATR), UV–Vis spectroscopies and colorimetric measurements. The results confirm that gamma irradiation at the applied doses does not cause any significant structural or chromatic alterations to the treated materials, while ensuring a substantial decrease in fungal and bacterial microbial load. These findings support the suitability of gamma irradiation as a non-invasive and efficient approach for the decontamination of delicate CH objects, such as parchment and paper documents.

1. Introduction and research aim

The preservation of archival, architectural, and literary Cultural Heritage (CH) is a milestone for safeguarding the tangible memory of human civilization [1–3]. In particular, the conservation of historical documents, such as manuscripts and books, is crucial to ensure accessibility for future generations. In this context, the restoration and long-term care of these delicate objects present significant challenges. Traditional conservation techniques often involve lengthy interventions and the use of chemical agents, which may pose risks not only to the materials themselves but also to conservators [4,5]. Furthermore, many libraries, archives, and monastic institutions store their collections under suboptimal environmental conditions, such as elevated humidity levels, that foster the proliferation of fungi, bacteria, and insects, accelerating the deterioration of paper and parchment materials [6–8]. Insects may also leave behind larvae or eggs, continuing the cycle of biodeterioration if not properly eradicated.

In this scenario, innovative methodologies based on ionizing radiation have increasingly been adopted by heritage institutions worldwide [9–15]. Among the various ionizing sources, gamma radiation exhibits distinct advantages, due to its high penetration capacity, allowing for homogeneous and large-scale disinfection without increasing temperature or leaving chemical residues [16–21]. Currently, gamma irradiation is already a well-established and widely applied technology for other material classes, medical devices and inorganic CH objects. Unlike chemical or thermal treatments, gamma radiation can penetrate deeply through the whole CH object, reaching not only the surface but also the inner layers of artifacts.

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The combination of such properties makes gamma irradiation techniques particularly suitable for the treatment of organic materials such as ancient documents, especially when microbial contamination is present. However, despite its proven effectiveness, the adoption of gamma irradiation in the field of CH remains limited in some countries, such as Italy [22,23]. Skepticism and regulatory hesitations have contributed to the underutilization of this method, specifically in the context of paper-based and parchment-based heritage objects. This is mainly due to the intrinsic sensitivity of these materials, which are highly susceptible not only to biodeterioration but also to potential radiation-induced chemical and structural modifications. This dual vulnerability makes paper and parchment particularly challenging substrates, requiring careful optimization of treatment parameters. Conversely, gamma irradiation could represent an attractive option, especially in situations involving large collections, where alternative methods may be more difficult to apply on a large scale and on objects with differing nature.

Initial studies carried out on both model systems and authentic historical samples have shown that gamma irradiation, when applied under controlled conditions, can eliminate microbial contaminants without altering the chemical, physical, or visual properties of paper-based artifacts [22,23]. In agreement with International Atomic Energy Agency (IAEA) guidelines [24–26], our recent study pointed out that a dose of 8 kGy was effective to achieve near-complete fungi eradication, while maintaining the structural integrity of the treated materials [27]. Ionizing radiation affects microorganisms through two main mechanisms: direct interaction with cellular components such as DNA, and indirect interaction via free radicals generated by water radiolysis. Here, hydroxyl radicals cause about 90% of DNA damage, inhibiting cell division [28].

On these bases, we previously demonstrated the efficacy of gamma exposure for heritage conservation applications, describing radiation-based treatments conducted on archival case studies [27]. These findings agree with recent studies of other groups. For example, Ashour et al. [29] and Jo et al. [30] have demonstrated the effectiveness of gamma irradiation in inhibiting fungal growth in archaeological manuscripts, even in the case of resistant species, while preserving the mechanical integrity of the paper. Similarly, Ergun et al. [31] showed how synergistic approaches, combining gamma irradiation with antimicrobial agents, enhance decontamination efficiency. Furthermore, Delgado Vieira et al. [32] disclosed the effectiveness of ionizing radiation not only against biological contaminants but also in degrading hazardous chemical residues, such as pesticides, without inducing significant alterations in the treated materials. Here, we present an application of gamma irradiation to original archival materials of significant historical value in Italy, a context in which such treatments are still rarely implemented. The tested items are part of the collection of the Archive of Montecassino Abbey (Cassino, Italy), one of the most emblematic monastic institutions in Europe. Founded by Saint Benedict in 529 CE, the Abbey has played a key role in the preservation and transmission of western cultural heritage for over fifteen centuries [33]. Despite the devastation it suffered during World War II, the monastic community has retained a substantial portion of its manuscript and documentary holdings, which include approximately 1150 codices, thousands of parchment fragments, and over 15,000 archival documents.

Within this preservation framework, the present study is based on a limited number of archival samples. Specifically, four representative samples, namely two paper-based and two parchment-based documents dating back between the 15th and 18th centuries, were selected from Abbey's private collection. These CH objects were affected by microbiological contamination detected by isolation and *in vitro* cultures. Therefore, the microbial strains isolated from the documents were subjected to gamma radiation and a substantial reduction of microbial load was achieved, as observed through subsequent *in vitro* tests after each absorbed dose. It should be noted that such microbiological assessments are mainly qualitative. To evaluate and ensure the integrity of the objects, several non-invasive and non-destructive techniques, including UV–Vis spectroscopy, micro-Raman and FTIR/ATR spectroscopies, along with colorimetry were carried out. Importantly, where possible, both the writing substrates and the organic and/or inorganic inks were also investigated to determine the treatment's overall impact. The experimental approach followed non-destructive protocols developed in previous research [27], ensuring the integrity of the original materials throughout the process. This study supports the potential of gamma irradiation as a non-invasive and effective approach for the conservation of historical documents. Although not novel in the broader context of CH conservation, this work represents a relatively original and advanced application within the Italian framework, where the use of radiation-based treatments on archival materials of significant historical value remains limited. This contributes to fostering greater awareness and acceptance of these techniques in contexts where their implementation is still uncommon.

2. Materials and methods

2.1. Samples description

Documents conserved in the Archive of Montecassino Abbey were labelled as Doc1, Doc2, Doc3 and Doc4 (Fig. 1a-b-c-d). Doc1 and Doc4 are parchment documents, while Doc2 and Doc3 are paper documents.

Doc1 (15th Century): A legal document dated July 31, 1448 (Sulmona). This animal-skin membrane (570×227 mm) exhibits significant mechanical damage, being severely lacerated and mutilated along the entire left margin and the lower edge. The surface shows signs of biological staining and uneven thickness typical of medieval manufacturing.

Doc2 (15th Century): A fragment of a paper leaf (102×170 mm) likely originating from a 15th-century incunabulum (Gospel of Luke). The paper is characterized by high cellulose purity but shows typical age-related yellowing and edge fraying.

Doc3 (16th Century): Detached leaves (numbered 185–192, 418×285 mm) from the legal treatise *In Digestum vetus interpretationes* (Lyon, 1552). These samples show critical preservation state, with extensive lacerations and structural weakening caused by historical exposure to high humidity levels and subsequent fungal activity.

Doc4 (18th Century): An altar *Cartagloria* (263×366 mm) used for liturgical celebrations. Compared to Doc1, this parchment is thinner and more refined, though it presents substantial mutilations at the bottom and localized brittleness. The ink coverage is extensive.

The samples consist of naturally aged, compromised documents originally stored in the historical archives of the Montecassino Abbey under fluctuating microclimatic conditions. Because of their already degraded state, they were selected for experimental irradiation studies to preserve intact archival heritage.

Specialized cultural heritage professionals handled the transport using custom-designed wooden crates. Prior to analysis, the samples were stabilized in a controlled environment ($T = (20 \pm 2)\ ^{\circ}C$; $RH = (50 \pm 5)\%$). To preserve the original degradation baseline, no chemical pre-treatments or cleaning were applied, and all handling was performed using lint-free cotton gloves. Detailed manuscript contents are provided in the Supporting Section.

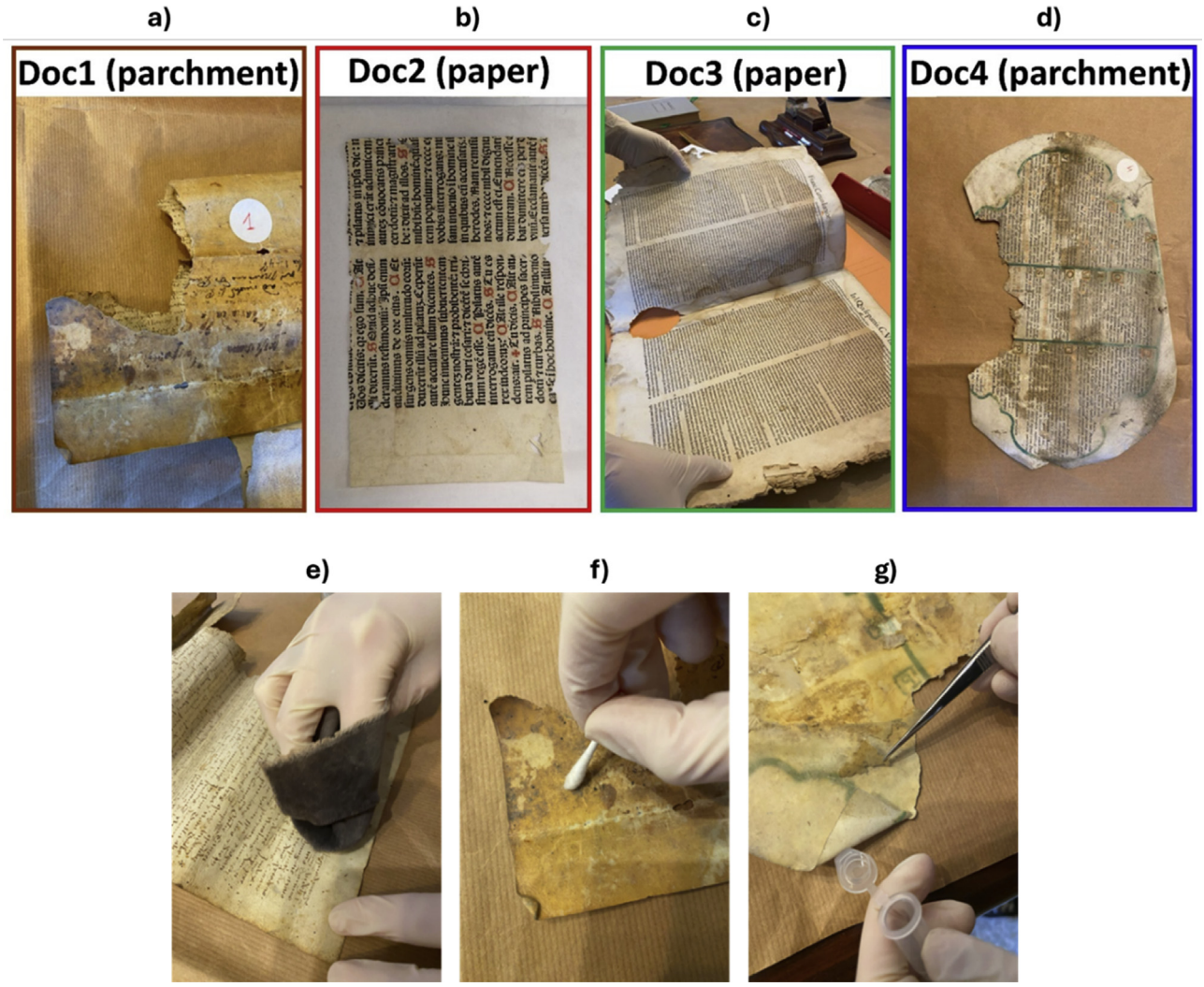


Fig. 1. Images of (a–d) documents from the Archive of the Montecassino Abbey and (e–g) the sampling tools used. The documents were sampled using (e) sterile velvet, (f) swabs, and (g) tweezers.

2.2. Microbiological analysis

2.2.1. Media

Petri plates containing Yeast Peptone Dextrose (YPD: 10 g/L of bacto-peptone, 10 g/L of yeast extract, 20 g/L of glucose and 21 g/L of agar) and Luria Bertani (LB: 10 g/L of bacto-tryptone, 5 g/L of yeast extract, 5 g/L of NaCl, 1 g/L of NaOH 1 N and 21 g/L of agar) were used for fungal and bacterial growth, respectively. All plates were incubated for two weeks at 28 °C and monitored daily.

2.2.2. Sampling procedure

Fig. 1 shows the documents and the sampling tools used. Microbiological samples were collected from visibly deteriorated areas of all documents using sterile velvet fabrics as described in [34] and swabs (Fig. 1e-f-g). Velvet fabrics were plated directly on plates while the swabs were soaked in a sterile solution of NaCl 0.8% and streaked onto different culture media. Detached fragments of the documents were also sampled with sterile tweezers.

2.3. Gamma irradiation tests

The gamma irradiation tests were carried out at the Calliope gamma irradiation facility [35], which is equipped with a ^{60}Co radioisotopic source array, as well as dedicated dosimetric and characterization laboratories. The dose rate was about 1.5 kGy/h, and all tests were performed in air at Room Temperature (RT).

Preliminary irradiation tests were conducted to evaluate the effects of the gamma irradiation on the microbial strains selected as representative contaminants. The microbial strains, grown on LB and YPD plates, were irradiated at different absorbed doses (2 kGy, 8 kGy and 10 kGy). All experiments were performed in triplicate. Control plates were not exposed to irradiation. Following gamma irradiation, the strains were transferred to fresh plates media and were incubated for two weeks at 28 °C, with daily monitoring to assess survival.

The effective dose for microbial inhibition was qualitatively defined as the lowest tested dose resulting in no visible microbial growth on the plates during the incubation period.

Based on the microbiological analysis, the documents were irradiated up to 8 kGy absorbed dose in two steps of 4 kGy. After the first irradiation step at an absorbed dose of 4 kGy, all the documents were characterized by non-invasive spectroscopic techniques. The samples were then further irradiated up to 8 kGy and analyzed again. This fractional approach was specifically designed to allow for intermediate non-invasive spectroscopic characterization.

To minimize the time interval between the two irradiation stages and prevent potential post-irradiation recovery or envi-

ronmental interference, a high-throughput analytical protocol was adopted. After the first 4 kGy step, the protective paper covers were removed, and the samples were immediately analyzed by multiple operators working simultaneously on different instruments.

The entire intermediate characterization phase was completed within approximately one hour. Following the measurements, the documents were promptly repackaged in their protective layers and returned to the irradiation cell for the final 4 kGy step. Given the short duration of this interval and the controlled laboratory conditions (RT), it is remarkably improbable that this interval was sufficient to trigger significant physico-chemical changes or microbiological regrowth.

2.4. Characterization techniques

All documents were characterized before and after each irradiation step using non-invasive and non-destructive techniques, applied to selected areas for each document. A Horiba XploRA Plus micro-Raman spectrometer was employed to acquire optical microscope images and Raman spectra by using 785 nm laser excitation, a 50 mW laser power and a diffraction grating of 1200 gr/mm. FTIR/ATR spectra in the range 4000–700 cm^{-1} were recorded using a Spectrum 100 Perkin-Elmer FT-IR spectrometer. By following the guidance of CIE 2000 system of colorimetry [36], a PCE-CSM 8 colorimeter was employed with 10 repeated measurements averaged per representative area to account for substrate heterogeneity; color differences were evaluated via the ΔE_{00}, assuming a perceptibility threshold of 1.5. Further details are reported in the Supporting Information section. Due to the high historical and artistic value of the documents, the instruments were configured to accommodate the documents in their entirety, ensuring that no cutting, or physical alteration were required throughout the entire analytical campaign. All measurements were conducted in air at RT. Specific points on each sample were selected and measured in triplicate before and after irradiation to ensure consistency and reliability of the results.

3. Results and discussion

3.1. Evaluation of the gamma irradiation efficacy through microbiological analysis

Microbiological samples were taken from areas of visible biodeterioration and dark areas resulting from ageing (Fig. 1). The aim of this qualitative microbial investigation was to assess resistance to gamma irradiation; it did not seek to determine microbial species at the molecular level. The microbial strains, which were grown on LB and YPD plates, were irradiated at different absorbed doses (2 kGy, 8 kGy and 10 kGy) at a dose rate of approximately 1.5 kGy/h. The effective dose for microbial inhibition was defined qualitatively as the lowest dose that resulted in no visible microbial growth on the plates during the incubation period.

Twenty-one bacterial and four fungal strains were isolated from the documents. These strains were selected based on their morphological features (*e.g.*, color of the colony, surface aspect, growth rate) and labelled with specific codes (Table S1).

Once the fungal and bacterial strains reached the appropriate level of growth, they were irradiated and subsequently re-plated onto fresh media, incubated at 28 °C and daily monitored for up to one week of growth to assess survival. The effective dose for microbial reduction was determined by evaluating growth in each experiment (Fig. 2), being qualitatively defined as the lowest dose that resulted in no visible microbial growth during the incubation period.

Fungal populations were unable to survive after the first exposure (2 kGy), indicating the high effectiveness of the gamma rays. In contrast, bacterial strains were more resistant. One strain (*Bacillus* sp.) remained viable after exposure to 10 kGy, a threshold commonly considered critical for the treatment of cultural heritage materials [26]. Some bacterial strains can be extremely resistant to gamma exposure [37], as they possess strong oxidative stress-resistance mechanisms that protect proteins from oxidative damage and highly efficient DNA repair systems that can accurately reassemble fragmented DNA. These mechanisms enable them to withstand extremely high radiation doses. Conversely, fungi lack these protective and repair mechanisms and are generally more sensitive to ionizing radiation [40]. An exception is melanized fungi [38], which can tolerate irradiation values approaching or even exceeding 1 kGy. In our case study, the analyzed fungal isolates were not melanized, which explains their higher susceptibility to gamma irradiation. Considering that 8 kGy is the dose recommended by the IAEA to treat biodeterioration on archival substrates while preserving their chemical and physical integrity, the 8 kGy dose was considered an excellent compromise between reducing almost the entire microbial load and avoiding damages to the material of the cultural artifact under examination. Based on these results, physical analyses of paper and parchments documents were carried out to ensure that the gamma ray doses active on biodeteriogens did not harm the samples, permitting effective microorganism reduction while preserving the cultural heritage.

3.2. Characterization of documents before irradiation

A preventive investigation into the molecular structure and chemical composition of the writing substrates and of selected decorated areas was carried out for the four historical documents before the irradiation. The Raman and FTIR/ATR spectra collected before gamma irradiation are reported in Fig. 3.

The Raman and FTIR/ATR spectra reported in Fig. 3a–b disclose that paper (Doc2 and Doc3) and parchment (Doc1 and Doc4) samples were mainly composed of cellulose and collagen, respectively.

As shown in Fig. 3a, the Raman spectrum of Doc1 (parchment) shows several sharp peaks attributed to calcite phases (blue asterisks) indicative of parchment degradation [39–41]. On the contrary, signals ascribable to calcite or other inorganic fillers are absent in the spectra of the paper documents, namely Doc2 and Doc3. As expected, these samples spectra display typical cellulose features [42–44]. Most notably, the prominent band around 380 cm^{-1} (yellow box) corresponds to the so-called "cellulose fingerprint line" [45–49]. Additionally, the broad signal between 1100 and 1200 cm^{-1} (magenta region) is attributed to β-glycosidic linkages (βCOC). It is worth noting that the paper materials used for Doc2 and Doc3 do not contain detectable amounts of lignin, as determined by the sensitivity of the micro-Raman instrument. Finally, Doc4 (parchment) exhibits a rather featureless Raman spectrum, which is consistent with the low scattering efficiency of collagen. In agreement with its more recent origin, the absence of calcite-related Raman signals in the spectrum of Doc4 indicates a lower degree of degradation for Doc4 than Doc1 parchment substrate.

The FTIR/ATR spectra in Fig. 3b provides complementary compositional information. Diagnostic signals of collagen or cellulose phases can be clearly identified in the spectra of parchment and paper documents, respectively. Specifically, the spectra of the parchment samples (Doc1 and Doc4) are characterized by amide I (AI) and amide II (AII) bands in the 1650–1550 cm^{-1} region, reasonably due to the proteinaceous composition of collagen [50–52]. As shown in Fig. 3b, the AI band overlaps the adsorbed water (Ads. H_2O) signal at around 1650 cm^{-1}. In addition, Doc1 also shows distinct carbonate absorption bands (blue asterisks), in agreement with the calcite signals observed in the Raman spectrum [51,53].

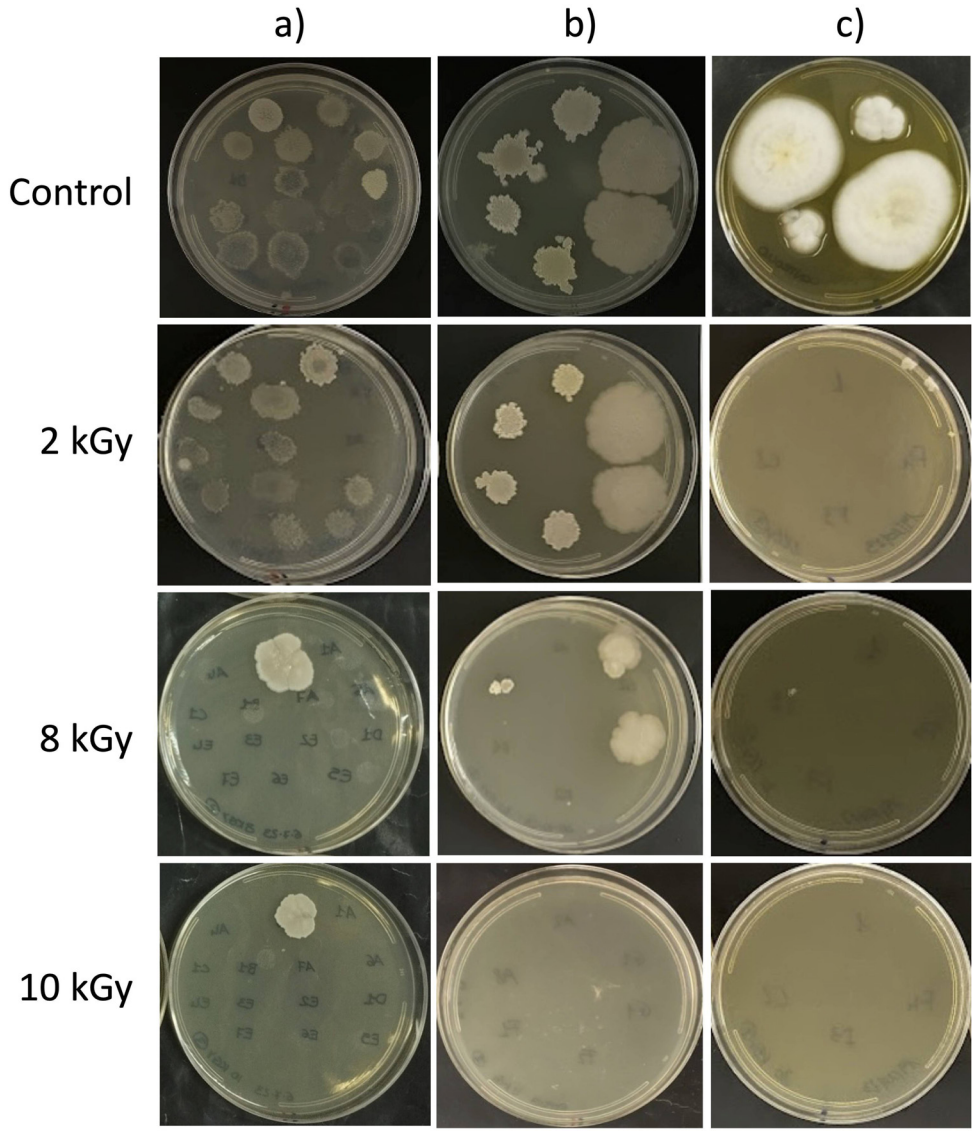


Fig. 2. Sensitivity of microorganisms isolated from Doc1, Doc2, and Doc3 of the Montecassino Library to three different gamma irradiation doses. (a–b) Bacterial strains grown on LB plates; c) fungal strains grown on YPD plates. Bacteria and fungi were isolated based on their macro- and microscopic characteristics. Each row shows the growth of the corresponding bacterial or fungal strain on the non-irradiated control plate and on plates irradiated at 2, 8, and 10 kGy.

Although calcite-related signals are not detected in the Raman spectrum of Doc4, their presence in the corresponding FTIR/ATR spectrum suggests trace amounts or a less crystalline form.

The paper documents (Doc2 and Doc3) display FTIR spectra dominated by cellulose features (gray box) [54–58]. Analogously to the Raman spectroscopy results, the FTIR/ATR spectra of Doc2 and Doc3 show no signals attributable to lignin or inorganic fillers.

Regarding the decorated areas, the spectral features of red ink in Doc2 are characteristic of cinnabar (HgS), a common historical pigment used in red inks [59,60]. For Doc4, the Raman spectrum of red ink exhibits the peaks diagnostic of red lead (Pb_3O_4) phase [61], while the broad band disclosed in the spectrum of the green ink suggests an organic nature for such a pigment. The selection of specific Raman intensity ratios was based on their diagnostic value. For inorganic pigments, we analyzed the ratios between primary lattice-mode peaks, that are sensitive to crystal lattice symmetry (specifically the ~250/~340 cm^{-1} bands for cinnabar and the ~130/~280 cm^{-1} lattice modes for red lead). Any radiation-induced deformation or phase transition would alter their relative intensities. For organic components characterized by broad spectral features, the ratio between the fluorescence intensity at 1100 cm^{-1} and the background noise ($I_{1110}/I_{background}$) and the was monitored. The specific point at 1100 cm^{-1} (located just before the maximum fluorescence peak) was selected because it offered a more reproducible signal. As the fluorescence profile is strictly dependent on the molecular environment, its stability may indicate the absence of significant oxidative degradation or chemical modification of the organic matrix at 8 kGy.

3.3. Characterization of documents after irradiation

Micro-Raman spectroscopy was used to thoroughly investigate the radiation-induced changes in terms of morphological and structural composition of the documents. Since the Raman spectra acquired on the printed areas revealed no significant signals attributable to the ink, the analysis of Doc1 was carried out on an unprinted region to evaluate potential alterations in the molecular structure of the parchment (Fig. 4).

Fig. 4a shows the analyzed section of Doc1 before and after gamma irradiation. As seen in the microscope images (Fig. 4b), the characteristic network of interwoven collagen fibers remains unaltered after exposure. Similarly, the Raman spectra (Fig. 4c) display

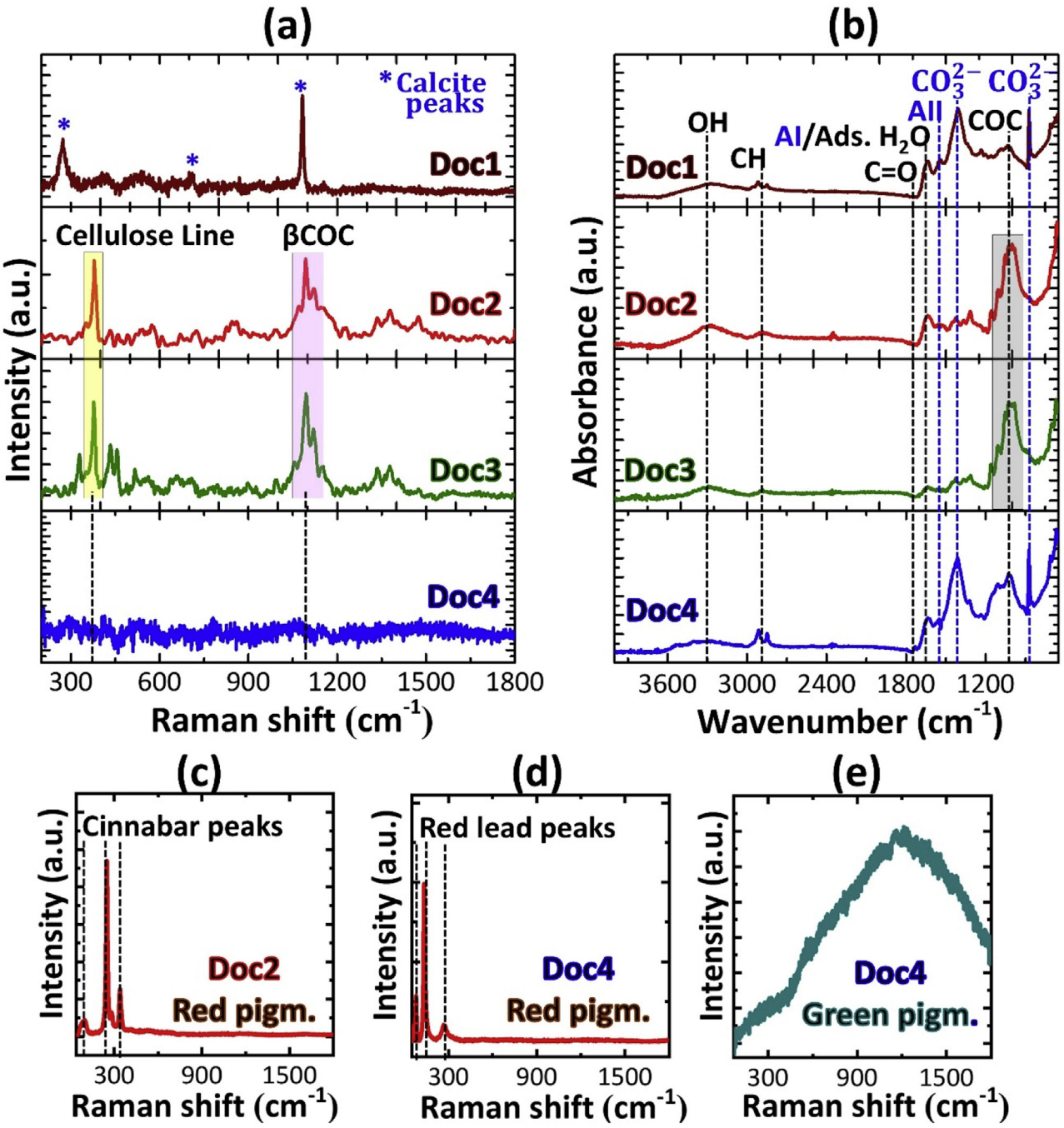


Fig. 3. Representative a) Raman and b) FTIR/ATR spectra acquired on the unprinted areas of Doc1, Doc2, Doc3 and Doc4 archival documents from the Montecassino Abbey before gamma irradiation. Representative Raman spectra for (c) red pigment of Doc2, (d) red pigment and (e) green pigment of Doc4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

persistent calcite peaks [39–41]. This stability after 8 kGy irradiation confirms that gamma treatment causes no significant changes to the parchment's micro-texture or mineral composition.

In contrast to Doc1, the printed area of Doc2 exhibited interesting Raman spectra; therefore, analysis focused on its red ink areas (Fig. 5).

Fig. 5a shows the portion of the document investigated before and after irradiation. A representative Raman spectrum collected on the "a" red letter (cinnabar [59,60]) before irradiation is presented in Fig. 5b

The I_{250}/I_{340} intensity ratio was calculated within the blue dashed square in Fig. 5a, with the resulting 2D Raman maps shown in Fig. 5c. Comparing these maps reveals no substantial spectral changes post-irradiation. When averaged over the mapped area, the I_{250}/I_{340} ratio shows no systematic variation, remaining consistent with the 0 kGy baseline at 2.3 ± 0.2 both before and after treatment. The small variations in the I_{25n}/I_{34n} distribution are likely attributable to common experimental factors, such as minor changes in the Raman microscope focus, rather than to radiation-induced chemical modifications.

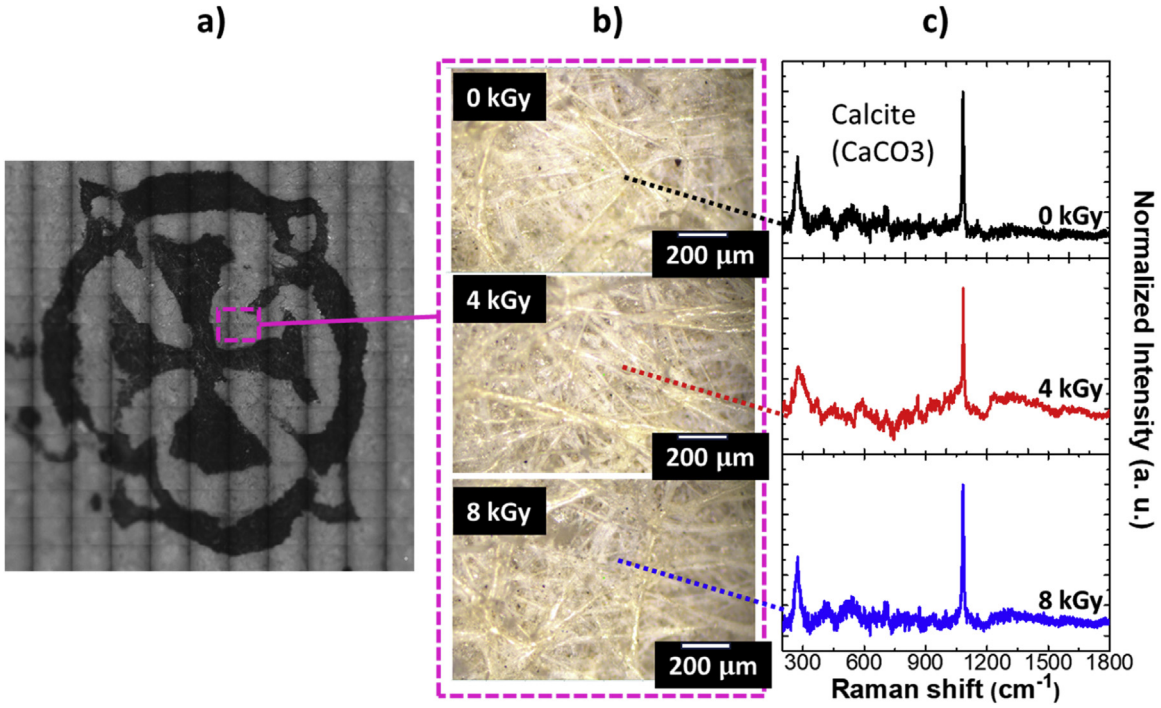


Fig. 4. a) Optical microscope image of a portion of Doc1 before irradiation and b) optical images (acquired in the area within the pink square of the picture) and c) Raman spectra of Doc1 before irradiation and after 4 and 8 kGy absorbed doses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

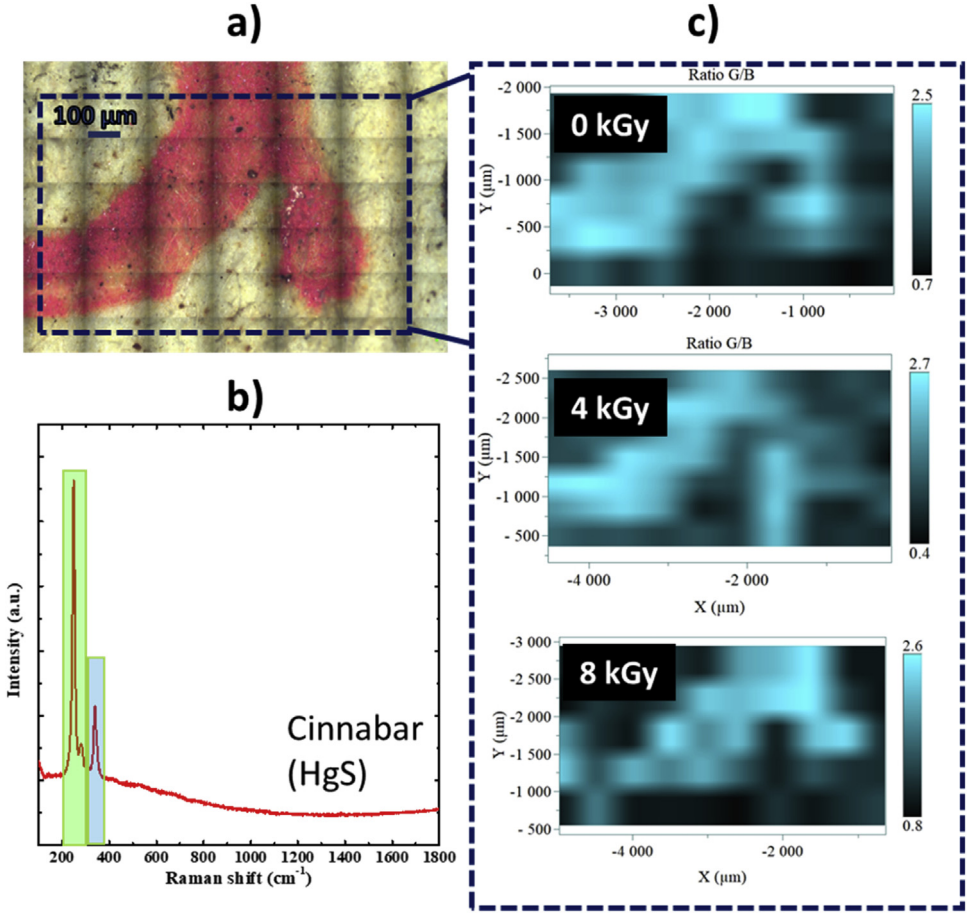


Fig. 5. a) Optical microscope image of a portion of Doc2 before irradiation, b) Raman spectrum of the red ink (cinnabar) and c) 2D Raman maps acquired in the area within the blue square of the picture a) before irradiation and after 4 and 8 kGy absorbed doses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

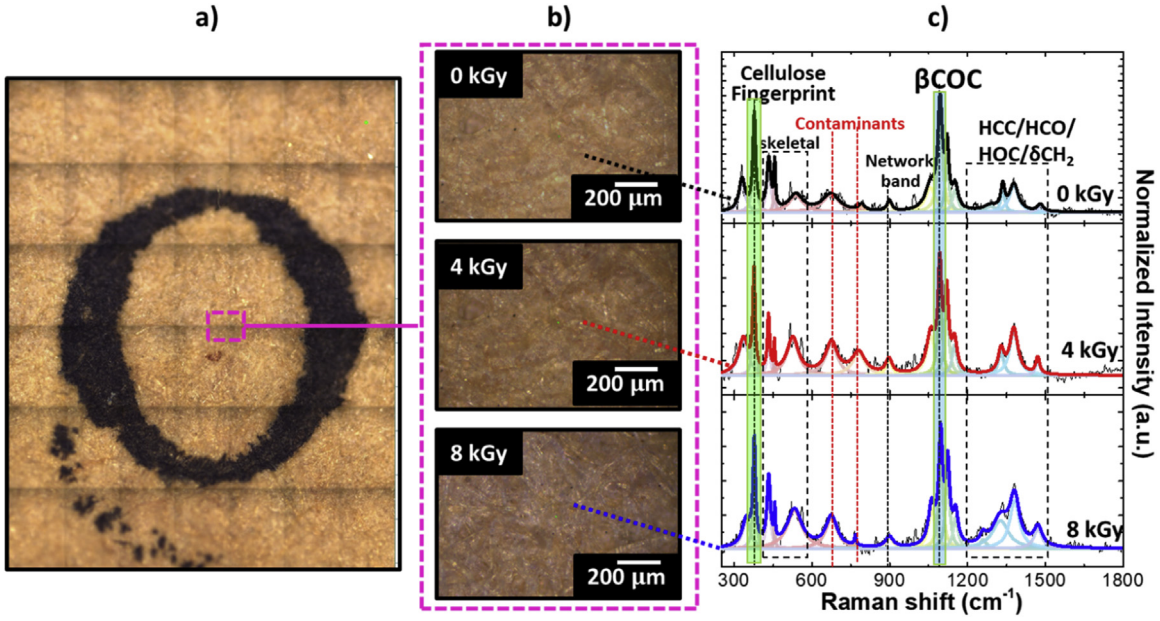


Fig. 6. a) Optical microscope image of a portion of Doc3 before irradiation and b) optical images (acquired in the area within the pink square of the picture) and c) Raman spectra of Doc3 before irradiation and after 4 and 8 kGy absorbed doses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

The preservation of this spectral pattern demonstrates that gamma radiation at the applied dose does not induce detectable alterations in the molecular structure of cinnabar ink. This confirms Raman mapping as a reliable, non-destructive technique for analyzing historical inks over extended surfaces.

Analogously to Doc1, the ink zones on Doc3 surfaces disclosed no specific Raman signals. Consequently, a blank area of Doc3 was analyzed to evaluate potential structural changes in the paper substrate (Fig. 6).

The portion of the document investigated is depicted in Fig. 6a. Microscope images (Fig. 6b) reveal a well-preserved fiber network characteristic of historical paper, which remains morphologically stable after exposure to gamma radiation. The corresponding Raman spectra (Fig. 6c) exhibit peaks attributable to cellulose (details in the Supporting Section). In agreement with other studies [45–49], the relative integrated intensity (RI) values between the cellulose fingerprint line and the βCOC band effectively evaluate the structural order of cellulose in paper matrices. For Doc3, RI values remained around 0.8 across all irradiation conditions, indicating that the treatments left the cellulose molecular structure largely unmodified.

Another interesting occurrence was found for Doc4, that was analyzed at a region where a decorative letter composed of both red and green ink appears on the parchment surface (Fig. 7).

As shown in Fig. 7a, Raman spectra before and after irradiation were acquired on specific points from the portion of the letter "O". The red ink is reasonably red lead [61], whereas the green ink contains organic pigments. The ratio I_{130}/I_{280} and $I_{1100}/I_{background}$ were used to evaluate the stability of red and green inks, respectively, within the area marked by the blue dashed square in Fig. 7a.

The resulting 2D Raman maps (Fig. 7c), generated before and after irradiation, show no significant structural changes in either pigment. This plausibly indicates the stability of both the inorganic and organic inks under the tested conditions. Consequently, Raman analysis supports the validity of controlled gamma irradiation for preserving delicate documentary heritage without significantly compromising ink integrity.

To investigate potential chemical modifications induced by gamma irradiation, the historical documents were analyzed using FTIR/ATR spectroscopy. Fig. 8 shows the representative spectra (Fig. 8b) acquired in the areas indicated by the yellow rectangles (Fig. 8a) for each document at three irradiation stages: 0 kGy (untreated), 4 kGy, and 8 kGy.

Across all samples, the spectral profiles of paper and parchment showed no significant changes after 4 or 8 kGy of gamma radiation. This confirms that irradiation under these conditions causes no detectable chemical alterations to either substrate. Further information on the paper samples' chemical composition was achieved by the indices of Total Crystallinity Index (TCI), Lateral Order Index (LOI), and Hydrogen Bond Intensity (HBI) [62–64]. The TCI reflects the overall molecular order of the material. The LOI is acknowledged as an empirical Crystallinity Index, while HBI reflects the degree of intermolecular regularity and the amount of bound water in the material. The indices were calculated using the ratios:

- TCI = A_{1376}/A_{2902}
- LOI = A_{1437}/A_{900}
- HBI = A_{3336}/A_{1336}

TCI, LOI and HBI as a function of the absorbed dose are shown in Fig. 9 for Doc2 and Doc3 samples.

Fig. 9 depicts that the indices show only limited changes in the cellulose supramolecular organization and hydrogen-bonding network. Specifically, as shown in Fig. 9a, the TCI values decrease lower than 20% in both samples after irradiation at 8 kGy, which may indicate a slight variation in the cellulose molecular organization. Since TCI is an indirect FTIR-based parameter, this variation does not provide direct evidence of changes in cellulose crystallinity. Considering the advanced natural aging of the historical documents, the observed decrease suggests that gamma irradiation induced only limited modifications in the cellulose structure, in line with the Raman findings, which also indicate the absence of significant molecular alterations after irradiation.

Conversely, the LOI values (Fig. 9b) remain relatively stable across all samples and doses, suggesting that the lateral order of the molecular network is not significantly disrupted by gamma irradiation, even at the highest investigated dose of 8 kGy.

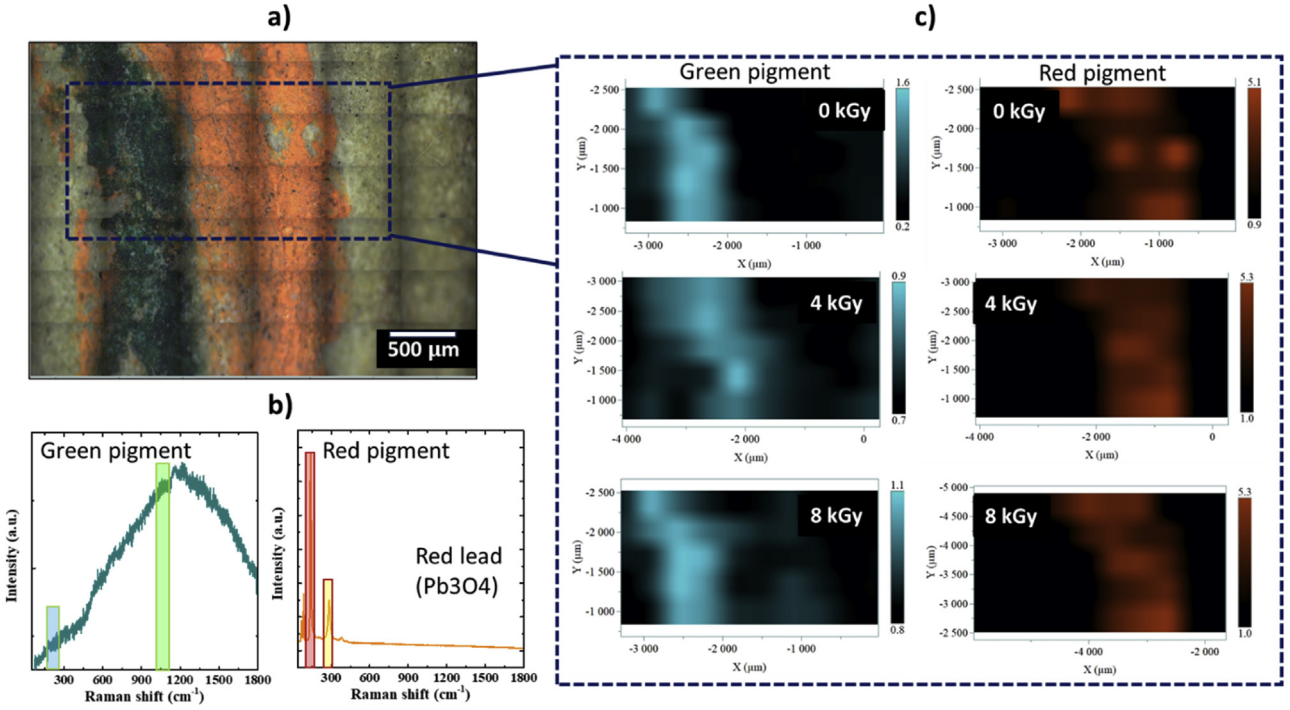


Fig. 7. a) Optical microscope image of a portion of Doc4 before irradiation, b) Raman spectrum of the green and red pigments and c) 2D Raman maps of the green and red pigments acquired in the area within the blue square of the picture a) before irradiation and after 4 and 8 kGy absorbed doses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

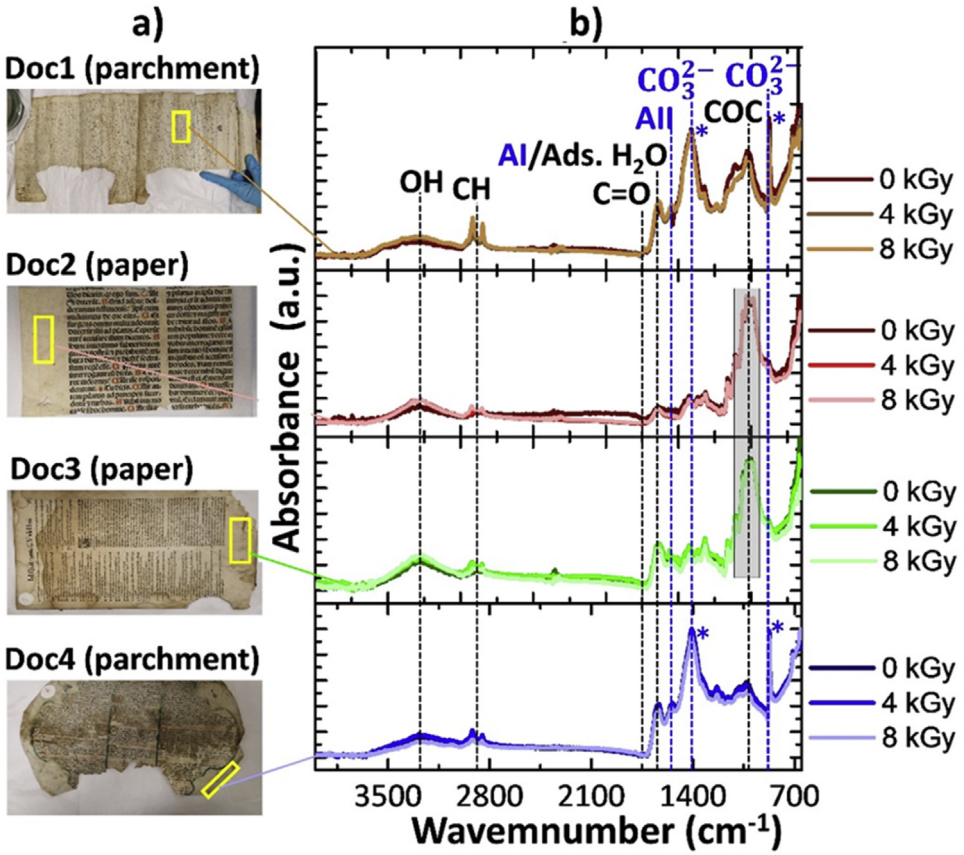


Fig. 8. a) Pictures of the four historical samples analyzed: Doc1 and Doc4 (parchment), Doc2 and Doc3 (paper). The yellow rectangles indicate the specific regions where FTIR/ATR measurements were performed; b) corresponding FTIR spectra before and after gamma irradiation at increasing absorbed doses (0, 4, and 8 kGy). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

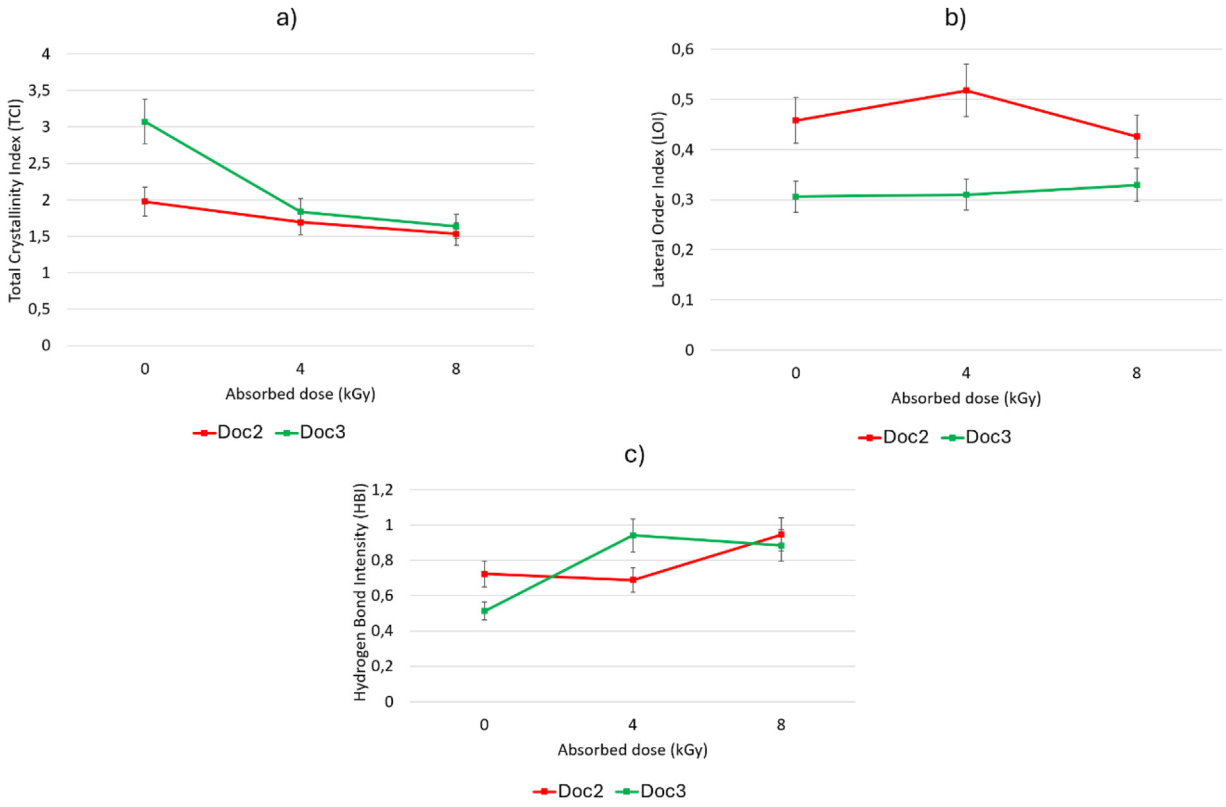


Fig. 9. Behavior of TCI, LOI and HBI as a function of the absorbed dose for Doc2, and Doc3 samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

More pronounced variations are observed in the HBI index (Fig. 9c), which gradually increases with rising radiation dose. This behavior may reflect changes in the hydrogen bonding networks and local cellulose organization induced by gamma irradiation. Overall, the results suggest localized modifications of the cellulose structure rather than extensive degradation.

Further information on the effects produced by gamma irradiation on the chemical composition of parchment samples (Doc1 and Doc4) are achieved by analyzing AI and AII bands, associated with C=O stretching and N–H bending/C–N stretching, respectively. In particular, the ratio between AI and AII signals intensities (I_{AI}/I_{AII}) provides insights into the hydrolysis degree of the polypeptide chains. Additionally, the position difference between the amide I and amide II bands, $\Delta\boldsymbol{\nu}$, is related to collagen denaturation [65]. The results summarized in Table S2 show that the I_{AI}/I_{AII} ratio and $\Delta\boldsymbol{\nu}$ values remain relatively stable across the different radiation doses, suggesting that the irradiation process did not significantly alter the hydrolysis or denaturation of the collagen structure in the parchment samples.

In agreement with FTIR spectroscopy results, the structural integrity of the collagen-based materials is further corroborated by UV–Vis spectroscopic analysis [66]. Details are reported in the Supporting Section.

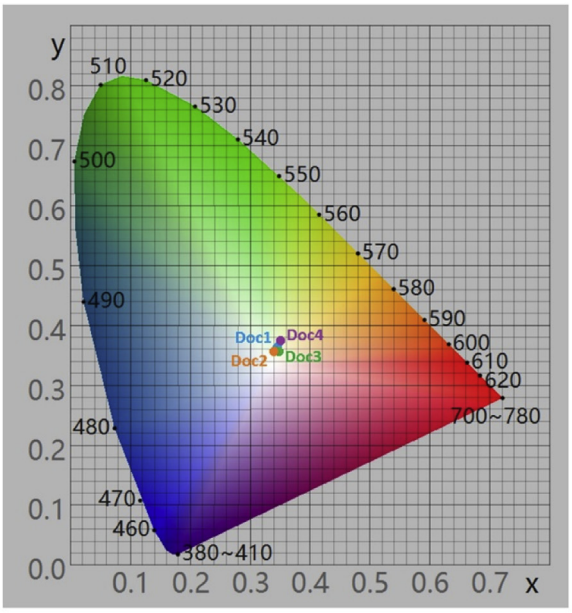
The combination of the results obtained from FTIR, Raman, and UV–Vis spectroscopic analyses provides consistent evidence that gamma irradiation up to 8 kGy does not induce substantial chemical or molecular structure alterations in the Montecassino documents. Despite a slight lowering in TCI parameter after irradiation, both the cellulose- and collagen-based substrates (paper and parchment, respectively) preserved their structural and chemical composition integrity. Moreover, the Raman analysis confirmed that the molecular signatures of the organic and inorganic inks remained unmodified by the irradiation, with no detectable degradation of key modes associated with their pigments.

The final step is to estimate the potential aesthetic impact of irradiation. In the context of radiation-induced secondary effects, color modification, which reflects the materials' oxidation state, is a notable concern for operators working with CH materials [27,67]. Colorimetric analyses were performed on unprinted areas of the documents before and after irradiation. The ΔE_{00} values quantified color differences, while sample colors were mapped in the CIE XYZ chromaticity diagram, as shown in Fig. 10.

The $\Delta E_{00}^{white\ ref}$ values for the samples before irradiation (0 kGy) relative to the white reference standard are 14.9, 14.4, 13.8 and 19 for Doc1, Doc2, Doc3 and Doc4, respectively, disclosing that the coloration of all samples before irradiation is significantly distant from the white reference. On the other hand, the irradiation process produced no color variation for each typology of writing substrate. As detailed in Fig. 10b, the ΔE_{00} values, representing the color difference of each irradiated sample with respect to its not irradiated (0 kGy) sample, remain below 1.5, confirming that any color change induced by gamma exposure is visually imperceptible, even at the highest applied dose [68–70].

Given the overall considerations, the combination between spectroscopic (FTIR, Raman, UV–Vis), microscopic, colorimetric, and microbiological results consistently demonstrate that gamma irradiation up to 8 kGy is valid for the decontamination of highly fragile cultural heritage items such as archival documents. The absence of significant alterations in the molecular structure of both the writing supports and inks, along with the remarkable reduction of biodeteriogenic agents, confirms the potential of ionizing radiation as a non-destructive and reliable tool for treating cultural materials without significantly modifying chemical and physical properties.

(a)



(b)

Sample	ΔE_{00}	
	4 kGy	8 kGy
Doc1	0.514	0.522
Doc2	0.861	0.790
Doc3	0.524	0.940
Doc4	0.554	0.738

Fig. 10. (a) CIE XYZ color space chromaticity diagram for Doc1 (light blue), Doc2 (orange), Doc3 (green) and Doc4 (purple) samples. The points correspond to the color coordinates of the samples before and after irradiation under various conditions. **(b)** ΔE_{00} values after irradiation at 4 kGy and 8 kGy, with respect to the relative not irradiated sample, for Doc1, Doc2, Doc3, Doc4. Error: 1%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

4. Conclusions

This study examined the effects of gamma irradiation on historical archival materials from the Abbey of Montecassino, with a focus on parchment, paper documents and inks. The microbiological results clearly demonstrate that gamma irradiation is highly effective in remarkably reducing microbial contamination at 8 kGy while the multidisciplinary approach, combining spectroscopic and colorimetric techniques, highlighted the stability of the materials after exposure to gamma doses of up to 8 kGy. Indeed, no significant structural or chemical degradation was observed in writing materials or inks. Both FTIR/ATR and Raman spectroscopies confirmed the preservation of the structural and chemical features of cellulose and collagen, as well as the integrity of the organic and inorganic ink constituents. Furthermore, UV–Vis spectra supported the stability of chromophoric groups in parchment samples, and minor variations in crystallinity indices were consistent with non-destructive effects.

Analogously, colorimetric analyses showed that chromatic changes remained below the threshold of visual perception, disclosing no major detectable alterations of chemical-physical properties and aesthetic appearance of the documents.

Therefore, up to a dose of 8 kGy, gamma irradiation represents a non-invasive, and chemically compatible approach for the conservation of fragile archival materials affected by microbiological contamination. Furthermore, the applicability of this method could potentially be extended to a broader range of organic cultural heritage materials. This represents a perspective for future research, offering a scalable strategy that may assist institutions in the preservation of biodeteriorated or at-risk artefacts. To strengthen the validation of this method, future studies may integrate non-invasive and molecular approaches to assess microbial persistence directly on treated documents.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used ChatGPT to adjust the text. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.culher.2026.07.013.

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