Cornelius Palmbach*, Sebastian Dobrusskin, Mathieu Jacot-Guillarmod, Olivia Raymann, Andreas Buder, Hans Hartmann, Guido Voser, Barbara Mordasini Voser, Gianclaudio Mordasini and Caroline Amberg

Disinfection of Mould-contaminated Archival Material by X-ray Irradiation – New Research Results on the Effect on Moulds and Cellulose

Desinfektion von mit Schimmelpilz kontaminiertem Archivgut mittels Röntgenbestrahlung – Neue Forschungsergebnisse zur Wirkung auf Schimmelpilze und Cellulose

Désinfection de fonds d'archives à l'aide de rayons X : résultat des études menées sur leur effet sur les moisissures et la cellulose

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***Corresponding author: Cornelius Palmbach**, Bern University of Applied Sciences – Bern Academy of the Arts, Fellerstrasse 11, 3027 Bern, Switzerland, E-mail: cornelius.palmbach@hkb.bfh.ch. https://orcid.org/0000-0002-8946-2310
Sebastian Dobrusskin, Mathieu Jacot-Guillarmod, Olivia Raymann and Andreas Buder, Bern University of Applied Sciences – Bern Academy of the Arts, Fellerstrasse 11, 3027 Bern, Switzerland, E-mail: sebastian.dobrusskin@hkb.bfh.ch (S. Dobrusskin), mathieu.jacot-guillarmod@hkb.bfh.ch (M. Jacot-Guillarmod), olivia@buchrestaurierung.ch (O. Raymann), andreas.buder@hkb.bfh.ch (A. Buder)
Hans Hartmann, STERIS Corporation – Synergy Health Däniken AG, Däniken, Switzerland, E-mail: hans_hartmann@steris.com
Guido Voser, Barbara Mordasini Voser and Gianclaudio Mordasini, docusave AG, Uetendorf, Bern, Switzerland, E-mail: guido.voser@docusave.ch (G. Voser), barbara.mordasinivoser@docusave.ch (B. Mordasini Voser), gianclaudio.mordasini@docusave.ch (G. Mordasini)
Caroline Amberg, Swissatest Testmaterialien AG, St Gallen, Switzerland, E-mail: caroline.amberg@swissatest.ch

Abstract: This research project examines the effects of X-rays on cellulose and some moulds frequently found on paper. The aim was to identify applications for X-ray irradiation that can be used as a disinfection method for archival material. The question was if X-rays are suitable as an alternative to gamma radiation and if they are less harmful. For this purpose, the minimum X-ray dose required to reduce the microbial count to a harmless level was determined. The material-altering effect was examined on samples treated with X-rays and gamma radiation. Spectrophotometric measurements showed that there is no noticeable colour change with either type of radiation. The determination of the molecular weight distribution, in turn, showed that the molar mass of the cellulose is considerably reduced with both treatment methods. Using mechanical tests, however, it could be demonstrated that this has no significant influence on the tensile strength. The examination of the oxidation behaviour also showed no significant difference between the differently treated samples. The studies demonstrated that both methods have an almost identical effect on cellulose. Thus, X-ray treatment is primarily a supplement to the known disinfection methods and is particularly suitable for objects that would not withstand treatment with alcohol.

Keywords: moulds, disinfection, X-rays, gamma radiation, cellulose degradation

Zusammenfassung: In diesem Forschungsprojekt wurden die Auswirkungen von Röntgenstrahlen auf Cellulose und einige häufig auf Papier vorkommende Schimmelpilze untersucht. Ziel der Studie war es, Anwendungen für die Röntgenbestrahlung als Desinfektionsmethode für Archivgut zu identifizieren. Dabei sollte geklärt werden, ob Röntgenstrahlen als Alternative zur Gammastrahlung geeignet, bzw. ob sie weniger schädlich sind. Dazu wurde die minimale Röntgendosis ermittelt, die erforderlich ist, um die Keimzahl auf ein unbedenkliches Maß zu reduzieren. An den mit Röntgen- und Gammastrahlen behandelten Proben wurde die materialverändernde Wirkung untersucht. Spektrophotometrische Messungen zeigten, dass bei beiden Strahlungsarten keine nennenswerte Farbveränderung auftritt. Allerdings wird die molare Masse der Cellulose durch beide Behandlungsmethoden deutlich reduziert. Mechanische Tests ergaben jedoch, dass dies keinen signifikanten Einfluss auf die Zugfestigkeit der Papierproben hat. Auch im Oxidationsverhalten gab es keinen signifikanten Unterschied zwischen den verschieden behandelten Proben. Die Untersuchungen zeigen also, dass beide Methoden eine nahezu identische Wirkung auf die Cellulose haben. Die Röntgenbehandlung ist in erster Linie eine Ergänzung zu den bekannten Desinfektionsmethoden und eignet sich besonders für Objekte, die für eine Behandlung mit Alkohol nicht geeignet sind.

Schlüsselworte: Schimmel, Desinfektion, Röntgenstrahlung, Gammastrahlung, Celluloseabbau

Résumé: ce projet de recherche a étudié l'effet de rayons X durs sur la cellulose et sur les spores de moisissures fréquents sur le papier, l'objectif étant de déterminer le potentiel des rayons X pour désinfecter des fonds d'archives infestés par des moisissures. Il s'agissait principalement d'analyser dans quelle mesure les rayons X constituent une solution de rechange aux rayons gamma, notamment en termes de préservation des matériaux. L'étude a ainsi défini le dosage minimal de rayons X pour réduire le nombre des germes à un niveau inoffensif. L'effet de transformation des matériaux soumis à ce dosage minimal a ensuite été examiné à l'aide d'échantillons de cellulose de coton exposés à des rayons X et gamma. La spectrométrie a permis de constater que les deux types de rayons ne provoquent pas de changements de couleur perceptibles à l'œil nu. La distribution de la masse moléculaire a montré pour sa part que les deux méthodes de traitement réduisent considérablement le poids moyen de la masse des molécules de cellulose. Des essais subséquents ont toutefois permis de prouver que cette réduction n'a pas d'impact sur la résistance à la traction. Par ailleurs, les essais réalisés pour étudier le comportement à l'oxydation permettent de conclure que les propriétés des deux échantillons ne présentent pas de différences significatives. L'effet des deux méthodes sur la cellulose est pratiquement identique. Le traitement aux rayons X est ainsi à prendre au sens d'une méthode de désinfection complémentaire par rapport aux méthodes éprouvées, notamment pour les objets qui ne résisteraient pas à un traitement à l'alcool.

Mots clés: moisissures, désinfection, rayons X, rayons gamma, dégradation de la cellulose

1 Introduction

Damage caused by mould to cellulose/paper-containing archival material and cultural property is a widespread problem for conservators. However, disinfection methods used to date often have serious disadvantages. For example, dry cleaning followed by treatment with 70% (w/w) ethanol or isopropanol in water is very time-consuming and expensive. Moreover, the typical short exposure time to alcohol is not sufficient to kill the spores but merely results in decontamination and reduction of the microbiological load to a level that is harmless to health (Meier and Petersen 2006, 144). Ethylene oxide fumigation instead is considered a health hazard and no longer allowed in some countries, as leaking of the carcinogenic gas can even occur several weeks after treatment (Arndt et al. 2010, 10). In addition, this method had

many negative effects on materials associated with the paper (Meier and Petersen 2006, 127f). Oxygen deprivation treatment indeed is less risky, but it is inefficient and only suitable for reducing the growth rate of existing moulds but does not destroy their spores (Meier and Petersen 2006, 142f). Gamma irradiation, on the other hand, is considered to be damaging because increasing doses lead to increased depolymerisation of the cellulose molecules and yellowing of the paper (Adamo et al. 1998, 55ff). Already in the 1970s, it was shown that gamma irradiation can have a fatal effect on the internal structure of paper with a high proportion of wood cellulose (Flores 1976, 27). In a later study, the long-term effect of gamma irradiation was examined. It was found that irradiation resulted in a reduction of the mechanical strength of the papers tested, similar to the effect of accelerated dry ageing. If the papers were both irradiated and accelerated aged, an even greater decrease in folding endurance and tear resistance was measured (Butterfield 1987, 181). The main cause of depolymerisation of the cellulose molecules is considered to be the formation of free radicals during irradiation. When examining the effects on cotton-cellulose, "a decrease in the degree of polymerisation from 1200 to 330 was measured after irradiation with a dose of 15 kGy" (Takács et al. 1999). It has also been shown that gamma irradiation – even if the cellulose remains largely intact – can have an effect on other ingredients of the paper, such as sizes, fillers, brighteners, blueing agents, and sodium chloride, which can lead to a change in colour (Adamo, Magaudda, and Omarini 2007, 41, 43; Bicchieri et al. 2016, 24). Colour changes have also been observed for several historical pigments. For all the pigments examined, however, these disappeared after about one month, except for marble dust, whose colour change could still be observed after three months (Negut, Bercu, and Duliu 2012).

While sterilisation using gamma rays, i.e., the complete elimination of all microorganisms on a surface or in the air, has been used for decades for other materials such as grains (Cornwell, Bull, and Pendlebury 1966), the use of X-rays with high photon energies is a newer technology. In the medical field, sterilisation of medical devices by X-rays with a photon energy of up to 7.5 MeV is already regularly used (Grégoire et al. 2003; Malinowski 2021), but for the disinfection of archival materials, it has only been used to a limited extent so far. In contrast to sterilisation, the aim of disinfection is not the complete elimination of all microorganisms, but the reduction of the microbial contamination to a harmless level. Studies on the side effects of X-ray irradiation as a disinfection method for cellulosic materials have not been published yet. However, there have been studies on the effects on paper when X-rays are used for analytical purposes. For XRF analyses it has been shown that "a typical measurement cycle with conventional tubes and energy dispersive systems does not cause visible harm" to office paper and industrial cotton. But wavelength dispersive XRF spectroscopy under the conditions required for this type of analysis for detecting light elements "can leave visible traces of permanent yellowing,

brittleness and even mechanical decomposition" on the paper and its binder (Mantler and Klikovits 2004, 16). Furthermore, in a study on the short- and long-term effects of X-ray synchrotron radiation with energies in the keV range on pure cotton paper, it was demonstrated that even in the dose range below 4 kGy, chain scission of the cellulose molecules, accelerated by a very low moisture content, and yellowing can occur (Gimat et al. 2020, 2804f). In a follow-up study, also the effect on artificially aged and historical archive papers containing additives was examined. The results show, among others, that depolymerisation in the aged papers was lower than in the unaged fully cellulosic papers and that there was no yellowing in the archive papers detectable. Furthermore, it was observed that "the papers with iron gallate ink showed limited degradation in the low doses range, most probably due to recombination of the free radicals produced". The presence of gelatine size and fillers ($CaCO_3$) also seems to have a positive effect, as there was slightly less depolymerisation in the samples containing these components (Gimat et al. 2022, 4347, 4362).

In the present study it was investigated if and under what conditions high-energy X-rays are suitable for the disinfection of archival materials contaminated with moulds and whether they might even have lower effects on cellulose than gamma rays. For this purpose, the irradiation dose was reduced to a minimum and both its effect on the mould (microbiological study) and on the cellulose (degradation study) were examined.

2 Material and Irradiation Set-up

2.1 Sample Material

To investigate the effects of X-rays and gamma radiation on the cellulose molecules, Whatman® paper no. 1 was used as sample material (Appendix). Due to its high purity and the standardised composition of 98% cotton cellulose (no lignin and no hemicelluloses), this paper was also used in many earlier studies on the effects of X-rays (Gimat et al. 2020, 2022) and gamma radiation (Adamo et al. 1998; Dupont and Mortha 2004; Jerosch, Lavédrine, and Cherton 2002; Magaudda 2004; Sequeira et al. 2017) on cellulosic materials. So, a comparison of the respective results is possible. In addition, samples of historical rag paper were also irradiated as an example of a material from conservation practice (Appendix). However, these were only examined with one of the analytical methods applied in the degradation study.

Since half of the germ carriers of the microbiological study contaminated with the spores of the tested moulds were to be irradiated in a frozen state, they were packed in polystyrene boxes filled with dry ice. To ensure uniform conditions during irradiation for all samples of both studies, also the non-frozen germ carriers of the

microbiological study and all the samples of the degradation study, which additionally were packed in sealable PE bags, were placed in identical boxes. During the irradiation process and afterwards until the analysis, the samples of the degradation study remained in these boxes. This way they were protected from any light that could affect the degradation of the cellulose. After irradiation and until analysis, the samples were stored under stable climatic conditions (ca. 22 °C and 55% RH).

2.2 X-rays and Gamma Radiation

Both X-rays and gamma radiation consist of high-energy photons. Depending on the type of radiation, however, these have different sources. The gamma rays used in industrial applications are emitted by nuclides artificially produced in a nuclear reactor. When these nuclides change from a higher-energy state to a lower-energy state, such as in the decay of cobalt-60 to nickel-60, gamma radiation is emitted. X-rays, on the other hand, can be generated in several ways. The X-rays used in the present study are produced by an electrically operated electron accelerator when strongly accelerated electrons collide with a tantalum plate in a high vacuum. In this process, the electrons are deflected or decelerated, releasing part of their energy in the form of X-rays (Bremsstrahlung).

Two parameters primarily play a role in the interaction of radiation with paper: the energy of the photons, which is generally speaking the spectrum of energies of the radiation beam, and the dose of the radiation, i.e., the total energy that can interact with the material. In addition, it is important to note that the spatial distribution of the dose (dose distribution) and the temporal distribution of the dose, i.e., the dose absorbed within a certain time (dose rate) are also decisive for the effect of the irradiation. Although the source of X-rays and gamma rays is different, theoretically they should have a similar effect on the irradiated material at the same energy and under the same experimental conditions. However, due to their origin, their energy spectra differ. Since gamma rays are produced by the alteration of atomic nuclei, which always involves the same predetermined reaction, they only have a discrete number of energy levels, while X-ray sources produce broader, continuous energy spectra. The photon energy of the gamma radiation produced by the unit used in the present study is 1.17 MeV and 1.33 MeV and that of the X-ray unit is 7 MeV (Appendix).

2.3 Doses

The samples of the degradation study were treated with seven different doses of X-rays and gamma radiation, while the germ carriers of the microbiological study were only treated with X-rays. The dose range studied was from 2 to 28 kGy (Table 1). The choice of the seven doses was based, on the one hand, on the doses used in earlier studies

Table 1: Doses recorded directly on the samples (min. and max. values) and average doses calculated.

Dose no.	X-rays (kGy)		Gamma radiation (kGy)	
	Recorded	Average	Recorded	Average
1	1.86–2.16	2.01	1.95–2.10	2.03
2	2.84–3.01	2.93	3.36–3.52	3.44
3	5.58–5.91	5.75	6.56–6.98	6.77
4	8.37–8.85	8.61	9.94–10.50	10.22
5	11.10–11.90	11.5	13.20–13.90	13.55
6	16.70–17.80	17.25	16.60–17.50	17.05
7	25.40–27.70	26.55	27.30–28.30	27.80

using gamma radiation for disinfection (Adamo et al. 1998, 43; Adamo, Magaudda, and Tata 2004, 166–67; Khan, Ahmad, and Kronfli 2006, 2303; Moise et al. 2012, 1046; Otero et al. 2009, 490) and, on the other hand, on the increment dose of the irradiation units used (Appendix). Since the increments of the X-ray and the gamma units differ slightly, the samples could not be treated with the same doses of X-ray and gamma radiation. During irradiation, two dosimeters (Appendix) were used in each of the boxes to record the dose applied to the material, one for the minimum dose and one for the maximum dose. This is necessary because the target dose set can deviate slightly from the dose actually absorbed by the samples due to the absorption of the polystyrene boxes and the increasing distance to the radiation source.

2.4 Irradiation Process

During irradiation in the X-ray plant, the material to be treated is transported past the fan-shaped exit opening of the radiation source on pallets using a roller conveyor. For each of the seven irradiation doses a separate pallet was used, on which the corresponding polystyrene box containing the samples was positioned. To ensure the most homogeneous irradiation possible, particularly with large and bulky material, the irradiation is done in several steps. In the first pass, only the upper half of the material to be disinfected is irradiated – first from one side and in the second pass, after turning the pallet by 180°, also from the opposite side. Then the pallet is lifted. In the third pass, the lower half is irradiated – again, first from one side and in the fourth pass, after another turning, finally also from the opposite side. The more often a pallet passes the radiation source, the higher the dose. The dose is thus determined only by the number of passes. The dose per pass is constant and is ca. 2.85 kGy for the X-ray unit and ca. 3.45 kGy for the gamma unit. These values correspond to the maximum dose per pass. For irradiation with the smallest of the doses applied, which

is less than a dose increment, the corresponding pallet has only completed half a pass. Also for gamma irradiation the material to be treated is placed on pallets, but here the pallets are placed on top of each other in a cabinet, which is transported on a conveyor belt through the irradiation chamber past the spherically emitting gamma radiation source.

During treatment in the X-ray plant, the average RH was 50.8% and the temperature ranged from 24.9 to 26.4 °C. In the gamma plant, the temperature was between 32.8 and 35.0 °C and the average RH was 34.2%. During gamma irradiation, the maximum temperature inside the boxes with the samples of the degradation study was also measured. The higher the dose, the higher the temperature. At the lowest dose (2.03 kGy) the temperature rose to 32.5 °C and at the highest dose (27.80 kGy) up to 45 °C.

3 Microbiological Study

The disinfecting effect of X-rays and gamma radiation is mainly the result of the irreversible damage it causes to the genetic material (DNA) of microorganisms. Due to the damage to the genetic code, the cells are hindered in their normal function so that they can no longer reproduce. But irradiation also damages other cell components, such as proteins. With increasing doses, however, not only the disinfecting but also the material-altering effect of the radiation on the substrate increases (Mantler and Klikovits 2004, 16; Gimat et al. 2020, 2804; Gimat et al. 2022, 4354, 4357). Therefore, when disinfecting paper, it is important not to use higher doses than necessary, as these would also damage the cellulose unnecessarily. The minimum dose of X-ray radiation at which enough spores are still eliminated was determined in a microbiological study. Only the disinfecting effect of X-rays was examined, as the effect of gamma radiation had already been the subject of earlier studies (Adamo et al. 2001, 124; Adamo et al. 2003, 147; Magaudda 2004, 116; Moise et al. 2012, 1047). These show that the microbial population decreases proportionally to the dose.

If the intended reduction of the microbial count (number of growing spores) can be achieved with a certain dose, the living mycelium of the moulds is also destroyed, as it is more sensitive to the radiation than the spores, which means the mould can no longer degrade the cellulose. Therefore, the present study was not carried out on the mycelia, but on the more resistant spores.

The aim was to reduce the microbial count by 3 $\log_{10}$ levels, which corresponds to a 99.9% reduction. This value was defined as sufficient, as it corresponds to a tolerable level of viable spores, but not to complete sterilisation. Finally, mould spores are ubiquitous, i.e., they are not only present on all surfaces – even cleaned ones, albeit in small numbers – but also in the air. The effect of sterilisation would therefore

be very temporary, as the sterilised materials would at once become contaminated again with individual spores, even if they were stored properly afterwards.

3.1 Selection of Moulds

The effect of X-rays was studied on five representative moulds frequently found on archival materials. Typical paper-degrading species were considered as well as species that pose a potential health risk, either due to the allergenic effect of their spores or due to their toxic metabolic products (Table 2). The selection is based on information in literature (Meier and Petersen 2006, 6).

Cladosporium sphaerospermum (IMI 170353) is a mould that is very commonly found indoors, both on surfaces and in the air.

Stachybotrys chartarum (DSM 2144, corresponds with *Stachybotrys atra*) is a species that grows mainly on cellulosic materials and requires elevated levels of moisture, which is why it is often found after incidents of fire when water was used to extinguish the flames.

Eurotium amstelodami (DSM 62629, main fruiting form of *Aspergillus glaucus*) is a quite common outdoor mould that can survive in dry locations and is often found in churches and castles or on other cultural property.

Chaetomium globosum (ATCC 6205) is a species that requires high levels of moisture and is commonly found indoors and in archives.

Trichoderma virens (IAM 5061) is a mould that produces cellulose-degrading enzymes, which are partly used in paper industry. It is also often found indoors and in archives.

Table 2: Properties of the tested moulds.

Species	Health risk*	Cellulose-degrading**
Cladosporium sphaerospermum	–	yes
Stachybotrys chartarum	A, T	yes (depends on type)
Eurotium amstelodami	A	–
Chaetomium globosum	+, A, p	yes
Trichoderma virens	A	yes

*Classification according to: Ausschuss für Biologische Arbeitsstoffe 2016, 5 ff: A = possible allergenic effect; T = toxin production; + = detected or suspected as a pathogen in individual cases, cases of illness mostly only in patients with reduced immune response. p = pathogenic to plants. **Classification according to: Meier and Petersen 2006, 6, appendix, IV–XI.

3.2 Sample Preparation and Disinfection Process

The resistance of the five moulds to X-rays was examined under two different conditions, using dry and frozen samples. Freezing the samples with the spores while still wet was intended to simulate a frequently practised procedure in case of real damage: after a flood or extinguishing a fire with water, the mould-contaminated, still wet paper objects are deep-frozen at first to prevent or stop mould growth. The frozen objects are then vacuum freeze-dried and subjected to disinfecting radiation treatment. Afterwards, the surface is cleaned mechanically. For statistical significance, five samples per mould were used and for each of the seven doses of X-rays, both for the dry and the frozen variant. In addition to X-ray irradiation, disinfection with alcohol was performed on separate samples for comparative purposes, which corresponds to a common procedure in conservation practice (Meier 2006, 25).

To check if cross-contamination between the samples occurred during transport and irradiation, several sterile germ carriers were placed directly with the samples contaminated with spores as negative controls – two per dose, for both the dry and the frozen samples. To examine if the number of germs had changed due to external influences during transport, additional contaminated germ carriers were included as transport controls. For each mould five dry transport controls, as well as five frozen ones, were used. These germ carriers always remained with the other samples, except during irradiation and vacuum freeze-drying. Later, the transport controls also served as reference samples on which the microbial counts before X-ray irradiation were determined.

When preparing the samples with the spores, 0.2 mL of the spore suspension (spores in 0.5% malt water with 10% glycerine) of the specific mould were applied to germ carriers made of Whatman® paper no. 1 (Appendix). The aim was to achieve microbial counts of >1E + 04 CFU (colony-forming units) per germ carrier. After contamination, the carriers were divided into two batches: the carriers of the first batch were dried at room temperature and then individually packed in airtight PE bags. The carriers of the second batch, on the other hand, were packed individually in Petri dishes immediately after contamination and frozen at −20 °C.

For disinfection with alcohol, five samples per mould were wiped by hand with a microfibre cloth that had previously been sprayed with 70% (w/w) ethanol in water. For reasons of reproducibility, this procedure was repeated three times. In the case of the frozen samples, the ethanol disinfection was done only after vacuum freeze-drying.

To ensure that the samples of the second batch remained frozen until later evaluation, i.e., also during transport to the X-ray plant and during irradiation, they were stored in polystyrene boxes filled with dry ice. The dry samples of the first batch were packed in identical boxes to ensure uniform conditions during irradiation.

The dry carriers were analysed in the laboratory immediately after irradiation. The frozen ones were first vacuum freeze-dried and then brought to the laboratory for analysis at ambient temperature.

3.3 Determination of Survival Rate

For the evaluation of the germ carriers, the survival rate of the moulds after application of the different disinfection methods was determined. To ascertain the microbial counts required, the samples were rinsed in 10 mL ¼ Ringer solution for 10 min on a vortex mixer. A dilution series in ¼ Ringer solution was prepared from the resulting spore suspension. The individual dilutions were in turn plated out for cultivation on a culture medium of oat malt or potato dextrose agar and the plates were incubated at 30 °C for 3–5 days. The microbial loads were determined by counting the colonies that formed during this process. The difference between the colony counts before and after disinfection was used to estimate the reduction of viable microbial contamination.

The microbial reduction of the moulds as a function of the irradiation dose was determined from the microbial counts of the transport controls and the microbial counts after irradiation. In addition, the *D* value (decimal reduction dose value) was calculated for each species. This describes the dose required to reduce the microbial count by one decimal power (1 $\log_{10}$ level = 9/10). With the help of the *D* values, it was finally possible to determine the minimum irradiation dose that is necessary for the intended reduction of the microbial count by 3 $\log_{10}$ levels.

$$D = \frac{x_1 - x_0}{\log(N_0) - \log(N)}$$

x_1 = higher dose, x_0 = lower dose, $x_0 - x_1$ = range for the calculation of the *D* value, N_0 = arithmetic mean of the microbial counts after dose x_0 (here transport control), N = arithmetic mean of the microbial counts after dose x_1

3.4 Results of Microbiological Study

3.4.1 Resistance to X-rays

Even at dose 3 (5.75 kGy), no surviving spores could be found in any of the moulds, neither on the dry nor in the frozen samples (Figure 1). The microbial reduction was higher than the 3 $\log_{10}$ levels required. However, the resistance of the moulds varied considerably. The most resistant was *S. chartarum*, where substantial fungal growth

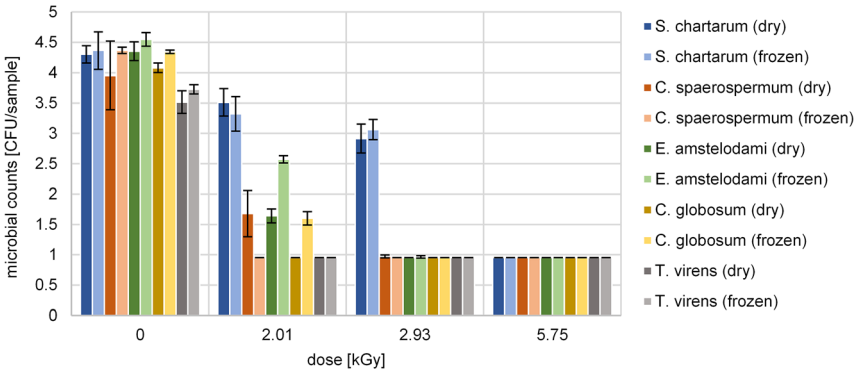

Figure 1: Reduction of the microbial counts of the five moulds tested with the first three doses of X-rays (0 kGy = transport control).

could still be observed after dose 2 (2.93 kGy), on both the dry and the frozen samples. With the two species *C. sphaerospermum* (only dry) and *E. amstelodami* (only frozen), on the other hand, it was only possible to cultivate individual moulds from one or two germ carriers after dose 2 (2.93 kGy). On the frozen samples, however, *C. sphaerospermum* was already eliminated after dose 1 (2.01 kGy). The most sensitive species was *T. virens*, for which no more moulds could be recovered after dose 1 (2.01 kGy) on both the dry and the frozen samples. This could be due, among others, to its very small (<5 µm) and therefore also very sensitive spores (Samuels 1996, 928). The spores of the most resistant species *S. chartarum*, on the other hand, are also much larger (>10 µm) (Samson 2004, 258).

3.4.2 Comparison of Microbial Reduction by X-rays on the Dry and Frozen Samples

The different treatment of the samples – air-dried, stored at room temperature and then irradiated on the one hand and wet-frozen, stored frozen and vacuum freeze-dried after irradiation on the other hand – led to different reduction rates depending on the species (Figure 1). On the frozen samples, the combined microbial reduction effect of X-ray irradiation followed by vacuum freeze-drying was registered. The microbial count of *C. sphaerospermum*, e.g., was reduced more effectively on the frozen than on the dry samples, so that dose 1 (2.01 kGy) already led to complete elimination. In the case of *S. chartarum*, on the other hand, there was no difference between the dry and the frozen samples regarding the disinfecting effect of the X-rays. It was of equal resistance in both cases. The two species *E. amstelodami* and *C. globosum*, on the other hand, were more resistant on the frozen samples.

E. amstelodami was also significantly less reduced by dose 1 (2.01 kGy) on the frozen than on the dry samples. However, this does not influence the *D* value, as this was calculated over the range "transport control – dose 2". For *T. virens*, no comparison between the dry and frozen samples is possible, as this species was already completely inactivated after dose 1 (2.01 kGy) in both cases.

3.4.3 Results of Alcohol Disinfection

The germ-reducing effect of the disinfection with ethanol is also species-dependent and varies between 0 and ca. 2.5 $\log_{10}$ levels for the dry samples. Remarkably, almost no germ reduction was observed for the three species *S. chartarum* (0.3 ± 0.3 $\log_{10}$ levels), *E. amstelodami* (0.0 ± 0.2 $\log_{10}$ levels) and *C. globosum* (-0.1 ± 0.4 $\log_{10}$ levels). *T. virens* (1.0 ± 0.8 $\log_{10}$ levels) was also only slightly affected by the treatment. Only the microbial count of *C. sphaerospermum* (2.4 ± 0.2 $\log_{10}$ levels) was significantly reduced. In the case of the frozen and vacuum freeze-dried samples, germination was even reduced by 3.3 ± 0.2 $\log_{10}$ levels.

Thus, the treatment with ethanol only achieved the intended germ reduction of 3 $\log_{10}$ levels in one of the five tested moulds, and this was only on the frozen samples, on which the effects of the alcohol and the vacuum freeze-drying add to each other. With X-ray irradiation, on the other hand, the spores of all five species were already eliminated from dose 3 (5.75 kGy) onwards, both on the dry and the frozen samples.

In the alcohol disinfection, the germ carriers were wiped with a microfibre cloth sprayed with 70% (w/w) ethanol in water, which presumably led mostly to a mechanical removal of the spores. Furthermore, the contact time of the alcohol is very short with this method.

In general, the moulds were reduced more on the frozen samples, which also passed through the vacuum freeze-drying process, than on the dry samples. E.g., *S. chartarum*, whose germ count remained almost unchanged on the dry samples, was reduced by 2.8 ± 0.7 $\log_{10}$ levels on the frozen samples. Here, however, it was not possible to distinguish between microbial reduction by vacuum freeze-drying and microbial reduction by treatment with ethanol. Rather, the effects of the two steps add to each other. The higher microbial reduction on the frozen samples was probably caused to a considerable extent by the vacuum freeze-drying.

3.4.4 Microbial Reduction by Vacuum Freeze-Drying

As with the disinfection with ethanol, the microbial-reducing effect of vacuum freeze-drying is also strongly dependent on the mould species. In some cases, vacuum freeze-drying led to a strong reduction in microbial counts. However, the

intended reduction of 3 $\log_{10}$ levels was not achieved for any of the moulds tested. The two species *C. sphaerospermum* (2.7 ± 0.4 $\log_{10}$ levels) and *S. chartarum* (1.9 ± 0.4 $\log_{10}$ levels) were reduced the most. Substantial microbial reduction also occurred for *T. virens* (1.4 ± 0.2 $\log_{10}$ levels). In contrast, the two species *E. amstelodami* (0.1 ± 0.1 $\log_{10}$ levels) and *C. globosum* (0.2 ± 0.1 $\log_{10}$ levels) were not affected by vacuum freeze-drying. When interpreting these results, however, it is important to note that the microbial reduction may also be partly due to spores being extracted by the negative pressure in the drying chamber during vacuum freeze-drying.

3.4.5 *D* Values and Required Minimum Dose

The *D* values give an overview of the resistance of the moulds to X-rays on the differently treated samples (Table 3). The most resistant species is *S. chartarum* with a *D* value of 1.7 kGy, for both the dry and the frozen samples. *T. virens* with a *D* value of 0.8 kGy, again, is the most sensitive.

Since a germ reduction of 3 $\log_{10}$ levels was intended, the future irradiation dose should be chosen in such a way that the germ count of the most resistant species tested, *S. chartarum* (*D* value 1.7 kGy), is reduced by this factor. Consequently, a dose of X-rays of at least 5.1 kGy is required for a safe microbial reduction by 3 $\log_{10}$ levels. This is also largely consistent with the results of earlier studies, in which a dose of gamma radiation of 5–7 kGy is recommended (Moise et al. 2012, 1049).

To ensure the effective dose is as close as possible to 5.1 kGy, the target dose in the X-ray unit should be set to a value 30–40% higher due to the loss of intensity caused by the absorption of the packaging and the volume of the material to be irradiated. In addition, it must be considered that the dosimeters have a measurement error of ±4%. In practice, the actual dose will therefore be slightly higher than 5.1 kGy.

Table 3: *D* values of the tested moulds.

Species	Dry, unrefrigerated	Frozen, vacuum freeze-dried
Cladosporium sphaerospermum	1.0 kGy	0.6 kGy
Stachybotrys chartarum	1.7 kGy	1.7 kGy
Eurotium amstelodami	0.9 kGy	0.8 kGy
Chaetomium globosum	0.6 kGy	0.9 kGy
Trichoderma virens	0.8 kGy	0.7 kGy

4 Material-altering Effects of Irradiation

The deterioration of paper by gamma and X-ray irradiation is the result of changes at the molecular level, which in turn result in macroscopic changes that affect its physical, optical, or chemical properties. The main ingredient of paper is cellulose, a polysaccharide consisting of a chain of thousands of anhydroglucose units (AGUs) that polymerise via β-1,4 glycosidic bonds to form a long macromolecule. If cellulose is irradiated, important molecular bonds within and between the cellulose molecules are broken, especially the bond between the AGUs, which – depending on the dose – leads to changes in the paper's properties.

To quantify the material-altering effects of the minimum irradiation dose determined in the microbiological study, samples of Whatman® paper no. 1 treated with seven doses of X-ray or gamma radiation (Table 1) were analysed using different analytical methods. Non-irradiated samples served as reference.

4.1 Colour Change

Radiation treatment of paper can cause the formation of conjugated double bonds, characteristic for chromophoric compounds, which results in yellowing of the paper. This effect was observed both with gamma radiation (Adamo et al. 1998, 55) and with X-rays, when used in the context of analytical techniques (Mantler and Klikovits 2004; Gimat et al. 2020, 2022). To objectively register such colour changes and the extent of the associated aesthetic deterioration, measurements were performed with a spectrophotometer (Appendix). Since it is known that degradation reactions initiated during irradiation – especially colour changes – may impact the paper with a delay and thus are not detectable immediately post-irradiation (Gimat et al. 2020, 2022), the samples were analysed 70 days after irradiation. In the study mentioned above, it took 62 days for the first measurable yellowing to develop (Gimat et al. 2020, 2803). For statistical significance, five separate measurements were used to calculate the arithmetic mean for each sample. From the CIELAB values determined, the quantitative colour difference ΔE to the untreated reference sample was calculated.

The results show that the brightness (L^* values) does not change, neither after X-ray irradiation nor after gamma irradiation (Figure 2). Also, on the red-green axis (a^* values), for both types of treatment no significant change was observed in the dose range up to 5.1 kGy (Figure 3). Only on the blue-yellow axis (b^* values) a significant change towards yellow could be measured, as shown by the increase of the values with increasing dose (Figure 4). Here, the change after X-ray irradiation was slightly smaller than after gamma irradiation. However, the calculated ΔE value in the dose range up to 5.1 kGy, which is relevant for conservation practice, is still

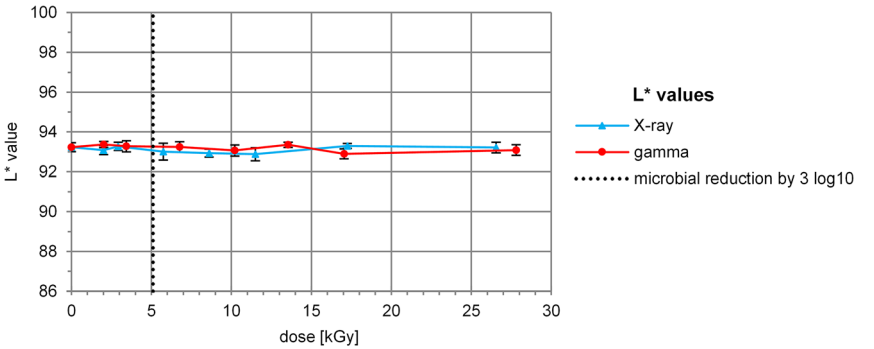

Figure 2: Dose-dependent development of the *L** values measured on Whatman® paper no. 1.

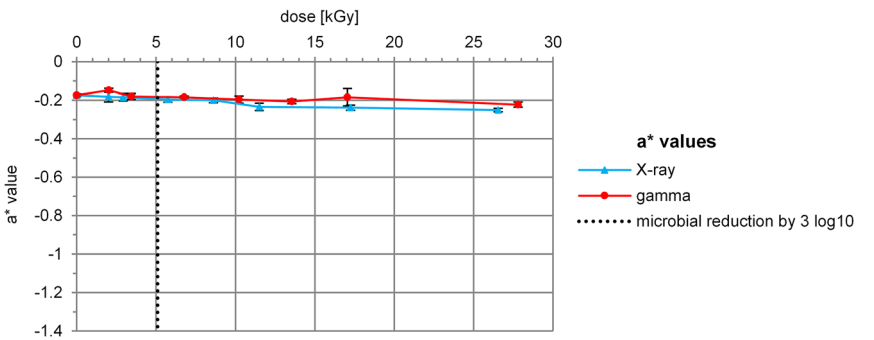

Figure 3: Dose-dependent development of the *a** values measured on Whatman® paper no. 1.

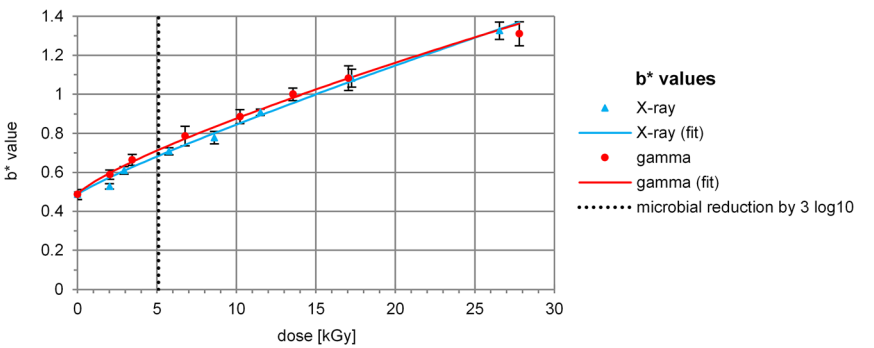

Figure 4: Dose-dependent development of the *b** values measured on Whatman® paper no. 1.

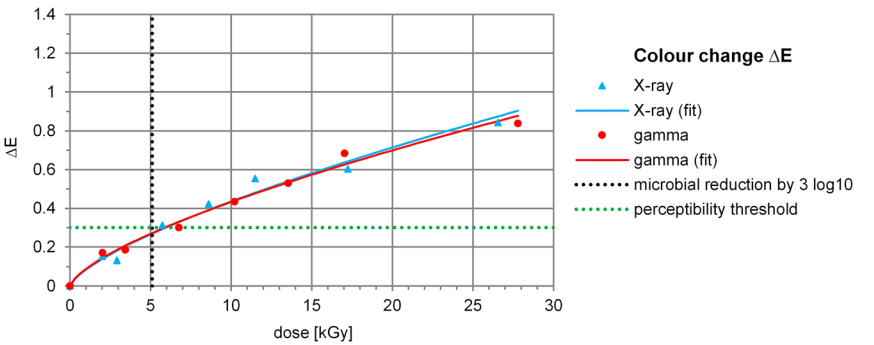

Figure 5: Dose-dependent development of the Δ*E* values (colour change) of Whatman® paper no. 1.

below the threshold value of 0.3, both for the samples treated with gamma radiation and X-rays (Figure 5). This indicates that the colour changes are not perceptible to the human eye. Furthermore, there is no significant difference between the Δ*E* values of the samples treated with gamma radiation and X-rays.

The results are thus consistent with those obtained in an earlier study on the effects of gamma radiation, which concluded "that low radiation doses (up to 10 kGy), as they are needed to disinfect/disinfest paper from biodeteriogenic organisms, cannot significantly damage its substrate [i.e., lead to no significant colour change]", with "*b**, indicating yellowing, giving the clearest result" (Adamo, Magaudda, and Omarini 2007, 44).

However, as a recent study demonstrated, it must be considered that it may take several months or even up to a year before a noticeable yellowing of the paper becomes apparent (Gimat et al. 2022, 4362).

4.2 Molecular Weight

A key parameter for describing the state of preservation of cellulose is the average molecular weight of the cellulose molecules (Dupont and Mortha 2004, 129; Jerosch, Lavédrine, and Cherton 2002, 222). However, the correlation between the molecular weight of the cellulose molecules and the mechanical strength of paper was already established in an earlier study (Zou et al. 1994, 401): the analysis of paper made from pure cellulose after accelerated ageing showed that the decreased paper strength and brittleness is mainly the result of the decrease in fibre strength. This in turn is due to the depolymerisation of the cellulose molecules by acid catalysed hydrolysis. The molecular weight distribution (MWD) determined by size exclusion chromatography showed that the depolymerisation is random. These findings could be confirmed in a

later study (Jerosch, Lavédrine, and Cherton 2002, 226): the smaller the weight-average molar mass (M_W), the lower the mechanical strength. 100 kg mol^{-1} were proposed to be the "critical M_W value" for cellulose. Below, the paper is considered very fragile, and its mechanical strength will decrease even faster at lower values. It should be noted, however, that the mechanical properties strongly depend on the specific composition of the paper, e.g., the presence of fillers or sizing, which is why the critical M_W value can vary between different papers (Jerosch, Lavédrine, and Cherton 2002, 234). There is a similar approach when considering the degree of polymerisation (DP) to characterise paper properties. Regarding its usability, a DP of 250–300 was suggested here as the lower limit (Strlič and Kolar 2005, 38).

As with accelerated ageing, radiation treatment also leads to a decrease in the DP, as the cellulose chains are broken into two or more polymers due to the partial destruction of the glycosidic bonds between the anhydroglucose units. However, it must also be noted that in addition to chain scission, cross-linking reactions between the molecular chains were observed in earlier studies on the effects of gamma irradiation on cellulose (Khan et al. 2006, 2305, 2308). But this phenomenon required much higher doses (up to 50 kGy) than those being applied in the present study.

Therefore, to examine the change in chemical properties of the samples, two months after irradiation a determination of depolymerisation was performed by analysing the MWD using size exclusion chromatography (SEC) with multi-angle laser light scattering/refractive index (MALLS/RI) (Appendix). The samples were treated according to the standard protocol for cellulose by the University of Natural Resources and Life Sciences, Vienna (BOKU) (Potthast et al. 2015). When determining the MWD using SEC-MALLS, the cellulose is brought into solution and chromatographically separated. Subsequently, the molar mass is determined by light scattering (Henniges and Potthast 2015, 294). First, the samples must be homogenised. Two samples (each ca. 20 mg) are taken from the resulting suspension for analysis, as two measurements are performed per sample by default. After the solvent exchange from water via ethanol to dimethylacetamide, the samples remain in the latter for 12 h. Afterwards they are dissolved in dimethylacetamide/lithium chloride 9% and diluted with dimethylacetamide before chromatography. For evaluation of the irradiated samples, the weighted mean molar mass (M_W [kg mol^{-1}]) was calculated from the measured MWD.

The results show that both gamma radiation and X-rays significantly affect the cellulose (Figures 6 and 7). Even in the low dose range of X-rays up to 5.1 kGy, which is relevant for conservation practice, the M_W of the cellulose molecules is considerably reduced – by more than 40% for Whatman® paper no. 1 and more than 30% for the historic rag paper (Appendix), which was also examined in this series of measurements (Table 4). Above 25 kGy, the highest dose used for both types of radiation, the M_W even drops below the critical value of 100 kg mol^{-1} mentioned above. However, a reduction in mechanical strength could not be detected even at the high doses, as shown by the results of the tensile strength tests (Figure 10).

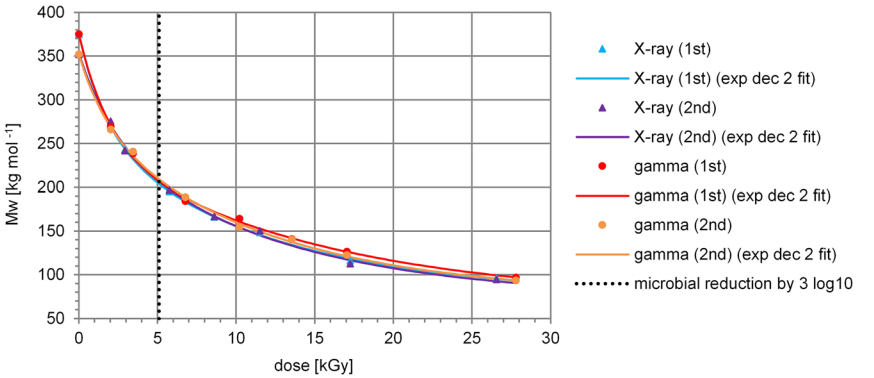

Figure 6: Dose-dependent reduction of the weight-average molar mass (M_W) of Whatman® paper no. 1 (1st = first measurement; 2nd = second measurement).

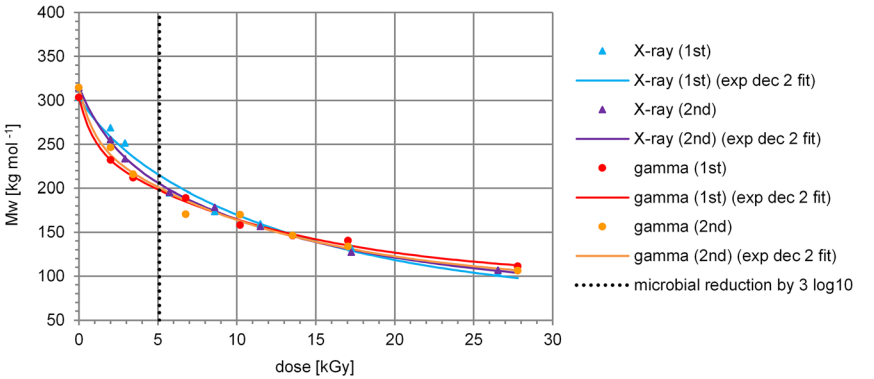

Figure 7: Dose-dependent reduction of the weight-average molar mass (M_W) of rag paper (1st = first measurement; 2nd = second measurement).

Table 4: Decrease of the weight-average molar mass (M_W) of the samples treated with doses 2 and 3.

	X-rays		Gamma radiation	
	Dose 2 2.93 kGy	**Dose 3 5.75 kGy**	**Dose 2 3.44 kGy**	**Dose 3 6.77 kGy**
Whatman® paper no. 1	33%	46%	34%	49%
Rag paper	22%	37%	31%	42%

For Whatman® paper no. 1, almost identical degradation can be observed with both types of radiation. There is no significant difference between the effect of gamma and X-ray radiation on the chain length of the cellulose molecules. For rag paper, however, degradation by X-rays at lower doses proceeds slightly slower than with gamma radiation, which may be due to the presence of hemicellulose or other additional ingredients. A recent study on the effects of X-ray synchrotron radiation also observed that historical and aged papers show a more complex behaviour, which differs from that of unaged paper made from pure cotton cellulose, as these contain other components in addition to cellulose and have already undergone an ageing process. Here, too, the investigations showed "that, overall, the historical papers resisted better the X-ray exposures than modern papers" (Gimat et al. 2022, 4362).

The curve of the M_W shows an ongoing flattening at higher doses (Figure 6). This indicates that with increasing irradiation dose the DP usually reaches the Levelling Off DP (LODP) at which all long molecular chains are already fragmented so that the DP does not decrease significantly with further increasing doses.

4.3 Oxidation State and Behaviour

The results of the measurement of the MWD of Whatman® paper no. 1 show that X-ray and gamma radiation reduce the chain length of the cellulose molecules to an almost identical extent (Figure 6). To clarify whether the oxidation state or the oxidation behaviour of the cellulose molecules is also affected by the irradiation and if there are differences between the effects of the two types of radiation, nine months after irradiation chemiluminescence (CL) measurements were performed (Appendix).

The measurement of CL is based on the emission of light quanta (photons), which can occur during the decay of excited metastable intermediates of molecules, e.g., radicals, 1,2-dioxetanes or other cyclic peroxides. The CL of cellulose among others is due to the decay of peroxides, whereby mainly alkyl radicals are converted to peroxyl radicals in an oxygen atmosphere. In this process, the decay of an intermediate stage leads to photon emission (Strlič et al. 2000, 2358). Since the CL signal is proportional to the peroxide concentration, the method is specific: the more photons quantitatively determined, the higher the peroxide concentration of the sample examined. In particular, the results of the *Papylum* research project prove that CL measurement is very characteristic and suitable for examining oxidation and degradation reactions of cellulosic materials. In the project, both deterioration and stabilisation possibilities were investigated, and important insights into the mechanism of cellulose degradation were gained (Strlič and Kolar 2005).

The CL measurements were performed on the samples treated with dose 3, which is 5.75 kGy for X-ray irradiation and 6.77 kGy for gamma irradiation, since this dose is closest to the minimum dose of 5.1 kGy relevant for conservation practice. The sample material needed was taken from the specimens in the form of circular punches (4 mm in diameter) with a net weight of ca. 1.7 mg and heated in a dynamic measurement procedure at different heating rates in a temperature interval from 30 °C to 200 °C. The CL is measured during the heating phase, whilst the sample chamber is flushed (60 mL/min) with either inert gas (nitrogen, N_2) or synthetic air (20% nitrogen and 80% oxygen, N_2/O_2).

The measurement in the inert gas atmosphere (N_2) only enables the compounds present in the sample that are capable of CL to react (termination reactions) and can thus be interpreted as thermal stability. The source of CL, especially in a nitrogen atmosphere, is the decomposition of charge-transfer complexes, which occurs between molecular oxygen and hydroxyl and/or ether groups (Strlič et al. 2000, 2358). In an inert gas atmosphere, the sample's degree or state of oxidation can thus be determined as the signal intensity, which is proportional to the concentration of peroxides.

Figure 8 shows the CL intensities of dynamic measurements in an inert gas atmosphere at a heating rate of 1.41 K/min. There is only a significant difference between the curve of the untreated reference sample (green) and the curves of the two irradiated samples (blue and red), which show lower chemiluminescence. The curves of the two irradiated samples, on the other hand, show almost identical characteristics. The quantitative differences between the samples treated with X-ray or gamma radiation are therefore negligible.

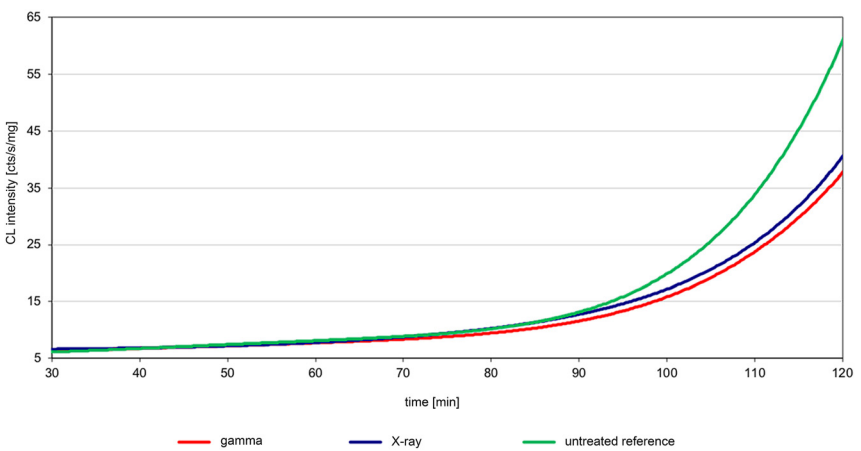

Figure 8: Chemiluminescence intensities of Whatman® paper no. 1 in an inert gas atmosphere (N_2).

All samples show a relatively strong increase starting at ca. 160 °C. This increase in light emission in an inert but also an oxidative atmosphere is an indication of chain scission followed by termination reactions of free radicals and can thus also be interpreted as a consequence of the thermal instability of saccharides. This effect requires a suitable acceptor, which is present in the system during this reaction (Strlič and Kolar 2005).

Assuming excited carbonyl compounds in the cellulose molecules are present, this means that the concentration of such compounds has decreased in the samples treated with gamma and X-rays compared to the untreated sample. However, earlier studies show that irradiation of cellulose, in addition to reducing the molar mass, also leads to oxidation reactions, i.e., an increase in oxidised functional groups in the molecule (e.g., carbonyl groups), whereby the increase in the carbonyl group content is linear with the increasing dose (Henniges et al. 2012, 4177). How this behaviour, which seems contradictory, can be explained will be the subject of further research.

The measurement in the oxidative atmosphere (N_2/O_2), on the other hand, interprets the oxidation stability during the heating phase and is mainly determined by termination reactions and the new formation of oxidation products. The intensity of the signal here is directly proportional to the speed of the oxidation reactions progressing in the sample.

Figure 9 shows the CL intensities of dynamic measurements in an oxidative atmosphere at a heating rate of 2.83 K/min. All three samples show almost identical characteristics. In contrast to the measurement in an inert gas atmosphere, however, a contrary behaviour of the untreated sample (green) can be observed, which shows

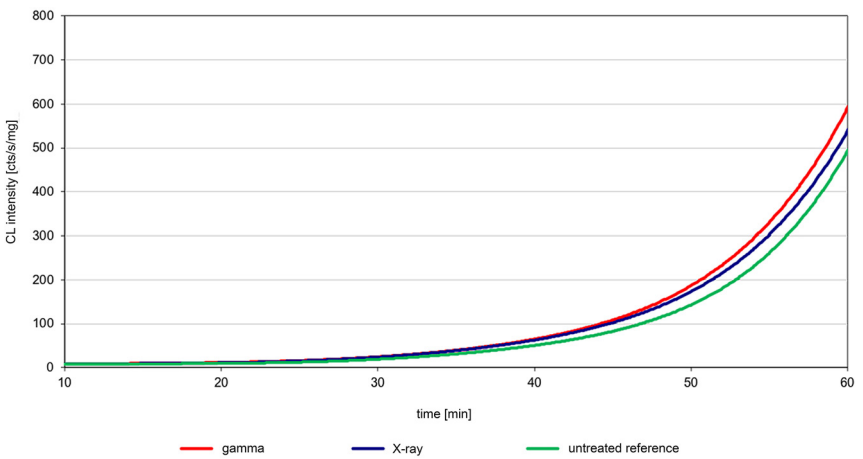

Figure 9: Chemiluminescence intensities of Whatman® paper no. 1 in an oxidative atmosphere (N_2/O_2).

a slight improvement in the oxidation stability compared to the irradiated samples (blue and red). The quantitative differences between the samples treated with gamma or X-rays are again negligible.

The most important finding of the CL measurements, however, is above all that the irradiated samples show hardly any measurable changes among themselves. This leads to the conclusion that no relevant differences between irradiation with X-rays or gamma radiation are to be expected regarding oxidation behaviour. However, since only three samples were examined, the results do not claim to be statistically significant.

4.4 Tensile Strength

Considering the results of the examination of the MWD, which show a considerable reduction in the molecular chain length, the question arises as to what degree the material change is still tolerable from a conservation point of view since the length of the molecular chains plays a key role in the physical properties of the paper (Zou et al. 1994, 401). Although there are no formal guidelines on material changes caused by radiation treatment, the usability of the irradiated paper must be guaranteed. To document any changes in the mechanical properties of the paper due to depolymerisation, which can become noticeable through increased brittleness, tensile strength tests were performed 12.5 months after irradiation.

For this purpose, strip-shaped samples with the standardised size of 15×210 mm were cut out of the specimens treated with X-rays and gamma rays and the untreated reference specimens, both in machine direction (MD) and cross direction (CD). If the samples are clamped in MD during the tensile strength test, both the cross bonds between the cellulose fibres and the fibres themselves break under the corresponding load. The results in MD, therefore, provide a direct indication of the brittleness or length of the cellulose fibres. If the samples are clamped in CD the paper breaks more easily because it primarily tears between the fibres, whereby the influence of the fibre length on the paper strength is less relevant.

The samples were preconditioned for three days at 22 °C and 55% RH. For each of the seven X-ray and gamma doses as well as for the reference samples, 10 strips were prepared for reasons of statistical significance and provided with a predetermined breaking point in form of a defined fold using a Bansa-Hofer folding device (Appendix). The measurement of breaking strength after folding is a widely used method, especially in the quality control of paper products. To determine the tensile strength, the samples were stretched in a material testing machine (Appendix) until they broke. The tensile strength was assessed by the tensile force measured at break (FB [N/mm^2]).

The results show that the treatment with gamma and X-ray radiation in the dose range up to 5.1 kGy, which is relevant for conservation practice, has no significant influence on the tensile strength, neither in MD nor in CD (Figure 10). How the reduced tensile strength in MD in the dose range 10–17 kGy can be explained, both for gamma and X-ray irradiation, could not be clarified conclusively yet.

As expected, the tensile strength in CD is generally lower than that in MD, since here the paper does not break in the fibre direction but crosswise, which requires lower forces. At 5.1 kGy, the samples treated with X-rays show an even slightly higher tensile strength than those treated with gamma radiation. Consequently, the irradiation has no negative effects on the mechanical properties and thus on the functionality of the paper. These results are largely consistent with earlier studies on the effects of gamma radiation, where also no significant decrease in mechanical properties was observed (Adamo et al. 1998, 45ff; Magaudda 2004, 114; Otero et al. 2009, 490, Figure 2a).

Although the measurement of the MWD proved that there was a considerable reduction in the molecular chain length due to irradiation, no significant change in the mechanical properties could be measured. This is probably because, at the low doses used, the mechanical effect of the shorter molecular chains cannot yet be detected with the Bansa-Hofer method due to its lower sensitivity, the comparatively slow reaction, and strong scatter. The determination of the MWD, on the other hand, has a very high sensitivity, so even very small changes in the cellulose molecules can be detected.

The observation that even a considerable reduction in the DP does not affect the mechanical properties has already been mentioned in earlier studies on the effects of gamma radiation on cellulose or paper (Calvini and Santucci 1978; Gonzalez, Calvo, and Kairiyama 2002, 265; Phillips and Arthur Jr 1985). A more recent study also proves that after disinfection of mould-contaminated paper by radiation treatment – at least in the case of gamma radiation – no negative long-term effect is to be expected

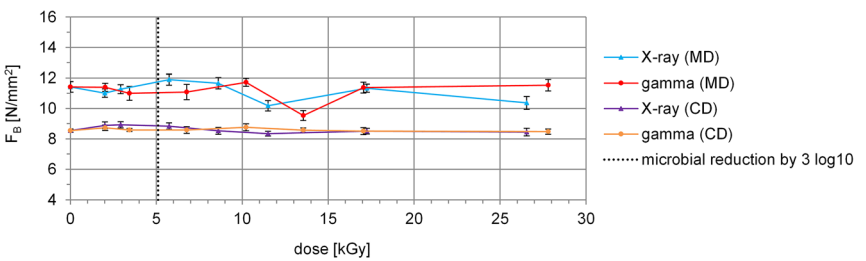

Figure 10: Dose-dependent development of the tensile strength of Whatman® paper no. 1 in machine direction (MD) and cross direction (CD).

(Pasternack et al. 2019). Even after 37 years of natural ageing, no significant change could be observed in comparison to untreated books, neither on the tensile modulus, the tensile strength, the folding strength, nor the colour of the paper. In another study, the influence of accelerated ageing on a sample treated with gamma radiation and an untreated sample was examined. The authors found no evidence that the treated sample degraded faster than the untreated one. Rather, it was shown that accelerated ageing had a much stronger effect on the mechanical properties of the paper than radiation treatment (Gonzalez, Calvo, and Kairiyama 2002, 265).

5 Discussion and Conclusion

5.1 Results and General Advantages of X-ray Treatment

In summary, no significant difference between the material-altering effect of gamma and X-ray radiation on cellulose could be found. The results show that both methods have approximately the same effect on cellulose. Thus, it was possible to prove that in the dose range studied, X-ray irradiation for disinfecting mould-contaminated archival materials is at least an equivalent method to gamma irradiation.

Even though the two types of radiation result in comparable material changes, X-ray irradiation offers several significant advantages: with the same dose, it has the same disinfecting efficiency as gamma irradiation, but with the X-ray unit, photons with higher energy can be produced that cause better material penetration, which in turn makes a shorter duration of treatment possible. This also reduces the time spent in the irradiation plant. It should also be noted that the intensity of the radiation decreases as it passes through a solid body, which is why it takes longer for a solid object to receive the same dose of radiation in its centre than on its surface. As a result, the surface receives a higher dose than the core. However, with a greater penetration depth, this dose difference can be reduced so that the centre can be disinfected without damaging the surface. This results in a better homogeneity of the X-ray irradiation compared to gamma irradiation, so that at a constant minimum dose, the maximum dose at the surface can be reduced, resulting in a lower material-altering effect.

Another advantage of an X-ray unit is that the dose, the dose rate, and the energy spectrum can be controlled more easily and thus also adjusted more precisely than with a gamma unit since the energy in the latter is dependent on the radiation source type and the maximum dose on the available mass of the radiation source – i.e., the amount of cobalt-60 – and its condition – i.e., the degree of decay that has already occurred. With an X-ray unit, on the other hand, the energy of the radiation can be

adjusted very precisely. This allows the penetration depth to be better adapted to the specific size and material of an object, which is one of the reasons why X-ray technology is safer and more efficient than gamma technology.

One more argument favouring X-ray treatment is that chemical reactions proceed more slowly during X-ray irradiation due to the lower operating temperature and the associated lower heating of material to be irradiated. In the X-ray plant used, the average temperature during irradiation is 20–25 °C, while in the gamma plant it is 30–45 °C. X-ray treatment also produces less ozone (O_3) in the irradiation chamber, which can also be extracted more easily in the X-ray plant than in the gamma plant because here it is easier to add a cooling and ventilation system. Ozone is a strong oxidant that is formed when oxygen molecules (O_2) are irradiated. It tends to bind to the cellulose fibres, which leads to a separation of the anhydroglucose units forming the cellulose molecules and thus to a reduction in the DP (Lemeune et al. 2004, 1221ff).

Finally, X-ray treatment has the great advantage that radiation is only produced while the unit is running. The cobalt-60 bars used as a radiation source in a gamma plant, on the other hand, continuously emit radiation and are thus depleted over time – even when the plant is not in use. As a result, the radiation power continues to decrease. The depleted but still radiating bars must be replaced regularly. This produces radioactive waste which is expensive to dispose of and the final disposal of radioactive material is still problematic without any long-term solution yet. For this reason alone, mould-contaminated archival material and cultural property should increasingly be treated with X-rays instead of gamma radiation in the future.

5.2 Applications for X-ray Irradiation

But why should archival material contaminated with mould be irradiated at all? Under proper climatic conditions – cool and dry – viable spores in small quantities usually do not pose a problem, since most of them do not form a mycelium that could damage the paper under these conditions. For people working with the contaminated material, however, the spores are problematic because they pose a health hazard. In addition, it should be noted that even spores eliminated by irradiation are potentially still allergenic and toxic. Therefore, manual decontamination must be done even in the case of irradiated objects to reduce the inactivated spores, the metabolic products, and the mycelia. Only in the case of irradiated archive stocks that are not used regularly but only in exceptional cases (e.g., tax records that are subject to a legally regulated retention obligation) the subsequent decontamination can be avoided. For safety reasons, however, such collections must be stored under quarantine conditions separately from the other, non-irradiated archive

material without previous mould contamination, as otherwise there is a risk of cross-contamination with the dead, health-hazardous spores.

Finally, X-ray treatment with a dose of 5.1 kGy is thus primarily a supplement to the time-consuming isopropanol or ethanol treatment of large mould-contaminated collections in archives and libraries, as irradiation is less time-consuming and even large quantities can be treated efficiently. It should also be used if the objects are sensitive to alcohol. However, irradiation has been shown to affect the cellulose, even though this does not seem to have any influence on the mechanical properties, there is no colour change perceptible to the human eye and no long-term consequences are to be expected regarding the oxidation behaviour – taking into account the general conditions under which the respective analyses were performed, such as the delay between irradiation and analysis. Furthermore, in the present study mainly the behaviour of unaged samples of pure cotton cellulose was investigated. Other studies have shown that aged papers and historical archive papers can show a more complex behaviour (Gimat et al. 2022). Therefore, irradiation is particularly suitable for those collections that are not used and only need to be stored for a limited time. If irradiated archival materials are nevertheless to be used, a precise procedure must be defined in advance, which gives the user the possibility, if necessary, to have the required documents subsequently cleaned of the inactivated components of the moulds to be able to work with them.

However, if a collection has been contaminated with faecal bacteria as well as mould, irradiation is the only way to eliminate the infestation. Here, mechanical decontamination by surface treatment with ethanol is not effective. Instead, the objects would have to be treated in an ethanol bath for at least 2.5 min, which can cause further damage (Meier 2006, 28).

To be considered successful, radiation treatment should not make the irradiated material more susceptible to re-infestation by microorganisms. In this regard, an earlier study concluded that moulds develop more quickly on cellulose with a lower DP. However, it was also shown that the decrease in the DP caused by gamma radiation in the dose range below 10 kGy is not sufficient to significantly increase the rate of mould growth (Adamo et al. 2003, 150).

The disinfection of highly valuable paper objects by X-rays with a high photon energy should only be applied in exceptional cases and after thorough consideration of the advantages and disadvantages due to the proven significant reduction of the molar mass of the cellulose molecules. It is also questionable whether radiation treatment of single objects such as loose leaves is reasonable, as dry cleaning followed by mechanical reduction of the spores with a microfibre cloth sprayed with alcohol is the more efficient alternative here. However, a reliable microbial reduction of the intended 3 $\log_{10}$ levels is difficult to achieve in this case, as shown by the results of the ethanol disinfection performed in the present study.

If irradiation is to be applied, it is recommended that this is preferably done at 50% RH or higher, as the moisture in the paper limits the degradation of the cellulose macromolecules during X-ray irradiation, as a recent study has shown (Gimat et al. 2020, 2804f).

Even irradiated archival materials must be stored under proper climatic conditions and in a previously decontaminated archive room, especially if it is the same room in which the infestation took place. Otherwise, if the relative humidity is too high, mould growth could recur due to the ubiquitous spores.

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Jacot-Guillarmod and Olivia Raymann made an important contribution to the success of the project with their research on the physical fundamentals of X-rays and gamma radiation as well as on alternative disinfection methods. Image credits: Figures 1–7 and 10 by Cornelius Palmbach, Figures 8 and 9 by Andreas Buder.

Appendix

List of materials

Whatman® filter paper no. 1 (WHA1001931, Sigma Aldrich): To determine the MD, the longitudinal and transverse edges of a piece of paper were moistened with water using a brush, causing the edges to become wavy. The CD and the MD can then be distinguished based on the pronounced waviness: the edge where the corrugation is less pronounced runs parallel to the MD (Teschner 2017, 906ff).

Rag paper: Cut-off, unprinted margins of the pages of an 18th-century book.

Instrumentation

X-ray radiation unit Rhodotron TT-1000 (IBA SA): Electron beam accelerator with tantalum target (Abs et al. 2004), photon energy: 7 MeV, power: 700 kW, increments: 2.8–2.9 kGy.

Gamma radiation unit (Nordion, Canada): Radiation is provided by bars made of the radioisotope cobalt-60. Photon energy: 1.17 MeV and 1.33 MeV, power: 3.5 MCi, increments: 3.4–3.5 kGy.

Dosimeter Alanine TapeTab (Harwell Dosimeters LTD): Alanine strip dosimeters read out using an electron spin resonance (ESR) spectrometer. The dosimeters were handled according to ISO 9001, ISO 13485, ISO 11137 and 21CFR Part 820 (FDA c GMP).

Spectrophotometer Spectrolino™ (Gretag Macbeth AG), colorimetry: CIE-L*a*b*, illumination: D65 (approximated daylight, colour temperature: 6504 K), observer angle: 10° normal observer, evaluable values: spectrum R (remission in the wavelength range 380–730 nm in 10 nm steps).

SEC-MALLS/RI instrumentation: The Agilent GPC system consisted of a MALLS (Dawn DSP 488 nm, Wyatt Corp.) and refractive index detector (Shodex RI-71) with automatic injection on four serial columns. DMAc/LiCl (0.9%, m/V, filtered through 0.02 µm) was used as the eluant. MWD and related polymer-relevant parameters were calculated by software programs ASTRA 4.73 and Grams, based on a refractive index increment of 0.140 mL/g for cellulose in DMAc/LiCl (0.9%, m/V) at 488 nm. GPC

parameters: flow: 1.00 mL/min; columns: four, Agilent PL gel, mixed A-LS, 20 µm, one pre-column, 7.5 × 300 mm; injection volume: 100 µL; run time: 45 min.

Chemiluminescence measuring device CL1.0 V.5 (ACL Instruments AG)

Bansa-Hofer folding device: The device consists of an inclined plane tilted at 20° to the horizontal and a 500 g cylindrical mass. After 30 cm, the strip-shaped sample clamped in a loop protrudes through a slot from below into the rolling path. After releasing the locking mechanism, the mass rolls down from the upper end of the inclined plane across the paper loop. Thereby, the loop is pressed flat with always identical force, providing the sample with a defined fold.

Tensile tester zwickiLine Z2.5/TN1S (Zwick Roell AG): Tests were performed following the DIN 53112 standard (meanwhile replaced by the DIN EN ISO 1924-2 standard). Initial force: 5 N/m, test speed: 20 mm/min.

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