



Application of gamma radiations and X-rays for disinfection of fungi in historical archives

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**Application of gamma radiations and
X-rays for disinfection of fungi in
historical archives**

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Chapter 1

General introduction

1.1 The state of fungi-contaminated on archive storage

Books, manuscripts, drawings, archive documents, and wooden artifacts are an important part of cultural heritage inherited from past generations and maintained for the benefit of future generations. To ensure the preservation of these objects in the best condition as much as possible and maintain for future generations, studying and conserving these object materials is a significant important work.

Books, documents, and wooden artifacts are composite objects made mainly of organic compounds, which is the major source of energy for microorganisms (Florian, 2002; Kim and Singh, 2000). The storage of books, documents, cultural objects in the museum, library, or archive storage where has created new, human-made environments for fungal and microbial species to inhabit (Nittérus, 2000). They are a part of the cause of the modifications of the cultural materials that occur over time at a different pace (Lucio C. Severiano et al., 2010).

The degree of biodegradation of cultural objects would vary depending on the organism contaminated on objects and the substratum. Among that microorganism, fungi play an important role in biodegradation because of its invasion altering the aesthetics of the artifacts such as discoloration and pigment accumulation causing stains on documents, woodblocks (Abd El Monssef et al., 2016; Mesquita et al., 2009; Nunes et al., 2013). Moreover, they are conspicuous due to the formation of mycelia and conidia.

The most critical fungal genera found in museums are *Alternaria*, *Aspergillus*, *Absidia*, *Acremonium*, *Cladosporium*, *Chaetomium*, *Chrysosporium*, *Eurotium*, *Fusarium*, *Geotrichum*, *Penicillium*, *Paecilomyces*, *Epicoccum*,

Phoma, *Cunninghamella*, *Emericella*, *Scopulariopsis*, *Stachybotrys*, *Trichoderma* and the yeast genus *Rhodotorula* with a high affinity to osmotic environments (Bensch et al., 2018; Carlo et al., 2016; Mesquita et al., 2009; Oetari et al., 2018; Sterflinger and Piñar, 2013). The fungi associated with water-damage consist of fungal species that require high water activity (Nielsen, 2003). These species can produce strong odors (*Trichoderma* spp.), colored stains (*Chaetomium* spp. and *Epicoccum* spp.) or toxic compounds (*Stachybotrys* spp.).

Depending on the fungal function, the fungi on cultural heritage items can be divided into two main groups: (i) opportunistic fungi that only require suitable humidity and unable to degrade the material, and (ii) real ‘material pathogens’ that are substrate-specific and able to degrade specific materials of works of art determining by each species. Both groups may cause serious deterioration, but only fungi belonging to the second group can decay. Conidia are the most important structure which helps fungi release, transport, and spread in the airborne. That can explain why fungal infection of cultural heritage materials is mostly airborne – with significant seasonal variations – and high numbers of conidia can accumulate in dust layers (Sterflinger and Pinzari, 2012). The fungal growth is determined by the material itself and the environmental factors such as humidity, pH, temperature. Among them, the humidity plays an essential role in the fungal bloom (Nittérus, 2000; Sterflinger and Pinzari, 2012). Unlike other organisms, many fungi (xerophilic and xerotolerant) are able to grow at much lower levels of humidity such as *Eurotium* sp., *Aspergillus* sp., or *Wallemia* sp. growing at water activity (aw) > 0.6. Water availability of above aw 0.8 already allows the growth of a wide variety of airborne fungi. In museums, the range of 55 % relative humidity is generally regarded as the borderline for fungal growth and thus, climate control is adjusted below this value.

Some fungi that damage paper documents can adversely affect users' health through their toxins. The fungus enters the body via inhalation of toxicogenic spores and direct dermal contact and may cause some diseases, including airway infections, fungal diseases, immune system problems, and asthma (Nielsen, 2003).

In conclusion, fungi-contaminated on archive storage is not only affecting archival materials but also threaten the health of the users and the restorers. Therefore, it is very important to remove fungi and their influence on archives by the appropriate methodology.

1.2 The current decontamination methods for preservation of cultural heritage

1.2.1 Conventional disinfection technique

◇ *Fumigation*

Sometimes, fumigation, which is the use of gases poisonous to living creatures, is used to sterilize the medical devices and the disinfect of cereals or fruits. Ethylene oxide ((CH₂)₂O) and methyl bromide (CH₃Br) are the gases most frequently used for these purposes. However, the crucial negative effectiveness of fumigation is dangerous to the user. For example, ethylene oxide is carcinogenic, extremely flammable and explosive, and unfriendly to the environment (phenyl phenol) (Nunes et al., 2013; Sterflinger and Pinzari, 2012).

◇ *Liquid treatment*

Cultural heritage artifact made of wood, paper, leather, or any other organic material would be effected when contacting any liquid prolonged. Therefore, their use must be following the procedures and standards for each material.

Ethyl alcohol and isopropyl alcohol are fungicidal and bactericidal and act very quickly, but these liquids do not destroy any conidia. Formaldehyde is also used especially formalin. It is a bactericide, fungicide, and sporicide. Unfortunately, formaldehyde is a carcinogen and can cause asthma-like respiratory problems, while exposure to the skin can cause skin irritation. Additionally, artifacts treated with formalin may emit formaldehyde over time, and formaldehyde in the air should be avoided (Nittérus, 2000; Ponta and Havermans, 2017).

The use of fungal sanitizers for the conservation of the cultural heritage should be considered, even under strictly controlled conditions, because of its health risk to staff and users (Nittérus, 2000).

◇ ***Dry cleaning***

It is sometimes mistakenly believed that dry cleaning is useful to disinfect the fungal infected on artifacts if they are stored afterward under suitable environmental conditions (e.g., at a temperature of 18°C and 45–50% relative humidity). However, dry cleaning only removes fungi on the surface, whereas in fact that fungi may still exist inside these objects. These fungi may grow in suitable humidity, and then the other biodeterioration may continue (Ponta and Havermans, 2017).

◇ ***Freeze drying***

Physical treatments such as thermal treatment and freeze-drying are often not recommended in cultural heritage conservation. However, in some urgent cases, one can use it as a temporary rescue. A commonly used method for rescuing wet cultural items is freeze-drying because it damages fungal cells (Nittérus, 2000),

inhibits mold growth. Freezing allows the time to determine if the original's value, use, and format are essential to de-access or purchase replacement materials or materials in a different format. Moreover, freezing will allow time to find space for air drying, determine if there are adequate staff and time to air dry, and to handle significant incidents in a smaller, more controlled atmosphere. However, conidia present inside the substrate may survive over the treatment, and mycotoxins cannot be removed by freeze-drying (Ponta and Havermans, 2017).

If water were able to be removed through sublimation (from solid to gas), then it would not change the artifact structure. The artifacts would be free of surface tension and capillary tension during drying. Although such sublimation can prevent collapses, it cannot prevent contraction, thereby creating stress and splitting. Additionally, water shows the cubical expansion of about 9% when it freezes at 0°C.

The vacuum freeze drying method is widely used in the manufacturing of instant food or medicine. Aqueous solutions or substances containing moisture are frozen rapidly and then sublimated under depressurized conditions for drying. As the vacuum freeze-drying method demands a special apparatus for the pretreatment besides a massive vacuum (drying) chamber, this method is not appropriate for the conservation treatment of large objects (National Research Institute of Cultural Heritage., 2012).

1.2.2 Ionizing radiation disinfection method

◇ *Gamma irradiation*

Since last century, gamma irradiation has been considered the most efficient method for cultural heritage decontamination. Gamma irradiation as disinfecting

treatment may damage cellular DNA directly and indirectly through radiolysis of cellular water and the formation of active free radicals. Thanks to the advantage of gamma-rays, such as guaranteed biocidal effect; short time process within a few hours; high degree of penetration to guarantee mass treatment without leaving any residues; easy handling (the material is irradiated in transport package); applicability to composite materials (paper, cardboard, wood, leather, textiles), this technology has been used for food and medical device sterilization for more than 50 years. It is already known as a commercial technology and an efficient quarantine measure (Choi et al., 2012).

◇ *Electron beams*

Similar to gamma radiation, electron beams can be used to disinfect a large number of objects in a short time. The treatment time for an individual object under the electron beam is a few seconds or several hours for vast collections of artifacts. Most materials making up cultural heritage objects that must be disinfected are not formulated for radiation stability. Some materials have demonstrated less degradation when processed with electron beam radiation as compared to gamma radiation. This is due to a significant difference in the dose rate between the two radiation technologies. In general, products processed with electron beam radiation experience shorter exposure time, resulting in a lower oxidative effect on certain materials. Some cellulose materials, for example, experience less breakdown and fewer long term aging effects from processing with accelerated electrons (Gluszewski, 2017).

In recent years, electron beam treatment is an established decontamination strategy for the sanitation of packaging materials and medical devices. However,

it is not yet a routine method for food treatment or cultural heritage because of the relatively high cost and only surface effectiveness (Etter et al., 2018).

◇ *Low energy X-rays*

Radiation processing by gamma-ray from ^{60}Co and electron beam / X-rays with high energy are commonly used for radiation sterilization and food irradiation. The benefits of radiation sterilization are high-speed, high efficacy treatments, without chemicals, and can be performed at room temperature. However, these facilities are required high shielding buildings with high costs.

The use of low-energy X-rays for sterilization of medical devices and food disinfection has lately attracted more interest. The merit of low-energy X-rays is its low shielding requirements. The irradiation device using an X-ray tube with low energy is reliable, compact, cost-effective. The compact low-energy X-ray irradiator is widely used in health-care blood irradiation (National Academies Press, 2008). In addition, the X-ray machine with a small size can be easily moved in the storage, which helps minimize the risk of damage that may occur during transportation. Even the machine can be transported to other museums to do conservation work.

1.3 Gamma radiation treatment of cultural heritage artifacts

In the 1960s, the bactericidal effect of gamma radiation began to be studied. Bletchley (1961) reported that gamma irradiation was an effective method for control wood insect infestation. Based on much research, there was a suggestion that ionizing radiation's biocidal effect can inhibit the biodegradation of cultural heritage artifacts (Lindsey, 1961).

Then, gamma radiation has been used for foods and medical device sterilization and is already known as a commercial technology and an efficient quarantine measure. International Atomic Energy Agency (IAEA) has been developing cooperation programs among countries worldwide to develop and apply nuclear methods in cultural heritage research. Ionizing radiation-based techniques are now recognized as important tools for examining characterizing and analyzing of art objects or other cultural heritage artifacts and their component materials.

Preservation of existing cultural heritage artifacts continues to pose a serious challenge, as various factors, such as improper storage conditions, climate change, or adversities like flooding lead to deterioration or loss of cultural heritage worldwide. Both chemical and physical methods have been developed for the treatment and restoration of cultural heritage artifacts. However, chemical methods may leave undesirable chemicals, and physical methods generally use extreme conditions that are not suitable for some types of material (Havermans, 2017; Nittérus, 2000; Sterflinger and Pinzari, 2012). Thanks to many gamma application achievements in the industrial branch and much research relating to the feasibility of using radiation in preserving the cultural heritage, the confidence in the use of radiation treatment for decontamination of cultural heritage artifacts increased.

The efforts of national and international research programs to develop appropriate radiation treatment methods have led to the acceptance of radiation technology in conservation cultural heritage artifacts. However, it should be noted that radiation (gamma, X rays, or electrons) has the potential of degrading organic materials, and those materials being irradiated successfully at present have theoretically short lifetimes (Havermans, 2017).

Therefore, it is necessary to strictly follow the regulations on the dose, irradiation time, and the possibility of deterioration of the exposed object. IAEA recommends that the treatment dose of a maximum of 10 kGy can be seen as a reference dose for the overall disinfection of cultural heritage artifacts. However, a dose higher than 10 kGy could be taken into consideration in special situations such as severely fungal contaminated, flood invasion, or poor storage conditions.

1.4 Advantages of radiation techniques

Cultural heritage preservation by using conventional methods either results in incompleting fungal disinfection or is harmful to user-health or is not environmentally friendly, whereas applying radiation techniques in this area have specific and indisputable advantages.

The first advantage is harmlessness. The irradiation is carried out in confined areas, strictly protected, in compliance with radiation safety regulations.

Radiation technology does not leave any residue in the treated item or release any substance that causes damage to the environment, so it will not harm the user.

Another critical advantage of radiation technology is high efficiency. The reason is that gamma radiation penetrates most of materials or has a high surface disinfection effect and can control the biocidal effects of radiation by calculation of absorbed dose. When using radiation disinfection, items can be irradiated without being removed from the package or container used for transportation.

A further advantage of decontamination by radiation is possible to irradiate oversized objects as effectively as smaller objects and takes significantly less time than conventional methods.

1.5 Effect of ionizing radiation on cultural heritage materials

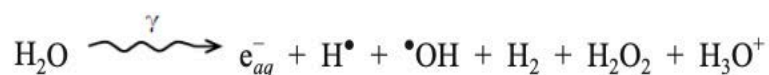
1.5.1 Biocidal effect and DNA modification

During irradiation, energy is transmitted by radiation to material and may cause modification of the chemical composition of both artifacts and organisms. Macromolecules in living organisms such as microorganisms, insects, the basic building blocks of an organism, are modified. Especially, DNA, the material is containing the genetic information of the cell. Under the action of radiation, the structure of the DNA is altered, leading to mutations or prevent replication of DNA and cell division. The result is cell death or the creation of genetically modified organisms.

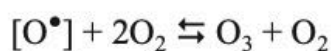
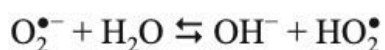
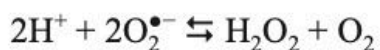
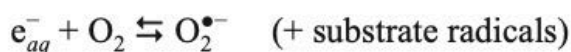
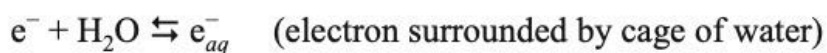
Ionizing radiation can interact with microorganisms directly or indirectly way.

- Direct interaction with cell components, such as DNA.

- The indirect modification produced by free radicals resulting from water radiolysis. It is the latter indirect effect that is the predominant pathway of the inactivation of microorganisms. Important free radicals like hydroxyl radicals ($\text{OH}\bullet$) are formed in the hydration shell of the DNA molecule. They are responsible for 90% of the DNA damage. Although many other hypotheses have been proposed on the mechanism of cell damage by radiation, it is universally accepted that the DNA in the chromosome represents the most critical 'target' for ionizing radiation as damage to it causes cell division inhibition.



In the presence of oxygen, other important radicals can be formed according to the following reactions:



Electron first is surrounded or captured by the water molecules to produce a hydrated electron, which reacts with oxygen to form the superoxide anion. The superoxide anion subsequently reacts with water, resulting in the formation of hydrogen peroxide. Oxygen, peroxide radicals, and ozone also may be formed.

The radicals formed are the cause of the deterioration of organic molecules. The impact of radiation varies depending on the evolution of the organism. The more complex the organic molecule, the less energy is needed to deteriorate it (Ponta, Havermans, Tran, et al., 2017).

1.5.2 Radiosensitivity of fungi

As mentioned in section 1.1, fungi play an essential role in the degradation of cultural heritage. With many advantages over traditional methods, the use of gamma radiation in preserving cultural heritage has been widely applied in many countries around the world. The use of gamma radiation is a promising treatment in the preservation field (Silva et al., 2006). The 10 kGy absorption dose is usually recommended for cultural items (IAEA). Many studies reported that the damage

in mechanical properties caused by gamma rays on paper was not significant. The doses of 10 kGy or even at a high dose of 14.4 kGy (Gonzalez et al., 2002), 15 kGy (D'Almeida et al., 2009), and 50 kGy (Choi et al., 2012) resulted in no significant change in some mechanical properties of the paper.

Fungi have been successfully inactivated in different materials, such as paper, wood, and soil with radiation dose ranging from 6 to 15 kGy (Choi et al., 2012; Etter et al., 2018; M. Silva et al., 2006). According to Tomazello and Wiendl, (1995), *Aspergillus* spp, *Cladosporium cladosporioides*, and *Alternaria tenuis* could survive at the dose up to 20 kGy, *Penicillium* spp. to 17.5 kGy.

Fumigation of wood-decomposing fungi usually requires a higher dose of radiation than fungal disinfection on paper or eradicated insects. The applied dose is usually ranging from 2 to 18 kGy depending on the fungus species. Freitag and Morrell., (1998) reported that a gamma radiation dose of about 15 kGy was adequate to sterilize Xylophagous fungi in wood and exterminate pests. Csupor et al., (2000) concluded that 12 kGy is sufficient for safe fumigation of wood. The European standard EN 113 (CEN 1996) requires a dose of 25 to 50 kGy for wood sterilization in lab testing procedures.

Unger et al., (2001); Hasan et al., (2008) revealed that the difference between the treatment time and the irradiation source's power has an insignificant effect on wood. In other words, there was no significant difference if the wood was irradiated with a weaker source for a longer time or with a stronger source for a shorter time. However, another study showed that the dose rate and the total dose of gamma radiation affected both flexural strength and some chemical components in the wood tested (Curling and Winandy, 2008).

However, it is not necessary to remove 100% of all mold spores from all cultural items because the result would only be temporary as new conidia will continuously settle on items (Zorila, 2014).

The killing effect of radiation in microorganisms is generally expressed by D_{10} value, a scientifically established term meaning the irradiation dose necessary to reduce the number of microorganisms by a factor of ten (an order of magnitude) (Fig 1.1).

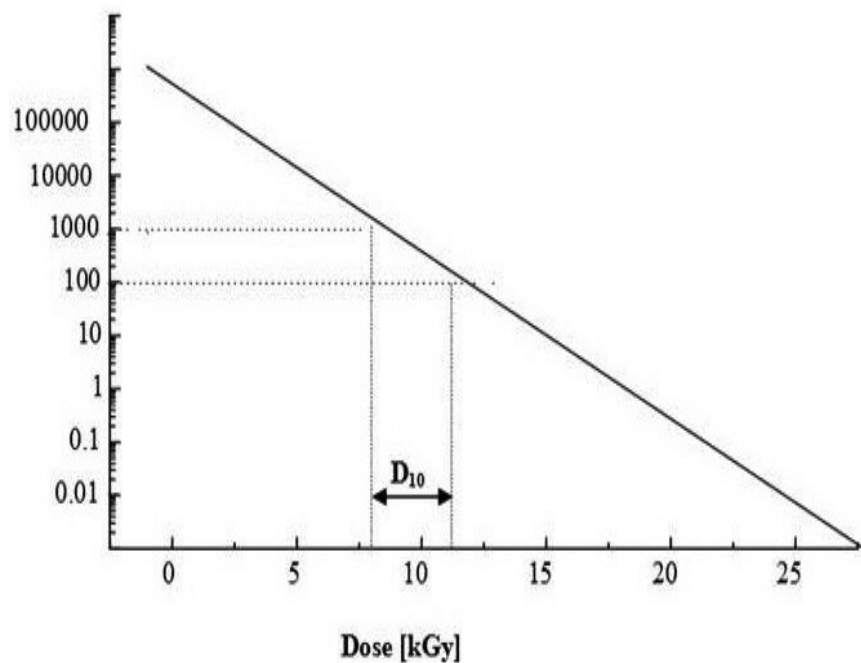


Fig. 1.1 Radiation inactivation of microorganisms, X-axis represents number of microorganism on a logarithmic scale (Ponta, Havermans, Tran, et al., 2017).

At a specific dose, the consequent treatment of artifacts can be applied, using D_{10} value, providing the desired reduction of microorganisms. The bioburden population is generally reported as the number of colonies forming units (or CFU). In order to establish the treatment irradiation dose, aspects like the degree of

degradation of the artifacts and the degree of contamination should be taken into account (Zorila, 2014).

The irradiation dose needed for effective biocide is best known in the fungi and bacteria cases, for which good statistics were available. D₁₀ value of some fungi conidia was reported by Saleh et al., (1988) (Table 1.1).

Table 1.1. Irradiation resistance of fungal conidia in water (Saleh et al., 1988).

Species	CFU/ml	D ₁₀ value (kGy)
<i>Aspergillus niger</i>	2 x 10 ⁶	0.42
	5 x 10 ⁷	0.42
<i>Aspergillus flavus</i>	1 x 10 ⁵	0.6
	4 x 10 ⁷	0.55
<i>Cladosporium cladosporioides</i>	1 x 10 ⁶	0.3
	2 x 10 ⁷	0.25
<i>Curvularia geniculata</i>	2 x 10 ⁴	2.42
	2 x 10 ⁵	2.9

Therefore, radiation decontamination of cultural heritage artifacts is essentially based on similar basic knowledge and can utilize the same equipment used in the two well established industrial areas of food disinfection and sterilization of medical devices.

1.5.3 Secondary effects of gamma radiation on paper, historical archives and woodend artifacts

Although the gamma radiation has been viewed as a promising method of preserving cultural heritage, it also has negative sides. The bactericidal effect of radiation may be accompanied by a change in the chemical composition and physical properties of the disinfected items. Radiation technology will be accepted if it does not lead to unacceptable alteration of the aesthetic and properties of cultural articles. The side effects of irradiation in cultural items may vary depending on the particular artifact, evaluation, and understanding of secondary effects is therefore essential.

When radiation interacts with an item, free radicals are formed. These free radicals then interact with the polymers causing chain scission and cross-linking reactions. Depending on the nature of the substrate, radiation dose, dose rate, and ambient conditions, one of these two reactions will become dominant. Chain scission is associated with the weakening of mechanical properties, while cross linking improves mechanical properties (Ponta, Havermans, Tran, et al., 2017).

During irradiation, oxidation can be seen as the leading factor in degradation, followed by acidification and finally, D-glucose is formed, along with several low molecular weight products. Moreover, many reactions may occur, including both oxidation and (acid-catalyzed) hydrolysis reactions because wood and paper do not contain 100% cellulose. During the process of degradation, hydroperoxide radicals are generated in the fibrous structure; lower mass polysaccharides are formed owing to the ionic hydrolytic degradation initiated via terminal groups in cellulose. Superoxide radicals play an important role in the oxidative degradation of cellulose (Kocar et al., 2012). In addition to being formed as a result of ionizing

radiation such as gamma radiation, radicals in (ligno)cellulose materials can also be formed owing to the presence of fungi (Fig. 1.2). Therefore, the deterioration of these materials by radicals is not only due to radiation but also because of the microorganisms contaminated them.

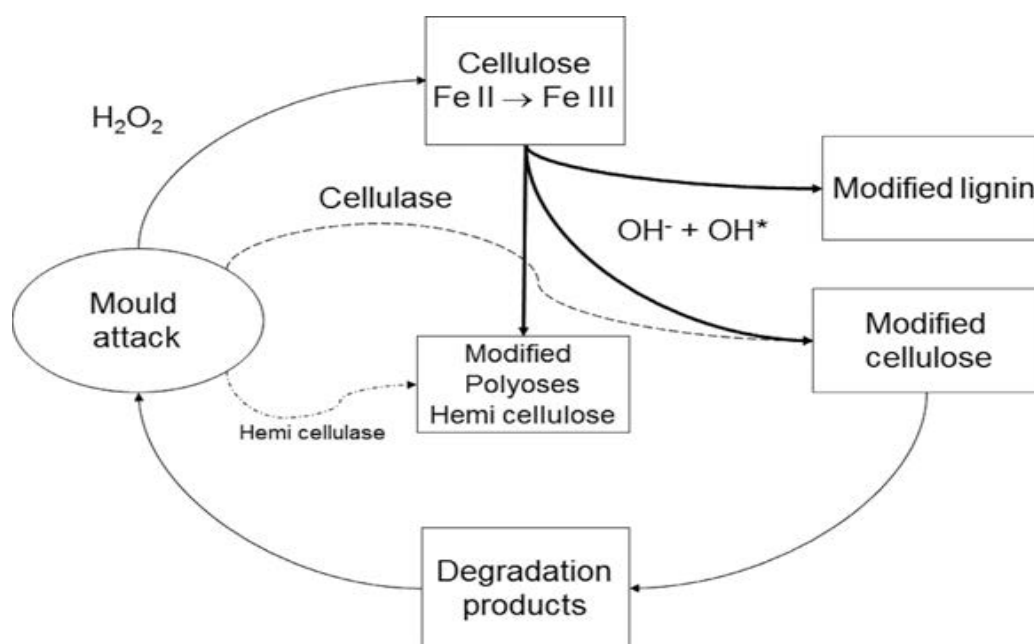


Fig. 1.2 Hypothetical model of the formation of peroxides and radicals by fungi initiating paper degradation (Ritschkoff and Mahlberg, 2001).

Most comprehensive research has been focused on the disinfection of paper using gamma irradiation. Tomazello and Wiendl., (1995) pointed out some possible paper damage related to irradiation even for doses from 5kGy up to 10kGy. Gonzalez et al., (2002) compared paper damage for accelerated aging and gamma irradiation and concluded that the former introduced more significant mechanical properties differences than the latter. The authors concluded that a radiation dose up to 14.4 kGy did not influence the properties of paper. In 2006, Silva et al. suggested that the minimum dose required to kill fungi isolated from the naturally contaminated books from a Brazilian public library and the environment was 16 kGy. Studies on the effects of a dose from 3 to 15 kGy on

pure cellulose matrix were also carried out by D'Almeida et al., (2009). They concluded that no significant changes were detected in paper samples irradiated up to 15kGy. One particular research on the traditional paper of Korea of Choi et al., (2012) investigated the sterilization effects of radiation and its effect on the mechanical properties of the traditional Korean paper-Hanji. Treatment doses of 9 kGy and 8 kGy of gamma irradiation inactivated 5 log units of *Aspergillus niger* and *Bacillus cereus* spores inoculated on the Hanji. The gamma irradiations up to an absorbed dose of 50 kGy resulted in no significant changes in the tensile strength, bursting strength, and appearance of the Hanji.

However, not all authors agree that gamma-ray does not significantly alter the mechanical properties of artifacts. An experiment on gamma irradiation of paper at 2–5kGy (Adamo et al., 2001) demonstrated an irradiation dose of 5 kGy caused significant variations on almost all the paper properties. The Whatman paper tended to yellow at that dose. In another study, in 2007, these authors concluded that commercial paper is sensitive to irradiation and darkened in color. Further, it could be shown that the paper samples after treatment with gamma radiation undergo more severe degradation during artificial aging (Adamo et al., 2007). Bicchieri et al., (2016) examined the effect of low dose gamma irradiation on cellulosic substrates. To simulate a real deteriorated document, which could need gamma-ray irradiation, a cotton paper and commercial permanent paper samples were submitted to a hydrolysis treatment to induces a degradation similar to that observed on original documents. The samples were then irradiated with 3-kGy gamma rays. The results showed the negative effects of gamma irradiation on the paper. Non-irradiated paper preserves better its appearance and chemical properties both in the short term and after aging, while the irradiated samples show appreciable color change and greater oxidation extent. Drábková et al., (2018) also

indicated that significant changes occur in the cellulosic and proteinaceous materials' structure on irradiation with the doses necessary for disinfection. Even the lowest radiation dosage (approximately 4.5 kGy) necessary for disinfection causes cleavage of macromolecules expressed by a decrease in the limiting viscosity number by 30–50% independence on the type of irradiated material. A post-irradiation effect was also demonstrated for cotton and silk samples; during four years after irradiation, there was an increase in the total color difference, and further cleavage of the macromolecules occurred. They also concluded that gamma radiation could be used for the disinfestation of cultural heritage. However, this method is not suitable for the disinfection of the objects made of cellulosic and proteinaceous materials.

There are many different opinions about the application of radiation technology in the preservation of cultural heritage, and thus the results obtained so far are controversial.

1.6 Objectives and outline of the thesis

1.6.1 Objectives of the thesis

As discussed in the previous part, cultural heritages such as ancient books, ancient documents, and wooden artifacts in libraries or museums often suffer from fungal infections of varying degrees. In many cases, the humidity in the storage changes in an undesirable direction, such as flooding by storms or tsunamis, an explosion of these harmful fungi will occur. These fungi not only degrade these cellulose items but also damage the health of the conservators and their users. The applying of appropriate methods to eliminate them is necessary. Nowadays, the

application of ionizing radiation technology to disinfect fungi from cultural artifacts is the most promising method.

In Japan, rescuing books and the old documents of the archives from natural disasters, such as floods, typhoons, and tsunamis, has been a severe problem. Many documents in a storehouse were extensively damaged by floodwater in Hyogo, Japan, in 2004 and a large volume of historical archives was extensively damaged by floodwater, following Typhoon Hagibis in Fukushima, Japan, in October 2019, for example. Preservation of the archives has been urgently required because the deterioration or loss of cultural properties would quickly occur, affecting the keeping of the unique local traditions and cultures in all the regions in Japan as well as in other Asian countries.

In Vietnam, woodblock, a vital cultural heritage, is the first candidate for fungal decontamination. Lime water and gas fumigation have been used as a conventional method for preserving this wooden artifact. No study using ionizing radiation for disinfection has been conducted because of the high cost, and the damage risk may occur during transporting to the radiation facilities. Portable irradiation, such as low energy X-rays irradiator, may overcome those drawbacks to essential to treat such archives with a museum.

In considering such a situation, we employed gamma rays for decontamination of archives and low energy X-rays for disinfection woodblock, a most important cultural heritage in Vietnam.

This thesis aimed to explore the effect of gamma radiation on fungal growth, particularly the radiation sensitivities of conidium-, germinating conidium, and mycelium-contaminated wet and dry paper, and the mechanical properties of the

traditional Japanese paper (Kohzo-gami) using its replica paper to evaluate the feasibility of the irradiation sterilization. The additional purpose of the thesis is to demonstrate the practical application of irradiation in fungal-damaged paper documents rescued from a flood in Japan by using industrial gamma radiation service. Moreover, the research also intends to investigate the effect of X-rays on the fungal contaminated wooden artifacts in Vietnam to find an effective way to disinfect the harmful fungal effect on the cultural heritage of Vietnam.

1.6.2 Outlines of the thesis

The thesis is divided into the following sections:

Chapter 1. A general introduction. The general state of fungi contaminated with the books, paper documents, and other artifacts is introduced. The current decontamination methods, such as the conventional method and radiation techniques applying to the cultural heritage is also presented. The advantages of radiation methods are reported to explain the reason for the acceptance widely in many countries of radiation technologies' application for preserving culture. Moreover, the ionizing radiation effect on the material is covered in this chapter.

Chapter 2. In this chapter, the effect of gamma irradiation on fungal growth stages and mechanical properties of the traditional Japanese paper, Kohzo-gami were investigated and discussed. In particular, the radiation sensitivities of conidium, germinating conidium, and mycelium-contaminated on wet and dry paper were carried out. Our findings provide valuable insights into disinfecting fungus-contaminated paper.

Chapter 3. Application of gamma radiation for disinfection of fungi in a large volume of historical archives damaged by flood following Typhoon Hagibis

2019, Japan. The result of sterilization on a large volume of paper documents degradation with fungi by flood following Typhoon Hagibis 2019 at a dose of 13 kGy to 16 kGy by using industrial gamma radiation service was reported in this chapter.

Chapter 4. Disinfection of the Nguyen Dynasty's Woodblock of Viet Nam by Low Energy X-rays. Radiation sensitivity of *Cladosporium* sp. isolated from woodblock was examined by using X-rays and gamma-ray. Decontamination of woodblock by 1mm aluminum filter (F1) X-ray and without filter (F0) X-ray irradiation was conducted at the top, the middle, and the bottom position of woodblock (17 mm thickness) to calculate the disinfection dose for woodblock by both side irradiation of X-rays (F1).

Chapter 5. Conclusion. The results of the thesis are summarized and the future perspectives are described.

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Chapter 2

Effect of gamma radiation on fungal growth stages and mechanical properties of traditional Japanese paper

2.1 Introduction

Sustained heavy rainfall has resulted in flash floods and submerged plains in Japan in recent years. The protection of historical paper documents from such flooding has become a severe problem for individual and organizational collectors. The prolonged exposure of paper documents to water is conducive for fungal growth. The fungal species that contaminate paper not only cause biodegradation of valuable books and documents, but also pose a threat to human health. These fungi can cause asthma and allergy in humans (Borchers et al., 2017; Mousavi et al., 2016).

Gamma irradiation has been used in recent times for mass disinfection of historical documents damaged by natural disasters because of its advantages of in-depth activity, homogenous effect, and rapid treatment of multiple objects (Calvo et al., 2017)(Moise et al., 2017). Several studies have demonstrated the use of gamma irradiation to disinfect paper contaminated with fungal species; however, the optimal dose and its effect on paper were variable (Choi et al., 2012; Gonzalez et al., 2002; M. da Silva et al., 2006).

The different stages of fungal growth include conidia germination, hyphal development, and conidia production (Fig 2.1). These stages can contaminate flood-damaged paper. In particular, mycelial growth can deteriorate paper quality by producing various catabolic enzymes, pigments, and toxic metabolites (Mesquita et al., 2009a; Sato et al., 2014; Sterflinger and Pinzari, 2012). While several studies have identified the effective radiation dose for the disinfection of fungal conidia, only a few studies have determined the effective radiation dose for the disinfection of fungal mycelia. This is because it is difficult to measure the effective radiation dose for each cell. That is, unlike conidia, mycelia are

multicellular and inseparable structures, making it challenging to quantify the number of mycelia using the common dilution plating techniques.

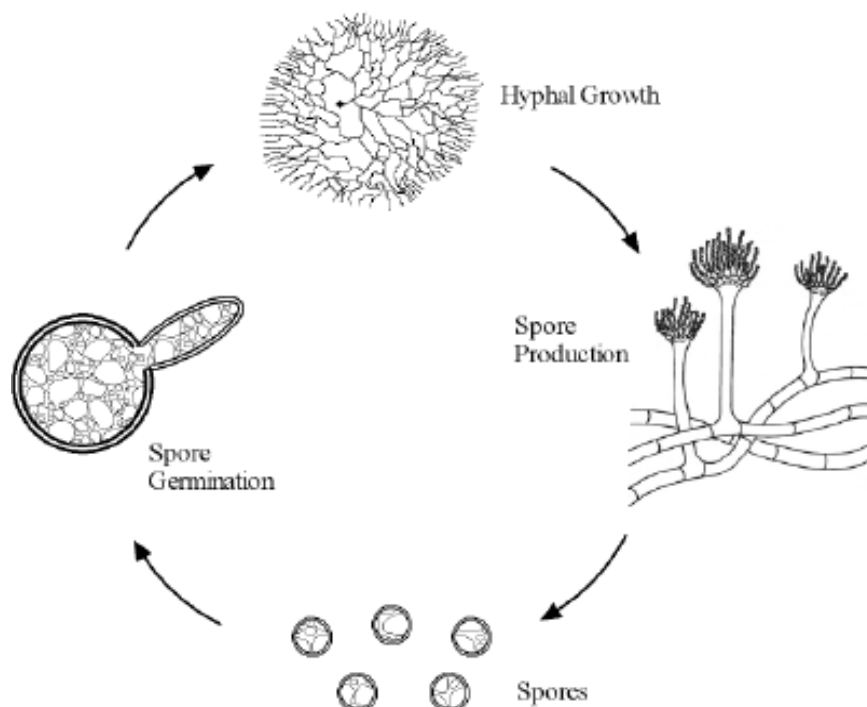


Fig 2.1 Life cycle of fung (<http://www.blackmould.me.uk>)

Water activity (a_w) is one of the most important factors for fungal growth, and reduced a_w can cause drought stress. In order to compare the radiation sensitivities of each fungal growth stage in both wet and dry conditions, we calculated the radiation dose capable of inactivating 50% (ID50) of a 30-sample population of dormant conidia, germinated conidia, and mycelia. The paper samples were artificially contaminated and cultured. From a microbiological perspective, we investigated the optimal irradiation dose to minimize the degradation of Kohzo-gami and maximize the efficiency of fungal disinfection.

The aim of this chapter was to investigate the effect of gamma irradiation on fungal growth and mechanical properties of the traditional Japanese paper, Kohzo-gami, using its replica. In particular, to compare the radiation sensitivities of each

fungal growth stage in both wet and dry conditions, we calculated the radiation dose capable of inactivating 50% of a 30-sample population of conidia, germinated conidia, and mycelia. The paper samples were artificially contaminated and cultured. From a microbiological perspective, we investigated the optimal irradiation dose to minimize the degradation of Kohzo-gami and maximize the efficiency of fungal disinfection.

2.2 Material and methods

2.2.1 Sampling procedure

Many documents in a storehouse were extensively damaged by flood water in Hyogo, Japan in 2004. After being rescued, two months later, these documents were frozen and freeze-dried for 6 months. Six Japanese flood-damaged books (approximately 120-years-old, from the Edo period) were sent to the laboratory for examination (Fig. 2.2; A–D). Deteriorating spots on paper representing color change, vulnerability, or foxing were randomly chosen and observed under a stereoscopic microscope. To isolate a wide range of fungi, potato dextrose agar (PDA) medium (for hydrophilic and mesophilic fungi) and M40Y medium (for xerophilic fungi) were used. A total of 35 spots with confirmed mold infection were directly inoculated on both media using sterile forceps, and incubated for 7 to 15 days at 25° C. Chloramphenicol, a broad-spectrum antibiotic, was used in all culture media to inhibit bacterial growth. All strains isolated from the water-damaged books were stored at -80° C until further experiments.

A replica of Kohzo-gami prepared by Mr. Ebuchi (Kochi Prefectural Center for Paper Industrial Technology) was also used. The replica paper was prepared under conditions similar to those used for raw material preparation in the Edo period. *Broussonetia papyrifera* (Kozo) and rice flour were mixed at a 1:1 weight

ratio, and this mixture was characterized by crosslinking of starch granules with Kozo fibers (Fig. 2.2; E).

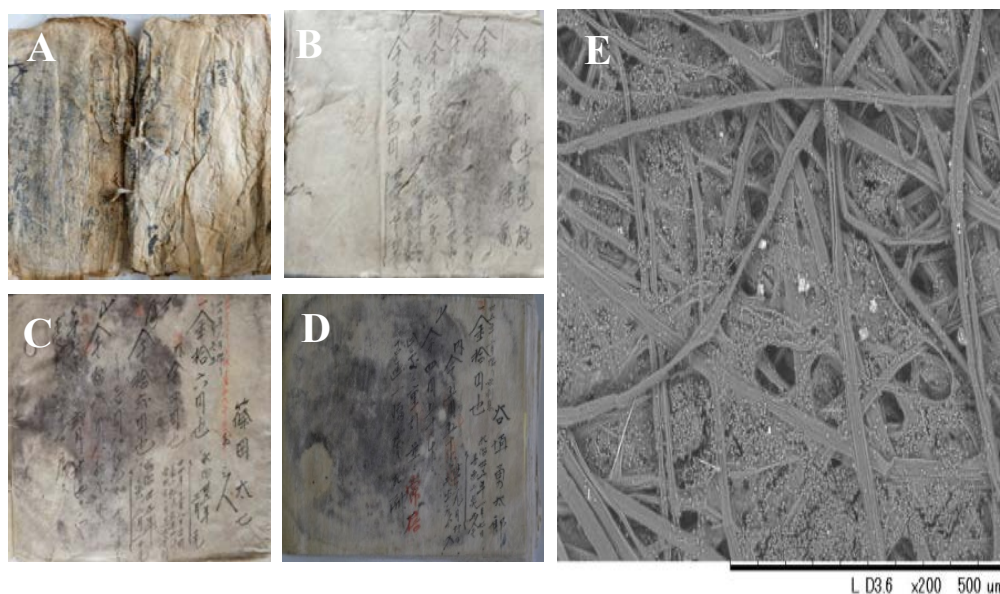


Fig. 2.2. Traditional Japanese books damaged by flood water in Hyogo (A, B, C, D); SEM micrograph of Kohzo-gami (E). Starch granules attached to Kozo fibers were observed.

2.2.2 Identification of isolated fungal species

The fungal species isolated from paper were identified using morphological (R. A. Samson et al., 2014) and molecular methods. DNA extraction, PCR targeting the ITS region, and sequencing analysis were carried out as described by Kumeda et al. (Kumeda and Asao, 2001; Kumeda, 2006). To identify the species of *Aspergillus* and *Penicillium*, we targeted β -tubulin (*benA*) and calmodulin (*CaM*) genes as well as the ITS region, as described by Samson et al. (Sommer et al., 1972; Visagie et al., 2014). The following primers were used: ITS1 and ITS4 (White et al., 1990), Bt2a and Bt2b (Glass and Donaldson, 1995), and CMD5 and CMD6 (Hong et al., 2005). All PCR products were sequenced at Macrogen Japan Corp. (Kyoto, Japan). The obtained sequences were aligned and compared with

existing sequences of a large database of fungal species using the nucleotide-nucleotide BLAST (blastn) tool.

2.2.3 Sample preparation and irradiation

◇ *Irradiation*

A Co-60 source was used for gamma irradiation (dose rate: 2.41 kGy/h) in the irradiation facility at Radiation Research Center (Osaka Prefecture University, Sakai City, Japan).

◇ *Radiosensitivity test for isolated fungal species*

To examine the radiosensitivity of fungal species contaminating paper documents, three representative isolates - *Penicillium chrysogenum*, *Aspergillus sydowii*, and *Cladosporium cladosporioides* - were selected from a pool of 35 isolates. The fungal isolates were grown on PDA for 7 days at 25° C. Conidia were harvested using phosphate-buffered saline (PBS, pH 7.0) with 0.05% Tween 80. After filtration, the conidia suspension was centrifuged and re-suspended in PBS. The density was adjusted to ca. 10^7 conidia/ml using an optical plastic plankton counter (Matsunami, Osaka, Japan). Five vials of aqueous conidia suspension (10^7 /ml) were prepared for irradiation. To prepare dried conidia samples, five (1 cm × 1 cm) pieces of Japanese paper were inoculated in 10 µl conidia suspension (10^7 /ml) and dried overnight in a biological safety cabinet at ca. 32% relative humidity at 25° C. Both aqueous and dried samples were irradiated under normal atmospheric conditions at 25° C with gamma rays from the Co-60 source. The irradiation doses for *P. chrysogenum* and *A. sydowii* were 0.5, 1, 1.5, and 2 kGy, and for *C. cladosporioides* was 1, 2, 3, and 4 kGy. The viability of irradiated and non-irradiated conidia was determined using the dilution plating method with

PDA. Dose-response curves were constructed and fitted through linear regression. The D_{10} value was calculated from the appropriate equation using the best fit value.

◇ ***Radiosensitivity test for dormant conidia, germinating conidia, and mycelia of *C. cladosporioides****

To determine the radiosensitivity of all growth stages of *C. cladosporioides*, a 5- μ l suspension of *C. cladosporioides* conidia (50 conidia) was inoculated on paper disks ($\Phi = 0.6$ cm) placed on PDA plates, and incubated at 25° C for 0, 12, and 24 h. The samples were divided into two groups: “dry”, where samples were dried overnight in a biological safety cabinet at ca. 32% relative humidity at 25° C, and “wet”, untreated samples. A total of 30 samples were subjected to each radiation dose. All samples were irradiated according to the following scheme: incubation time, 0 h: 1, 2, 3, 4, 5, 6, and 7 kGy; incubation time, 12 h: 1, 2, 3, 4, 5, and 6 kGy; and incubation time, 24 h: 7, 8, 9, 10, 11, 12, and 13 kGy. After irradiation, all samples were placed on PDA plates and incubated at 25° C for 1 to 4 days. The ability to form a visible colony was accepted as the criterion for survival. Survival percentage (%) = (number of paper samples showing colony formation / total number of paper samples) \times 100. The ID50 of *C. cladosporioides* population was calculated by interpolation.

◇ ***Statistical analysis***

All irradiation experiments were conducted independently in triplicate. The data are presented as the mean \pm S.D. Statistical analysis was performed with student's *t*-test using the Excel 2016 software (Microsoft Corp., USA), and statistical significance was set at $p < 0.05$.

2.2.4 Measurement of gamma irradiation-induced deterioration of paper

The replica of Kohzo-gami was cut in 15 mm × 180 mm strips according to JIS P 8113:2006 (ISO 1924-2:1994, 2006). Ten probes of the sample were gamma irradiated with 10, 30, and 40 kGy, and subjected to tensile test and colorimetry. The tensile strength was measured for both irradiated and control samples (n=10) using a tensile tester machine (Shimadzu AG-1 5KN) at 10 mm/s. A spectrophotometer (Konica Minolta, CM-2600d) was used to obtain L*a*b* coordinates, as described by ASTM E1347-06, 2015 (ASTME 1347-06, 2015). The color difference (ΔE^*) before and after irradiation was calculated using the formula: $\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$, where ΔL^* , Δa^* , and Δb^* are differences in L*, a*, and b* values before and after irradiation, respectively. Moreover, ΔE^* values were converted into the National Bureau of Standards (NBS) unit using the equation: NBS unit = $\Delta E^* \times 0.92$ (D. L. da Silva et al., 2013).

Both tensile test and colorimetry were performed in Kyoto Municipal Institute of Industrial Technology and Culture at 59% relative humidity and 20° C. The results are a mean of 10 samples. Student's *t*-test was used to analyze results.

2.3 Results and discussion

2.3.1 Isolation and identification of fungal species from old deteriorated Japanese paper

A total of 35 fungal species from 16 genera were identified based on the molecular and morphological characterization (Table 2.1). The most frequent genus was *Penicillium* (22.9%), followed by *Aspergillus* (14.3%), *Cladosporium* (14.3%), and *Chaetomium* (14.3%). Other less frequent genera were *Epicoccum*, *Rhodotorula*, and *Paecilomyces* (2.85%). The results showed that a large number

of fungal species found on paper belonged to some of the most important genera found in museums (Sterflinger and Piñar, 2013), such as *Penicillium*, *Aspergillus*, *Cladosporium*, *Chaetomium*, *Paecilomyces*, and *Epicoccum* (Calvo et al., 2017; Carlo et al., 2016; Lugauskas and Krikštaponis, 2004; Tomazello and Wiendl, 1995)

Table 2.1. *Fungal species isolated from traditional Japanese books damaged by flood water in Hyogo*

Species	Number
<i>Aspergillus</i> spp.	5
<i>A. sydowii</i>	1
<i>Others</i>	4
<i>Penicillium</i> spp.	8
<i>P. chrysogenum</i>	2
<i>P. thomii</i>	1
<i>Others</i>	5
<i>Cladosporium</i> spp.	5
<i>C. cladosporioides</i>	4
<i>Other</i>	1
<i>Chaetomium</i> spp.	5
<i>C. globosum</i>	2
<i>C. subaffine</i>	1
<i>C. coarctatum</i>	1
<i>Other</i>	1

Species	Number
<i>Coniochaeta</i> sp.	1
<i>Cutaneotrichosporon dermatis</i>	1
<i>Diplomitoporus rimosus</i>	1
<i>Epicoccum</i> sp.	1
<i>Nectriaceae</i> sp.	1
<i>Rhodotorula</i> sp.	1
<i>Peniophora</i> spp.	2
<i>P. incarnate</i>	1
<i>Other</i>	1
<i>Paecilomyces formosus</i>	1
<i>Pallidocercospora crystalline</i>	1
<i>Phaephlebiopsis ignerii</i>	1
<i>Riopa metamorphosa</i>	1
Total	35

In this study, no xerophilic fungal species was identified. Almost all fungal isolates from the paper samples were mesophilic, including *P. chrysogenum*, *A. sydowii*, and *C. cladosporioides*. The growth rate of mesophilic fungi at 0.85 aw (water activity) is slow, as they reach the highest growth rate at 1.0-0.99 aw (Pitt and Hocking, 2009). It has been assumed that mesophilic fungal strains that inhabit soil and adhere to water-damaged paper can germinate and grow only for a few days or weeks. However, the results of our study indicate that mesophilic fungi could survive for relatively long periods after freeze-drying.

2.3.2 Irradiation of paper samples with gamma rays

To investigate the effect of gamma irradiation on fungal viability, we selected *A. sydowii*, *P. chrysogenum*, and *C. cladosporioides* from the 35 identified species for the subsequent experiments. These fungal strains are ubiquitously found and can cause severe degradation of paper constituents (Mesquita et al., 2009b)(Magaudda, 2004). The dormant conidia of these three isolates were gamma irradiated in aqueous suspension (wet condition) as well as in their dried form on paper (dry condition). Fig. 2.3 and Fig. 2.4 show the survival curves fitted through linear regression. The radiation sensitivity of *C. cladosporioides* was lower than that of *A. sydowii* and *P. chrysogenum* under both conditions. The D_{10} values for all isolates are shown in Table 2.2. For the dry condition, the D_{10} value for *C. cladosporioides* was significantly higher than those for *A. sydowii* and *P. chrysogenum* ($p < 0.01$). Several studies have demonstrated that different fungal species possess varying degrees of radiosensitivity (Saleh et al., 1988). *C. cladosporioides* is one of the most radioresistant species because of their thick cell wall that contains melanin. Therefore, *C. cladosporioides* was used in subsequent experiments.

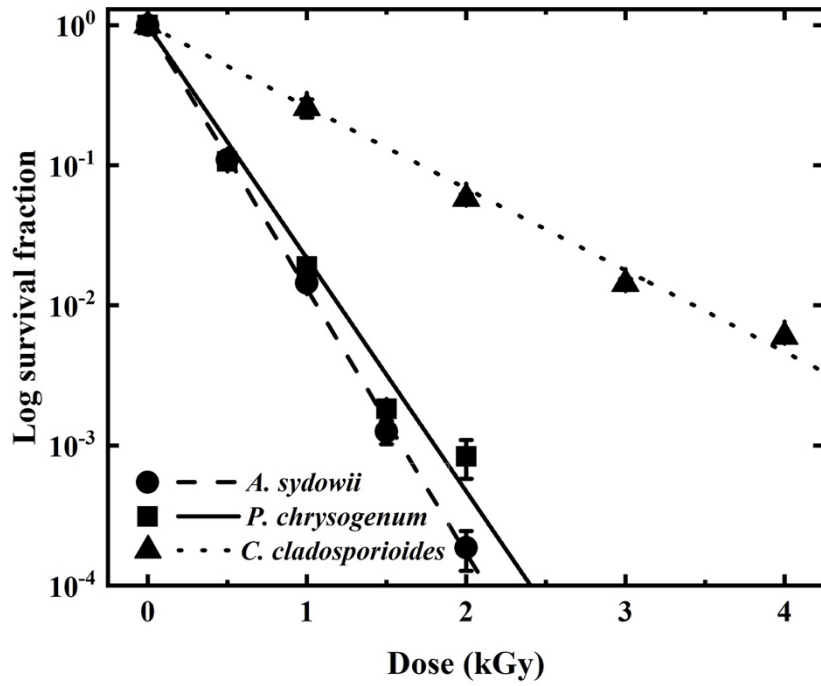


Fig. 2.3 Survival curves of gamma irradiated dormant conidia of *A. sydowii*, *P. chrysogenum*, and *C. cladosporioides* in aqueous suspension

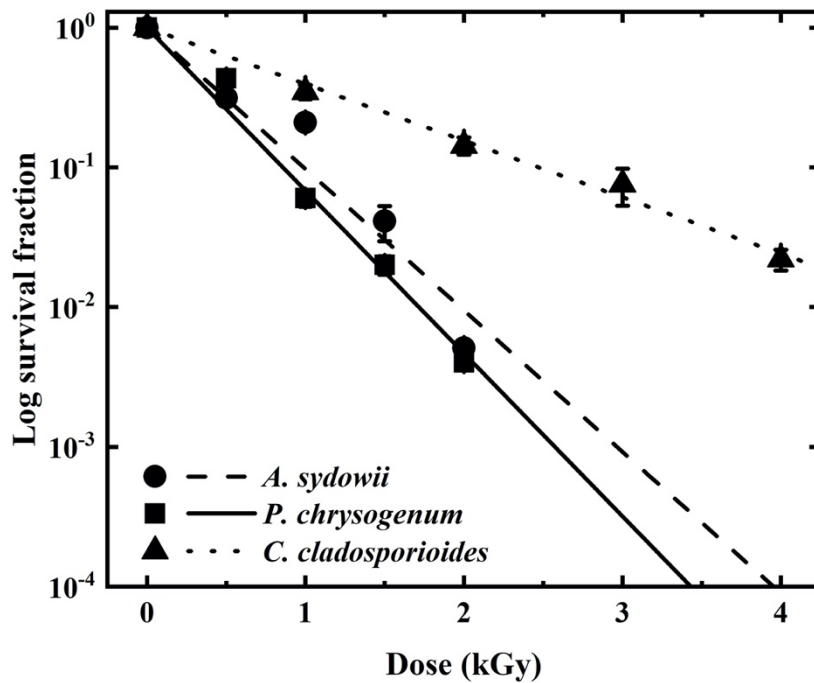


Fig. 2.4 Survival curves of gamma irradiated dormant conidia of *A. sydowii*, *P. chrysogenum*, and *C. cladosporioides* in dried on paper

Table 2.2. Radiation sensitivity of conidia of the fungal strains from traditional Japanese books

Species	D ₁₀ (kGy)	
	Aqueous suspension	Dried on paper
<i>Penicillium chrysogenum</i>	0.55 ± 0.01	0.89 ± 0.2 ^a
<i>Aspergillus sydowii</i>	0.53 ± 0.01	0.99 ± 0.9 ^a
<i>Cladosporium cladosporioides</i>	1.68 ± 0.04	2.45 ± 0.16 ^b

¹⁾ The D₁₀ values were determined by calculating the survival slope as shown in Fig. 2.3 and 2.4

²⁾ Different letters in same column represent significant differences ($p < 0.05$).

To determine the radiation sensitivity of dormant conidia, germinating conidia, and mycelia of *C. cladosporioides* on wet and dry paper, a modified end-point method was used (Sommer et al., 1972). Briefly, the 30-sample population of each stage was exposed to a selected radiation dose. After all the samples were cultured on PDA, the survival percentage was plotted against the radiation dose. The ID50 of the population was determined by interpolation, and their radiation sensitivities were compared. As shown in Fig. 2.5 and Table 2.3, under the wet condition, the radiation resistance in fungi was increased as their growth stage advanced. The ID50 for germinating conidia (3.71 ± 0.23) was slightly higher than that for dormant conidia (2.57 ± 0.05). Moreover, the ID50 for mycelia (11.94 ± 0.67) increased considerably. This result was expected because it is known that the number of fungal cells increase exponentially with an increase in the incubation time (Fig. 2.5; A1 to C1). However, under the dry condition, the ID50 for germinating conidia was lower than that for dormant conidia, although the number of germinating conidia was slightly higher than that of dormant conidia.

Table 2.3. Radiation sensitivities of conidia, germinating conidia, and mycelia of *C. cladosporioides* under wet and dry conditions

<i>C. cladosporioides</i>	50% inactivation dose (ID50) (kGy)	
	Wet condition	Dry condition
Conidia	2.57 ± 0.05	2.70 ± 0.24
Germinating conidia	3.71 ± 0.23 ^a	0.98 ± 0.04 ^b
Mycelia	11.94 ± 0.67 ^a	9.17 ± 0.15 ^b

¹⁾ The 50% inactivation dose (kGy) was determined by calculating the survival slope.

²⁾ Different letters in same row represent significant difference ($p < 0.05$).

The ID50 for dormant conidia did not significantly differ between the wet and dry conditions ($p > 0.1$). However, the survival percentage of dry dormant conidia was higher than that of wet dormant conidia at 4–6 kGy (Fig. 2.5; A2). In contrast, the ID50 for dry germinating conidia and dry mycelia were significantly lower than that of wet germinating conidia and wet mycelia ($p < 0.01$, $p < 0.05$), respectively (Table 2.3). Remarkably, there was a marked decrease in the radiation resistance of dry germinating conidia (Fig. 2.5; B2).

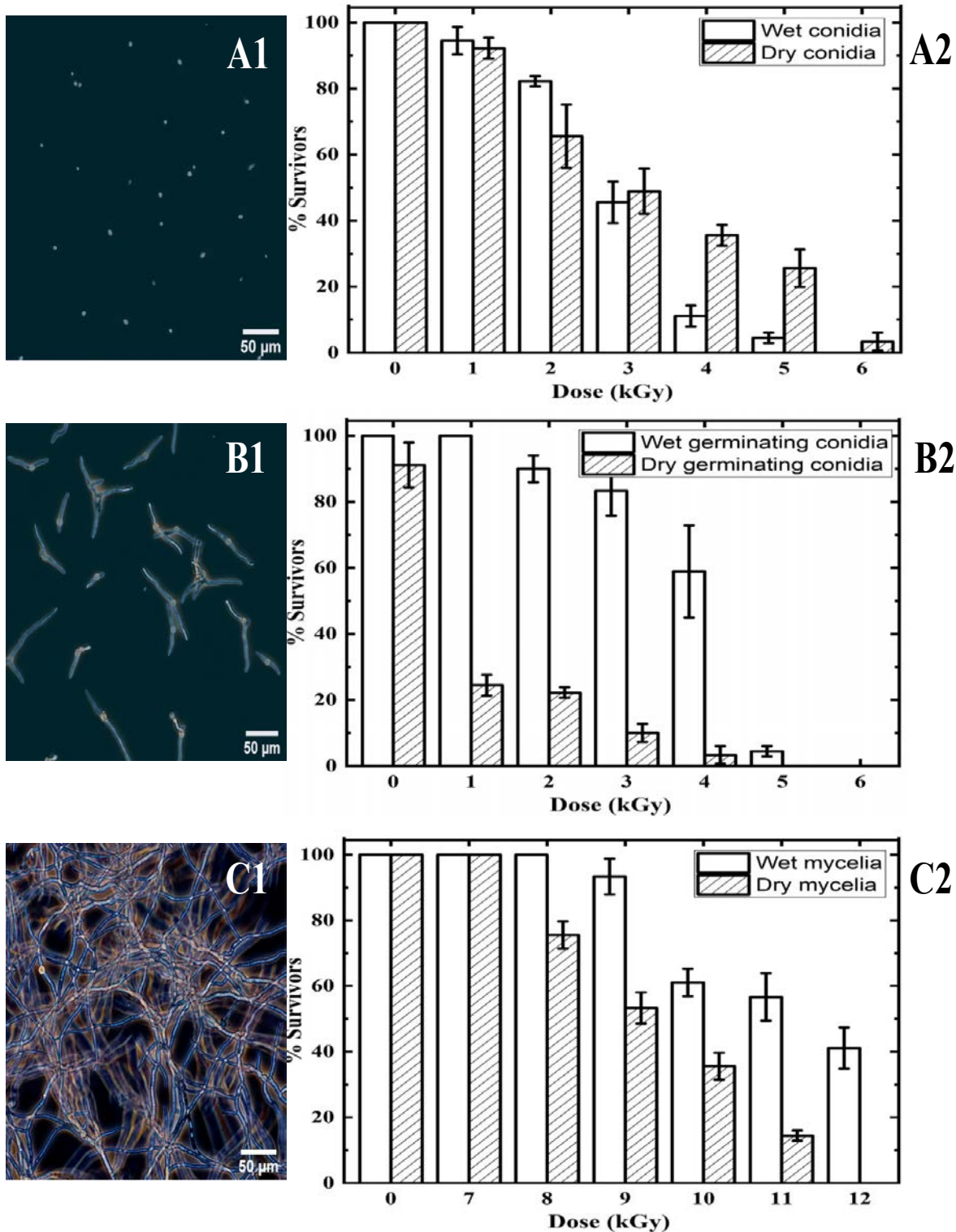


Fig. 2.5. Survival percentages of dormant conidia, germinating conidia, and mycelia of *C. cladosporioides* after exposure to gamma radiation under wet and dry conditions. Optical microscopic photographs of dormant conidia, germinating conidia, and mycelia are shown in A1, B1, and C1, respectively. The survival percentage for each stage is shown in A2, B2, and C2.

Dry dormant conidia are thought to be more resistant to radiation than wet dormant conidia because the radiation sensitivity of microorganisms varies according to the water content of the solvent. Gamma irradiation of water generates reactive free radicals that indirectly affect radiation sensitivity (Azzam et al., 2012). Therefore, the radiation sensitivity of dormant conidia on low-water-content paper was lower than that of dormant conidia on wet paper. The same explanation was applied to the results presented in Table 2.2. Additionally, dormant conidia can easily adapt to dry conditions and are highly resistant to drought stress (Krijgsheld et al., 2013). Conidia formation can facilitate the distribution of fungi in space and time. The metabolic rate of stable conidia is low, and their contents are protected by a thick, often pigmented cell wall containing accumulated compatible solutes (Dijksterhuis, 2019). This defense mechanism is thought to enhance resistance of dormant conidia to radiation. Mohyuddin et al. (Mohyuddin et al., 1972) have reported that the radiation sensitivity of germinating conidia is higher than that of dormant conidia. This increased radiation sensitivity is attributed to cell development processes during germination and active DNA synthesis. In the presence of water, germinating conidia extend hyphae from the tips after swelling. Germinating conidia are sensitive to environmental conditions including a reduction in water activity (Grindle and Good, 1961; Kim et al., 2016). Such sensitivity to drought stress was apparent from our results, which showed that the survival rate of control samples (without irradiation) under the dry condition was already approximately 90% (Fig. 2.5; B2). Fungal mycelia are also more sensitive to drying than dormant conidia because they require water to proliferate. Our results showed that drought stress can significantly enhance radiation sensitivities of germinating conidia and mycelia.

2.3.3 Mechanical properties

The tensile strength of paper refers to the ability of paper to endure tension. Factors including fiber resistance and fiber network formation can affect tensile strength. The tensile strength of Kohzo-gami exposed to different radiation doses is shown in Fig. 2.6. The order of tensile strength of Kohzo-gami relative to that of control samples was as follows: 10 kGy ($p < 0.01$) > 30 kGy ($p < 0.01$) > 40 kGy ($p < 0.01$). The tensile strength would increase due to the increase in fiber durability, which is a result of the adhesion between lateral fibers, at a low dose (10 kGy), while the tensile strength would gradually reduce, due to the decrease in fiber durability, with increasing radiation dose (40 kGy), owing to the increasing degradation of the fiber structures. This result suggests that even a 10 kGy radiation dose can affect the mechanical properties of paper.

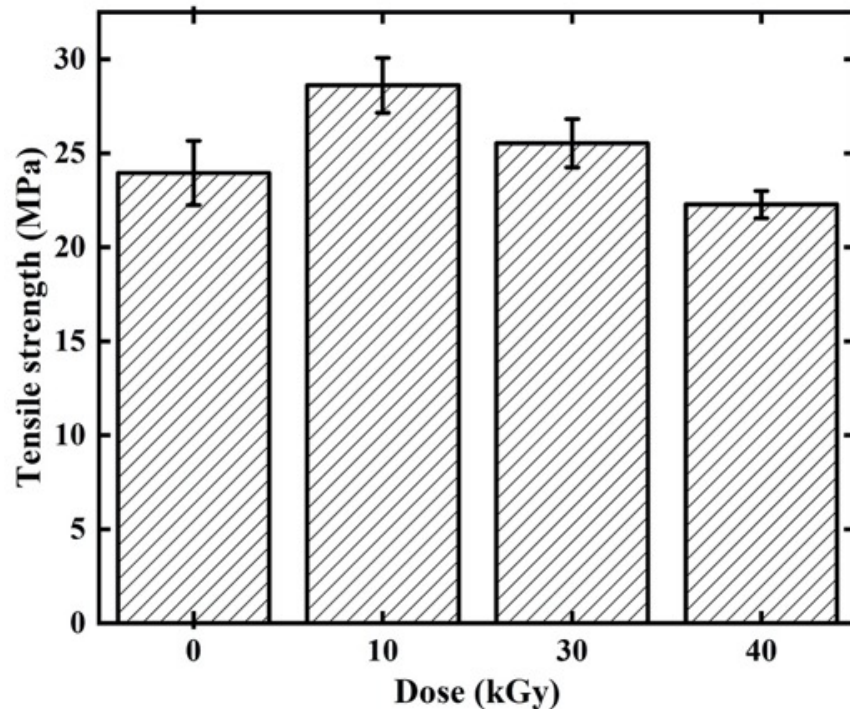


Fig. 2.6. Tensile strength of the Japanese paper (Kohzo-gami) after exposure to gamma radiation at 10, 30, and 40 kGy.

The color differences among the irradiated samples of Kohzo-gami are shown in Table 2.4. An increase in color change was observed at all radiation doses, although the increase was not significant. The NBS rating showed the “slight change” remark at all doses. The major contribution to color change was a shift on the a-axis towards negative values ($p < 0.05$), corresponding to greening of the sample. The paper mulberry (Kozo) fibers are thought to confer substantial resistance to irradiation by increasing the crosslinking density and cellulose polymerization. Moreover, excessive interstitial starch granules can enhance a chemical change, such as hydrolysis.

Table 2.4. Color differences in traditional Japanese paper (Kohzo-gami) after exposure to gamma radiation at 10, 30, and 40 kGy.

Absorbed dose (kGy)	$\Delta L^*(D65)^{1)}$ (Mean \pm SD)	$\Delta a^*(D65)^{1)}$ (Mean \pm SD)	$\Delta b^*(D65)^{1)}$ (Mean \pm SD)	$\Delta E^*(D65)^{1)}$ (Mean \pm SD)	NBS rating	
					NBS unit ²⁾	Critical remark
10	0.18 \pm 0.41	-0.40 \pm 0.26	-0.12 \pm 0.45	0.73 \pm 0.34	0.67	Slight
30	0.50 \pm 0.38	-0.24 \pm 0.11	-0.31 \pm 0.31	0.70 \pm 0.41	0.65	Slight
40	0.09 \pm 0.42	-0.09 \pm 0.19	0.57 \pm 0.46	0.80 \pm 0.34	0.74	Slight

¹⁾ L* indicates lightness and darkness, a* indicates redness and greenness, b* indicates yellowness and blueness, and $\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$

²⁾ NBS unit = $\Delta E^* \times 0.92$; NBS; National Bureau of Standards

Several studies have showed that high radiation doses of 14.4 (Gonzalez et al., 2002b), 15 (D’Almeida et al., 2009), and 50 (Choi et al., 2012) kGy did not significantly change the mechanical properties of paper. In contrast, Bicchieri et al. (Bicchieri et al., 2016) and (Drábková et al., 2018) have recently demonstrated that gamma irradiation can cause depolymerization and degradation of paper

substrates even at minimum radiation doses (3 and 4.5 kGy) required for fungal disinfection, and thus, the results obtained are controversial.

As shown in this study, the radiation dose required to disinfect fungus-contaminated papers varies according to the fungal species and total number of fungal cells on contaminated paper. Our results suggest that it is difficult to determine a standard radiation dose for the treatment of contaminated papers. However, it is important to immediately arrest fungal growth to minimize the spread of infection (Pitt and Hocking, 2009). While sterilization of paper materials may not be required, a sterility assurance criterion of 10^{-6} ($6 \times D_{10}$ value) is widely accepted in the medical industry. Moreover, a radiation dose of 8 ± 2 kGy has been recommended by several researchers for the treatment of contaminated paper (Bratu et al., 2009; Havermans, 2017b). In this study, the examined radiation dose was approximately equal to $6 \times D_{10}$ value for *C. cladosporioides* (Fig 2.5). In cases of low bioburden, the radiation dose can be reduced below 5 kGy. We suggest that books and documents that are damaged by flood water should be dried at the earliest, gamma irradiated, and conserved in an appropriate dry condition (<0.55 aw). Drought stress can not only increase radiation sensitivity of fungal mycelia, but also facilitate their degradation during the conservation period. A combination of irradiation and dry storage conditions can efficiently kill fungal mycelia on contaminated paper. Further investigation is needed to put our findings to practical use.

2.4 Conclusion

Traditional Japanese books damaged by flood water are mainly infected by mesophilic fungi, including *A. sydowii*, *P. chrysogenum*, and *C. cladosporioides*. The radiosensitivities of dormant conidia, germinating conidia, and mycelia of *C. cladosporioides* on wet and dry paper were measured using the ID50 calculated using a modified end-point method. The ID50 for dormant conidia did not significantly differ between wet and dry paper; however, the ID50 for dry germinating conidia and dry mycelia were significantly lower than those for wet germinating conidia and wet mycelia, respectively. These results indicated that drought stress can increase the radiation sensitivity of fungal mycelia, especially germinating conidia. Furthermore, even a 10 kGy radiation dose had a slight effect on the mechanical properties of paper. A combination of irradiation and dry storage conditions can be used to minimize the degradation of paper and maximize the efficacy of fungal disinfection.

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Chapter 3

**Application of gamma radiation
for disinfection of fungi in a large
volume of historical archives
damaged by flood following
Typhoon Hagibis 2019, Japan**

3.1 Introduction

A large number of historical archives were extensively damaged by flood water, following Typhoon Hagibis in Fukushima, Japan, in October 2019. They were recovered several months later, however, the prolonged exposure of the paper documents to water caused severe biodegradation caused by fungal growth (Fig. 3.1). These damaged paper documents will, in future, be cleaned by hand with tap water, frozen for temporary storage, and subsequently freeze-dried for long-term preservation. Before workers can carry out this task, disinfection of fungi that has grown on the documents is necessary, as contaminated fungi pose potential health risks (Borchers et al., 2017; Mousavi et al., 2016), as well as the risk of spreading fungal contamination to other objects.



Fig. 3.1 *The historical paper documents heavily contaminated with fungi.*

Several methods have been developed to disinfect fungi-contaminated heritage objects. Most methods involve the use of toxic chemicals, including methyl bromide and ethylene oxide (Nittérus, 2000; Ponta and Havermans, 2017), potentially ozone layer depleting and/or carcinogenic fumigants are now banned

in a number of countries (Barry et al., 2012; Dowdy, 2003). Gamma radiation draws attention to an effective alternative method in the field of conservation of cultural heritage, which has been used successfully for food and medical device sterilization for more than 50 years. Recently, several studies on the treatment of deteriorated books and archives using radiation have been reported (Bratu et al., 2009; Choi et al., 2012; M. da Silva et al., 2006). However, there are few case reports on the application of radiation to a large number of historical archives with fungal damage caused by natural disaster. In the present study, we demonstrated the successful practical use of irradiation in fungi-damaged paper documents using an industrial gamma radiation service.

3.2 Material and methods

Samples were public documents made from Japanese paper with starch paste during the Meiji era. Total packaging consisted of 29 carton boxes (ca. 560 x 400 x 400 mm) (Fig. 3.2). Twenty-three samples were wet and 6 samples were dry. The total weight was 375.6 kg. Many samples were heavily contaminated by fungi. On the most wet samples, fungi produced various color pigments and unusual musty odors.



Fig. 3.2 Packages of the fungi-contaminated paper documents for irradiation.

All samples were exposed to gamma radiations emitted by a source of Cobalt 60 at Koga Isotope, Ltd. (Shiga, Japan). Before irradiation, several samples with severe fungal contamination were used for fungal isolation. The fungal conidia or hyphae on the spots representing color change were directly inoculated on two sets of petri dishes containing either potato dextrose agar (PDA) medium or M40Y medium (for xerophilic fungi) with sterile forceps, and one of the sets was subjected to gamma-irradiation and the other was incubated for seven to fifteen days at 25°C. All experiment procedure was shown in the Fig. 3.3. Representative fungal species isolated from both wet and dry samples were identified using morphological (Samson et al., 2010) and molecular methods. The molecular analysis targeting ITS regions (Schoch et al., 2012) was conducted at Macrogen Japan Corp. (Kyoto, Japan).

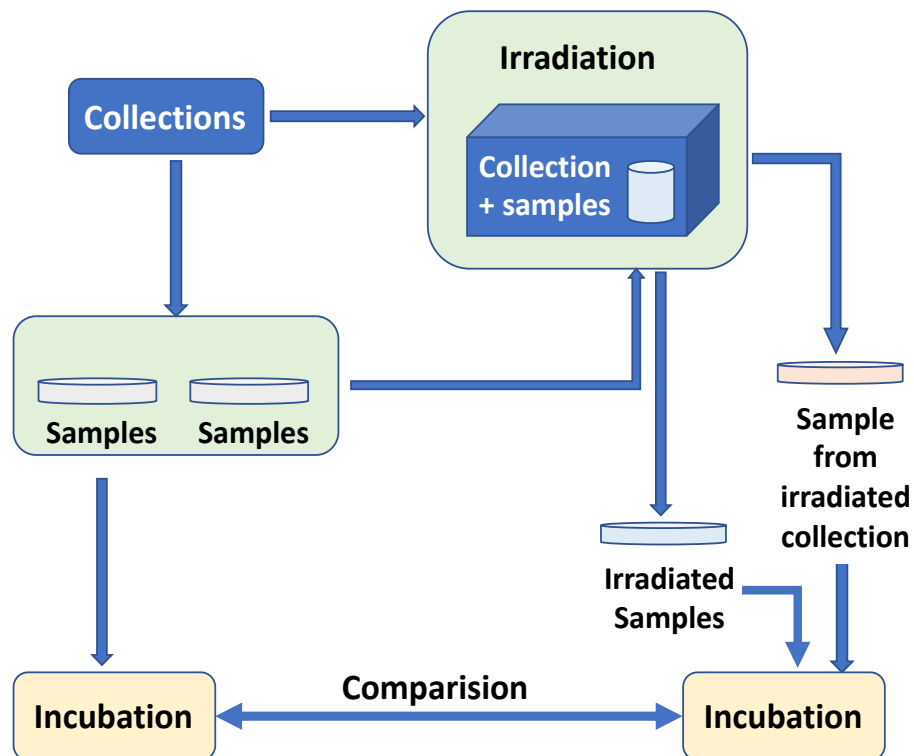


Fig. 3.3 Procedure of the experiment

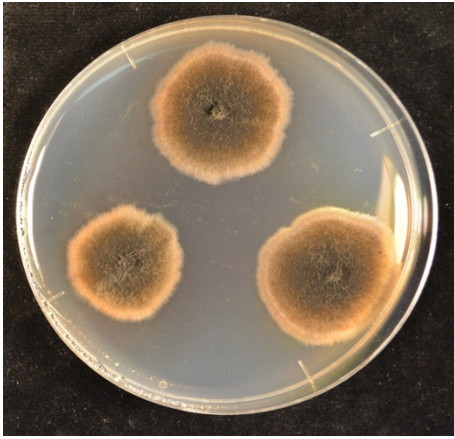
3.3 Result and discussions

Results can be found in Table 3.1. As expected, the wet samples were contaminated with hydrophilic fungi, including *Trichoderma*, *Stachybotrys*, and *Fusarium*, and the dry samples were contaminated with mesophilic fungi, including *Penicillium*. *T. harzianum* and *S. chartarum* are well-known cellulolytic fungi, commonly isolated from high cellulose contents, such as fiberboard, gypsum board, and paper when there is moisture from water damage, water leaks, or water infiltration (<https://www.cdc.gov/mold/stachy.htm>) (Fig. 3.4). As *S. chartarum* was also detected in heritage objects that were water-damaged by the Great East Japan Earthquake in 2011, the Tokyo National Research Institute for Cultural Properties issued a warning with regards to the appropriate handling of such artifacts on their website to avoid the health risk of the fungus to the personnel rescuing the damaged objects (<https://www.tobunken.go.jp/japanese/rescue/20120319.pdf>). However, at present, an association between *S. chartarum* and acute idiopathic pulmonary hemorrhage has not been proven (Borchers et al., 2017).

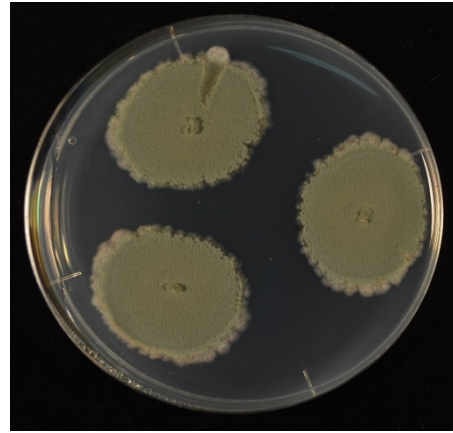
Table 3.1. Representative fungal species isolated from paper documents damaged by floods

Color change of the paper	Condition of the paper	State of the contaminated fungi		Species	Identity (%)
		Conidia production	Hyphae		
yellow	dry	++ ^a	+	<i>Penicillium commune</i>	99%
blue	wet	++	++	<i>Trichoderma harzianum</i>	99%
yellow	dry	+++	+	<i>Penicillium commune</i>	99%
black	wet	+++	+	<i>Stachybotris chartarum</i>	99%
blue	wet	++	++	<i>Trichoderma atroviride</i>	100%
blue	wet	+	++	<i>Trichoderma harzianum</i>	99%
purple	wet	—	++	<i>Fusarium</i> sp.	99%
black	wet	++	+	<i>Stachybotris chartarum</i>	99%
black	wet	+++	+	<i>Stachybotris chartarum</i>	99%

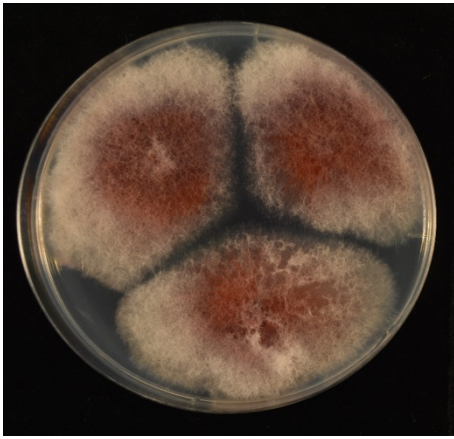
^a The level of conidia or hyphae; (+) little, (++) medium, (+++) many, and (-) no



Stachybotrys chartarum



Penicillium commune



Fusarium sp.



Penicillium commune



Trichoderma atroviride



Trichoderma harzianum

Fig.3. 4 Some fungi isolated from the paper documents damaged by floods

All 29 packages of paper documents were put into 14 totes (aluminum alloy irradiation container) for gamma irradiation and forwarded to the shielded irradiation room through a maze in the concrete, thereafter the totes were indexed around a source of radiation (Fig. 3.5).

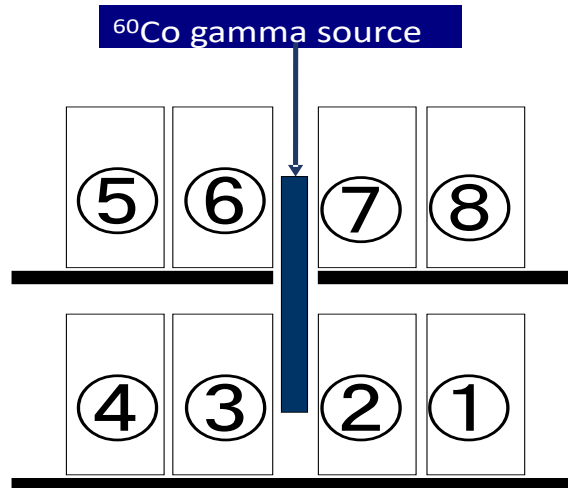


Fig. 3. 5 Cross section of the assembly of the totes during ^{60}Co -gamma irradiation at the Koga Isotope irradiation facility.

The radiation from the source penetrates through the totes to deliver the required dosage to the packages within the totes. The absorbed dose range was estimated using alanine dosimeters placed at the two areas of each tote, one expected for the highest dose (maximum dose) and the other expected for the lowest dose (minimum dose) (Fig. 3.6).

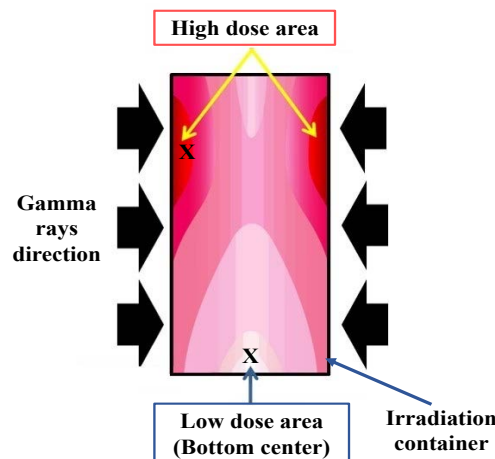


Fig. 3. 6 Gamma-ray dose distribution and position of alanine dosimeter within one tote.

Each value of the dose was measured with an e-scan alanine dosimetry system (Bruker; Massachusetts, United States) based on the ISO/ASTM 51607:2013. Depending on the position of the container, the absorbed dose varied from 11.9 to 16.5 (Table 3.2).

Table 3.2. Radiation dose distribution of the packages of paper documents in totes

Totes	Package No.	Minimum dose (kGy)	Maximum dose (kGy)
1	001 - 002	13.8	15.9
2	003	13.2	16.0
3	004 - 005	12.8	16.5
4	006 - 007	13.2	16.3
5	008 - 009	13.3	16.1
6	010 - 011	13.7	16.1
7	012 - 013	13.0	16.5
8	014 - 015	13.2	16.3
9	016 - 017	13.0	16.2
10	018 - 3019	13.7	16.0
11	020 - 3021	13.1	16.3
12	022 - 023	13.2	16.3
13	024 - 025	13.0	16.1
14	026 - 029	11.9	15.6
Average		13.1	16.1

After irradiation, the fungal conidia or hyphae on the same spots of the same samples were directly inoculated once again on PDA and M40Y, and incubated for seven to fifteen days at 25°C. The petri dishes inoculated with fungal isolates, followed by irradiation were also incubated for seven to fifteen days at 25°C. As a result, no fungi were detected on any of the media plates. The results indicated that absorbed dosages between 13.1 kGy and 16.1 kGy were sufficient to disinfect paper documents heavily contaminated with fungi, including *Stachybotrys*. All the samples after irradiation looked the same as before, and unusual musty odors were almost the same as before. However, the erosion and the spread of contaminated fungi on the samples stopped after irradiation.

The effective dose to disinfect fungi was considerably different from the previously reported dosage from 5 Gy to 20 kGy (Tomazello and Wiendl, 1995; Gonzalez et al., 2002; Silva et al., 2006), because it varies according to the fungal species (Choi et al., 2012; Saleh et al., 1988), in addition to the total number of fungal cells exposed, for example, the conidia suspension (Jeong et al., 2015) or colonies grown on media plates (M. da Silva et al., 2006). Moreover, it is apparent that high doses of gamma radiation cause depolymerization and degradation of paper substrates, and significantly change the mechanical properties of paper (Adamo et al., 2001, 2007; Bicchieri et al., 2016; Drábková et al., 2018). Thus, it is difficult to determine the radiation dose for the treatment of contaminated papers.

Previously, we investigated the effect of gamma irradiation on fungal growth and the mechanical properties of traditional Japanese paper, Kohzo-gami, infected by mesophilic fungi such as *Aspergillus sydowii*, *Penicillium chrysogenum*, and *Cladosporium cladosporioides*, and revealed that they were inactivated at a dose of 10 kGy without significantly deteriorating the mechanical properties of the paper (Chapter 2). In 2017, the International Atomic Energy

Agency (IAEA) recommended a standard radiation dose of 8 ± 2 kGy on paper materials infected with fungi caused by moisture and water (IAEA, 2017). They indicated that no serious changes were found in paper substrates from evaluation with SurveNIR spectrophotometry at the aforementioned dose. In this work, we determined the dose to be more than 10 kGy. The reason is that the bioburden seems to be significantly higher than that recommended by the IAEA report; there is an urgent need to stop fungal growth in the paper documents, and the paper degradation caused by more than 10 kGy of gamma radiation is minor compared to the degradation caused by fungal contamination.

Gamma radiation has the advantages of in-depth activity, homogenous effect, and high throughput treatment of multiple objects (IAEA, 2017; Nittérus, 2000). Additionally, gamma radiation can be used to disinfect fungi, even for objects submerged by flooding or a tsunami; chemical fungicides cannot be used for wet objects. Owing to these advantages, radiation sterilization has been re-evaluated in historical archives or heritage objects damaged by natural disasters in recent times.

3.4 Conclusion

In this work, we demonstrated that a radiation dose of 13 kGy to 16 kGy had a sterilizing effect on a large volume of paper documents degraded with fungi, caused by flooding following Typhoon Hagibis in 2019. We expect that this case report will provide useful information about the restoration of heritage materials damaged by fungal growth caused by fungi-favorable environmental conditions in future natural disasters.

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Chapter 4

**Disinfection of Woodblocks of
the Nguyen Dynasty of Vietnam by
Low-Energy X-rays**

4.1 Introduction

Cultural heritage that is inherited from past generations is an integral part of each society and country. The inherited artefacts such as wooden structures, wooden furniture, and woodblocks are a large group of the wooden cultural heritage artefacts. Among them, woodblocks play an important role. Woodblock was used widely throughout East Asia to print text, images, or patterns and originating in China. Vietnamese culture is one of the oldest in Southeast Asia, and the many intangible cultural heritage of Vietnam has been presented to the world. There are more than 34,000 plates of woodblocks from the Nguyen dynasty in Viet Nam that engraved in classical Chinese, as well as Chu Nom (the old Vietnamese scripts), record historical and literary works in the Nguyen Dynasty from the middle of the 17th century to the beginning of the 20th century. These woodblocks also evidence of the development of the woodblock carving and printing profession in Viet Nam in that time. The woodblocks of the Nguyen dynasty of Viet Nam was recognized by UNESCO as Memory of the World in 2009. From 2009, these woodblocks are preserved and displayed at the National Archive Center IV (Dalat, Vietnam). These woodblocks are damaged by insects such as termite and various fungi, which is known to be the main cause of the color change and cellulose biodegradation (Severiano et al., 2010). Fungi are the most important and conspicuous organisms capable of digesting wood products. Some fungi not only affect the quality of the cultural objects but also may be dangerous to professionals and users, due to the production of mycotoxins (Nielsen, 2003).

The lime water or gas fumigation is mainly used to preserve woodblocks (Luong, 2017; Havermans, 2017), but these woodblocks were still damaged by insects and fungi. These conventional methods are toxic to humans, and sometimes the disinfection effect is low. Therefore, the disinfection of fungi by the effective

method is strongly required for the conservation of woodblocks.

The application of radiation technologies for preserving cultural heritage has been accepted widely in many countries (Katusin-Razem et al., 2017). Radiation processing by gamma-ray from ^{60}Co and electron beam / X-rays with high energy are commonly used for radiation sterilization and food irradiation. The benefits of radiation sterilization are high-speed, high efficacy treatments under room temperature. However, these facilities require radiation shielding for irradiation buildings to protect personnel from radiation damages, which is highly expensive. The use of low energy X-rays for sterilization of medical devices and food irradiation has lately attracted more interest. The merit of low-energy X-rays is its low shielding requirements. The irradiation device using an X-ray tube with low energy is reliable, compact and cost-effective. The compact low energy X-ray irradiator has been widely used in health-care blood irradiation. In addition, the X-ray machine with a small size can be easily transport to storage places such as the museum, library.

This study aims to investigate the irradiation effect of low energy X-rays on fungi and find a suitable irradiation condition for the disinfection of woodblocks.

4.2 Materials and method

4.2.1 Materials

Woodblock samples were obtained from National Archive Center IV (Dalat, Viet Nam) (Fig. 4.1), and the small pieces (ca. 2 x 3 cm) were used for the irradiation test.

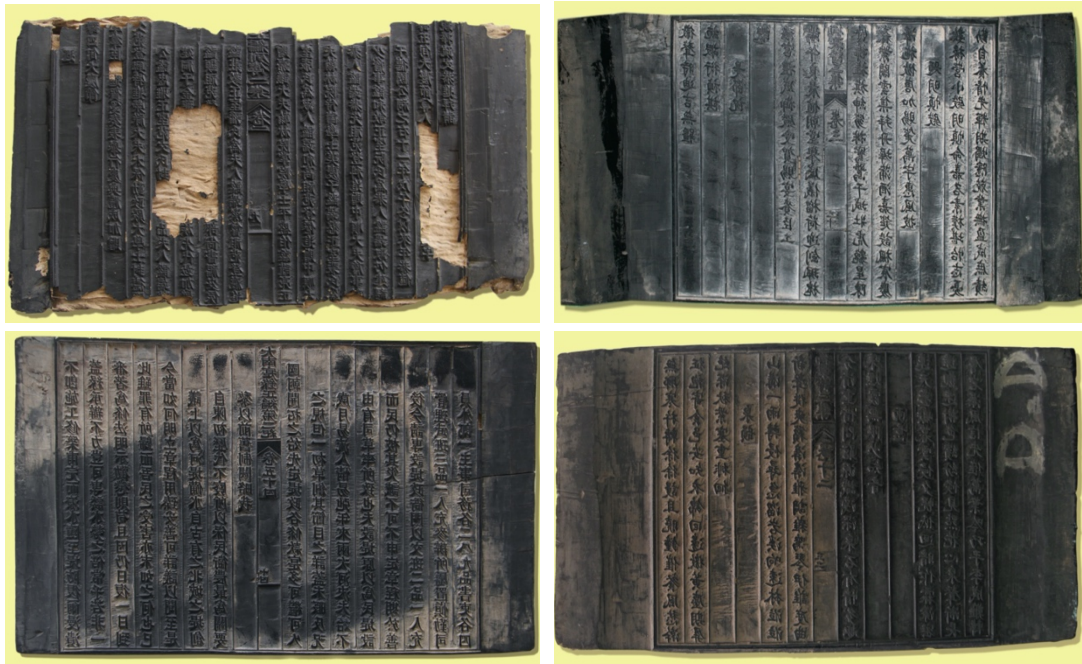


Fig. 4. 1 Some degraded woodblocks by fungi

Standard strains of fungi, *Aspergillus niger*, *Cladosporium cladosporioides*, and *Aureobasidium pullulans*, were purchased from NBRC (Biological Resource Center, NITE).

HD-V2 Gafchromic film purchased from Ashland, USA, was used for the dosimetry of X-rays. This film dosimeter is thin (ca. 110 μm) with an active layer (12 μm) coated on a polyester substrate (97 μm) and the dose range from 10 Gy up to 1000 Gy.

4.2.2 Microbiological analysis

The swab method was used to collect the fungi from woodblocks. The sterile swabs were directly rubbed on the woodblock to collect fungal cells and transferred to potato dextrose agar (PDA) medium and M40Y medium (for xerophilic fungi). Chloramphenicol, a broad-spectrum antibiotic, was used in all

culture media for inhibition of bacterial growth. Isolated fungi were identified by the morphological method (Samson et al., 2010).

The isolated fungi were incubated on PDA for 7 days at 25°C, the conidia were harvested using phosphate-buffered saline (PBS, pH 7.0) with 0.05% Tween 80. Conidia density was adjusted to ca. 10^7 CFU/ml by using a disposable Optical Plastic Plankton Counter. Japanese paper (1cm x 1cm, 0.1 mm) was inoculated with 10 μ l of the conidia suspension (10^7 CFU/ml) and dried overnight in a biological safety cabinet at room temperature and subjected for irradiation. Viable cell count after the irradiation is expressed as an average \log_{10} value with a standard deviation of triplicate experiments.

4.2.3 Irradiation

X-ray irradiation system model MBR-1618R-BE (Hitachi Power Solutions, Japan) installed at the Faculty of Physics and Nuclear Engineering (Dalat University, Dalat, Viet Nam) was used in this research. The irradiator can be changed the tube voltage (35 to 160kV), tube current (1 to 30mA), and irradiation time or irradiation dose to emit X-rays. Five types of filters are equipped to cut the very low energy part of X-rays. X-rays at 160 kV, 18.6 mA (3kW) with non-filter (F0), and 1 mm aluminum filter (F1) were used in this experiment. The distance from the X-ray focal point to the sample is 150 mm (Fig. 4.2). HD-V2 film dosimeter calibrated (Agematsu et al., 2008) with a Fricke dosimeter (Kume et al., 1976) was used to measure the dose rates of X-rays.

In case of gamma irradiation, Cobalt-60 sources were used (dose rate: 1.87 kGy/h) in the irradiation pool at Radiation Research Center of Osaka Prefecture University, Sakai City, Japan). The dose was determined with an ion chamber (Applied Engineering Inc.) and PMMA dosimetry (Yamamoto et al., 1987).

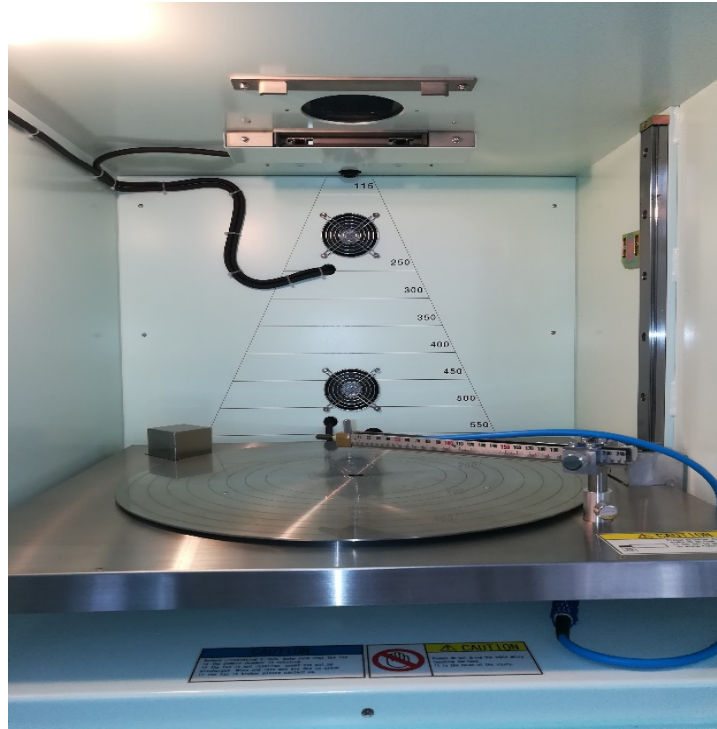


Fig. 4. 2 The distance from X-ray focal point

4.2.4 Dose uniformity ratio (D.U.R)

Dose uniformity ratio (DUR) measures the range of doses delivered to the product and is important to optimize for irradiation sensitive materials in order to minimize degradation. DUR is defined as a ratio of the maximum to the minimum dose received by anyone product (IAEA - International Atomic Energy Agency, 2013). The DUR of a radiation sterilization process is always greater than 1.0. In this experiment, D.U.R is the ratio of the absorbed dose of the surface and middle for both side irradiation.

4.3 Results and discussion

4.3.1 Isolation of fungi from woodblock samples

The contaminated fungi on woodblock samples were detected before the

study of radiation disinfection. Deteriorating spots on the woodblocks, which represent color changing, were randomly chosen by the naked eye. 14 fungal strains (hyphomycetes) were isolated from woodblocks and labeled from VN1 to VN14. The isolated fungi were incubated on PDA for 7 - 10 days at 25°C, and the conidia were harvested. These 14 strain's conidia were irradiated with gamma-ray at dose ranges from 3 to 5 kGy to select the highest radiation resistance strains. Eight strains were survived at 3 kGy, but only three strains of VN1, VN3 and VN7 were survived at 5 kGy as shown in Fig. 4.3. All of these 3 strains were belonged to the genus *Cladosporium* based on morphology identification. The highest radiation resistant VN3 (*Cladosporium* sp.) was selected for subsequent experiments.

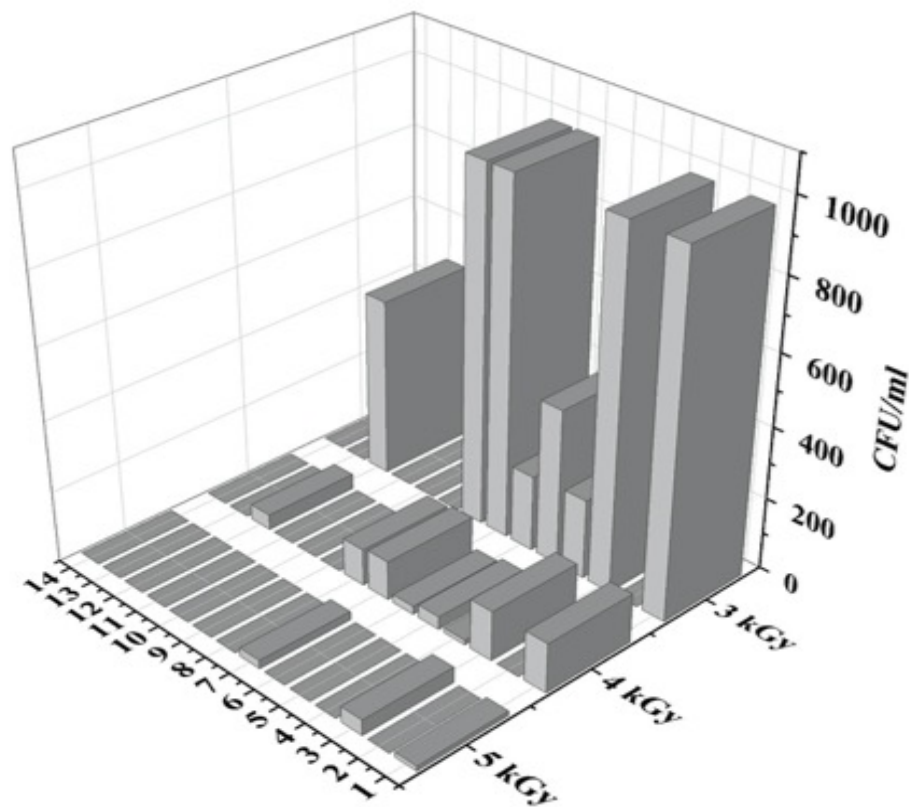


Fig. 4.3 Radiation sensitivity of fungi isolated from woodblocks. The isolated strains were label from VN1 to VN14. The conidia of 14 strains were irradiated at 3, 4 and 5 kGy by gamma-ray.

4.3.2 Radiation sensitivity of fungi

Radiation sensitivity of *Cladosporium* sp. isolated from woodblock was examined by using X-rays and gamma-ray. For X-rays, *Cladosporium* sp. conidia were exposed to 1mm aluminum filter (F1) X-ray and non filter (F0) X-ray with cut-off energies of 160 keV. Fig 4.4 shows the X-ray spectra with and without a 1mm aluminum filter for the 160 keV electron beam. Filtration absorbs the lower-energy X-ray photons, so the low energy part of 1 mm aluminum F1 decreases comparing to F0.

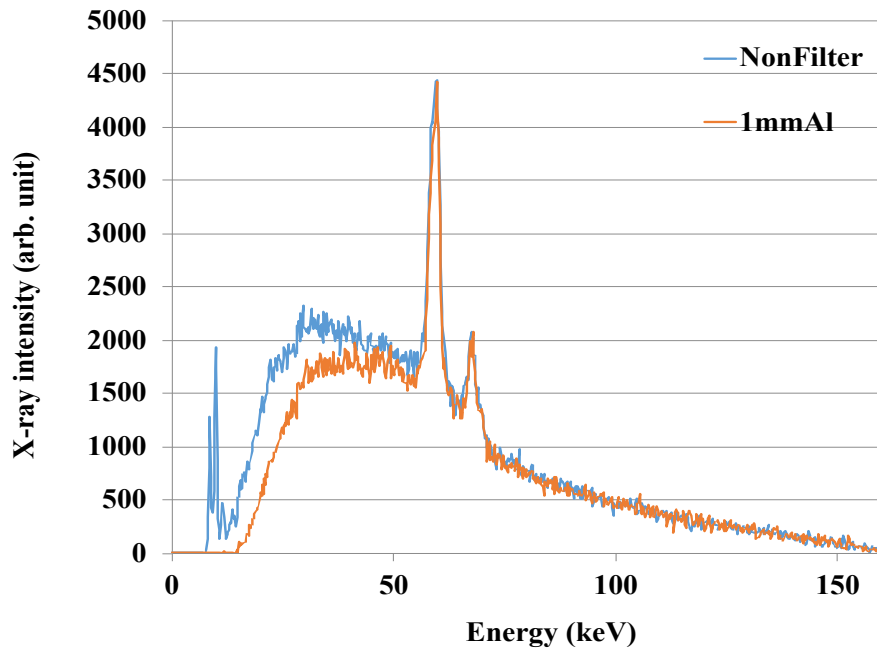


Fig. 4. 4 The X-ray spectra of 1mm aluminum filter (F1) and non filter (F0) for 160 keV (provide by Hitachi compapny)

Figure 4.5 shows the survival curves of F0 (non-filter) X-rays, F1 (1 mm aluminum filter) X-rays, and gamma-ray. Dose rates for X-rays (F1), X-rays (F0) and gamma-ray were 1.10, 4.65 and 1.87 kGy/h, respectively. All 3 survival curves of *Cladosporium* sp. showed an exponential curve. The D_{10} values for X-rays (F1),

X-rays (F0) and gamma-ray were 0.79, 0.57 and 0.89, respectively. Radiation sensitivity for X-rays (F1) and gamma-ray were close, but it was higher for X-rays F0. Ha et al., (2017) reported that the reduction of *Bacillus pumilus* spores was increased by X-ray irradiation with cut off the energy of 50-150 keV. Miura et al., (2011) also reported that the low energy component of X-rays was remarkably removed by using the filter, and the thinner filter will bring a greater effect on the cell. Further study is necessary to clarify the effect of low energy parts of X-ray by using different energy and different cut filters.

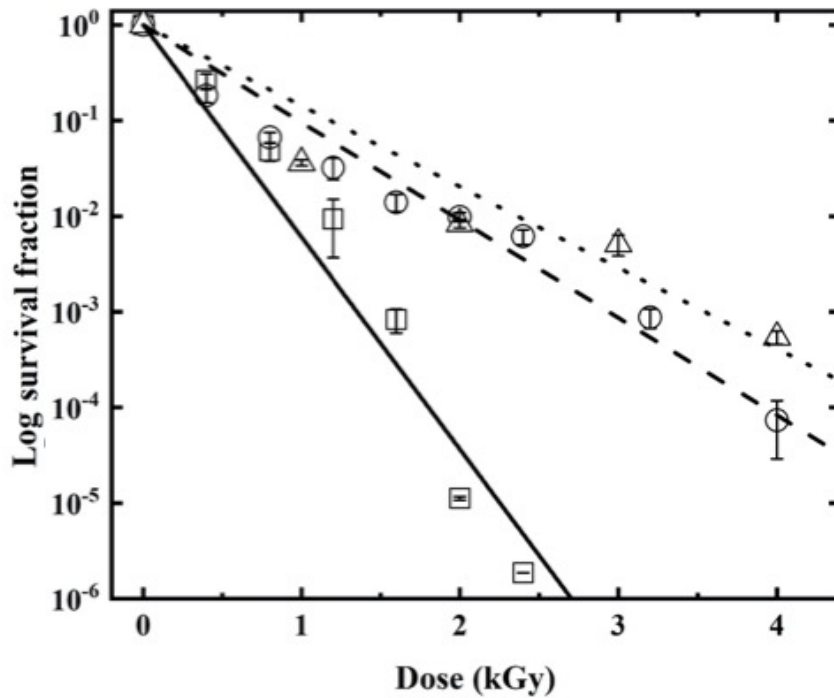


Fig. 4. 5 Radiation sensitivity of *Cladosporium* sp. □; X-ray F0 (non-filter), ○; X-rays F1 (1mm aluminum filter), △; gamma-ray. The conidia were dried on paper and irradiated.

The radiation sensitivities for gamma-ray of *Cladosporium* sp. isolated from woodblock were compared with three NBRC strains of *A. pullulans*, *C. cladosporioides*, and *A. niger* to better understand the radiation resistance of isolated strain. The dry conidia on paper were irradiated by gamma-ray, and the

survival curves of 4 strains were shown in Fig. 4.6. Both survival curves of *Cladosporium* sp. and *C. cladosporioides* show an exponential curve, and the D_{10} values were almost the same. *A. pullulans* was radioresistant with a large shoulder. Tolerance to gamma-ray decreased as follows: *A. pullulans* > *C. cladosporioides* (*Cladosporium* sp.) > *A. niger*. The D_{10} value of *A. pullulans* was about 5 times and 11 times as high as those of *A. niger* and *Cladosporium* genus, respectively. *A. pullulans*, ubiquitous fungi that can be found in environments with background radioactivity, is one of the most radioresistant species because of their stress protector trehalose and melanin production (Saleh et al., 1988). These results suggest that *Cladosporium* strain belongs to the medium level of radioresistant fungi species.

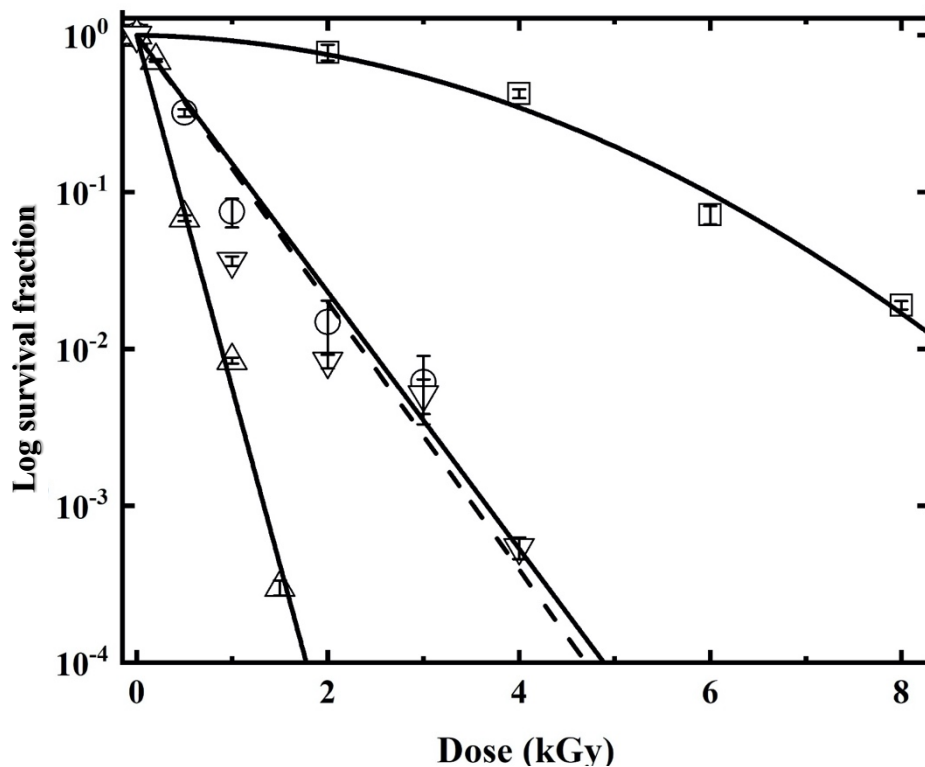


Fig. 4. 6 Survival curves of fungi by gamma irradiation. ; *A. pullulans*, ○; *C. cladosporioides*, △; *A. niger*, ▽; *Cladosporium* sp. (VN3). The conidia were dried on paper and irradiated.

4.3.3 Decontamination of woodblock by X-ray irradiation

Woodblock samples (ca. 2 cm x 3 cm, thickness 17 mm) were cut half and used as a model to investigate the necessary dosage for fungal disinfection in woodblock. Table 4.1 shows the dose rates for X-rays (F1) and X-rays (F0) at 3 positions of the top, middle (8.5 mm depth), and bottom (17 mm depth) of each woodblock. The dose rates of F1 and F0 at the surface of woodblock were 1.14 and 4.64 kGy/h. The result means about 75% of the low energy part of X-rays was cut by a 1mm aluminum filter. At the middle position of woodblock, the doses of F1 and F0 decreased to 76% and 20% of surface doses.

Table 4. 1 Dose rates in woodblocks for X-ray (F1) and X-ray (F0)

Position	Thickness (mm) from top to bottom	Dose rate (kGy/h)	
		F1	F0
Top	0	1.14 (1.0)	4.64 (1.0)
Middle	8.5 ± 0.8	0.87 (0.76)	0.94 (0.20)
Bottom	17 ± 2.1	0.65 (0.57)	0.69 (0.15)

F1: with 1mm aluminum filter, F0: non-filter. The ratio to the dose of top were shown in parences.

Paper sheets contaminated *Cladosporium sp.* with 10^7 CFU/ml were put at 3 positions of the woodblock and irradiated by X-rays (F1) and X-rays (F0) at various doses. The paper sheet samples (thickness ca. 0.1mm) were covered by plastic bag (thickness ca. 0.05 mm) and used for irradiation in woodblock. The doses at each position were shown in Table 4.2.

Table 4.2 Dose distribution in woodblocks

	Thickness from top (mm)	Dose (kGy)					
		F0			F1		
Top	0	4.0	7.9	18.5	4.1	6.2	8.3
Middle	8.5 ± 0.8	0.8	1.6	3.7	3.2	4.7	6.3
Bottom	17 ± 2.1	0.6	1.2	2.7	2.4	3.6	4.7

Figure 4.7 shows the survival rate of *Cladosporium* sp. in woodblock irradiated by X-rays (F1). At the surface dose of 4.1 kGy, 1.8×10^4 at the top, 4.4×10^5 at the middle, and 8.6×10^5 CFU/ml at the bottom were detected. More than 2 log reduction at 4.1 kGy and 4 log reduction at 6.2 kGy in the middle of woodblock were observed, whereas no survival conidia could be found at 8.3 kGy.

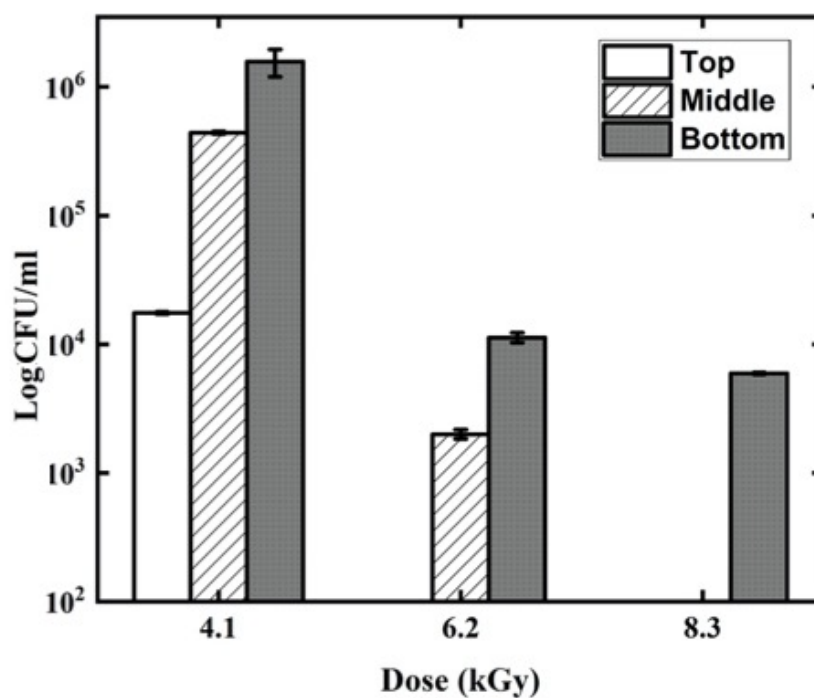


Fig. 4.7 Effect of X-ray with 1mm aluminum filter (F1) on *Cladosporium* sp. conidia in woodblock. The effect was examined at 3 positions of woodblock (Top, middle and bottom)

In the case of F0, no conidia were detected at all doses at the top of woodblocks (Fig. 4.8). More than 10^6 CFU/ml were detected at the middle and bottom position of the woodblock at 4.0 kGy and 7.9 kGy surface irradiation. Even at the dose up to 18.5 kGy of the top, nearly 10^3 conidia were survived at the middle position.

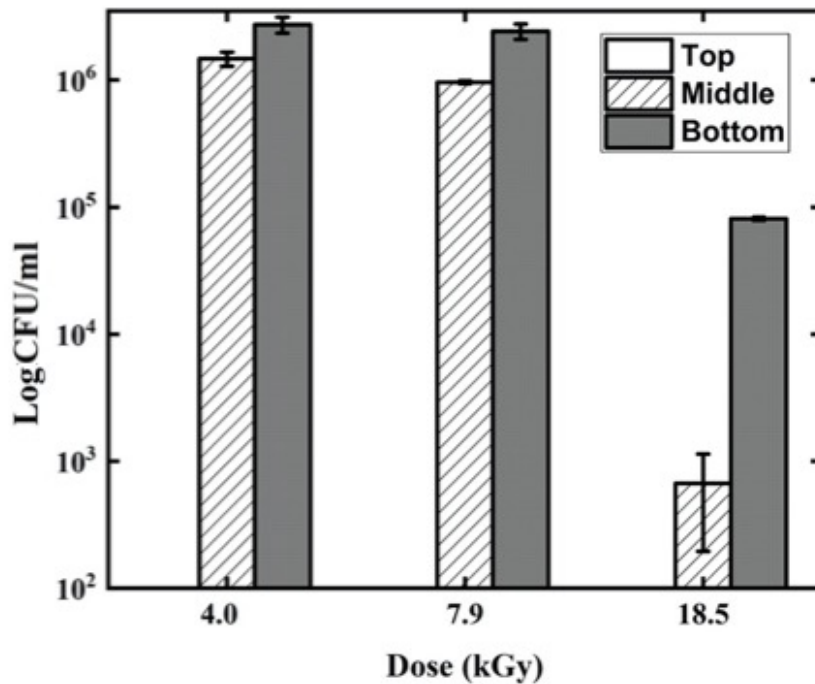


Fig. 4.8 Effect of X-ray non-filter (F0) on *Cladosporium sp.* conidia in woodblock. The examined position similar to F1.

For the disinfection of fungi in woodblock by low energy X-rays, both side irradiation is necessary. In the case of 6.2 kGy irradiation by X-rays (F1) shown in Fig. 4.7, more than 4 log reduction was obtained in the middle of woodblock. The doses of surface and middle for both side irradiation were calculated as 9.8 (6.2 + 3.6) kGy and 9.4 (4.7 x 2) kGy (see Table 4.2). The results show that more than 8 log reduction can be obtained by both side irradiation of X-rays (F1) at 10 kGy with a dose uniformity 1.04. Dose uniformity in woodblock is very important in radiobiological work as dose uniformity affects fungal conidia survival

probabilities.

The radiation dose required to disinfect fungus-contaminated woodblocks varies according to the fungal species, the population of contaminants, the surface area, and the thickness of objects (Sengupta et al., 2020). The sterilization assurance level, SAL (10^6), is commonly used for the sterilization of medical devices, and IAEA recommended the dose of 10 kGy for safe sterilization of wooden artifact against fungi (Ponta et al., 2017). Moreover, the European Standard EN 113 (1997) recommended doses between 25 and 50 kGy for wood sterilization in laboratory testing procedures. In this study, the sterilization dose of woodblock can be calculated as 9.5 kGy by 0.79 kGy (D_{10} value obtained in Fig. 4.5) x 12 log reduction. In addition, more than 8 log fractions can be reduced by 10 kGy of both side irradiation. Considering the contamination of fungi at the middle position of woodblock is usually lower, it can be concluded that the necessary dose for the disinfection of fungi in woodblock is less than 10 kGy.

Wooden cultural heritage object is treated for insect eradication with a dose of 2 kGy (Katusin-Razem et al., 2017). Therefore, the insect would be eradicated while the woodblock was exposed to 10 kGy for the fungi's disinfection. The disinfection dose of 10 kGy obtained in this study could also protect woodblocks from the negative effects of high dose irradiation (Kalawate and Mehetre, 2015). The use of a cut filter for low energy parts of X-rays is essential to avoid the high surface dose by X-rays (F0).

Recently, Haff et al., (2016) designed the in-line system of X-ray tube-based irradiators for fruit irradiation. The merit of low-energy X-rays is its low shielding requirements and thus the design of the mobile system is recommended for the irradiation of cultural heritage.

4.4 Conclusion

Low-energy X-rays combined with a 1 mm thick aluminum filter showed similar effects with gamma rays when studying their effects on fungal spores. 160 kV (F1) applicable for the disinfection of woodblock (thickness 17mm) was obtained for both sides irradiation, demonstrating feasibility for laboratory-based irradiators for research purposes. This study also demonstrated that low-energy X-rays could pass through wooden objects and reach to in deep them to sterilize fungal conidia contaminated inside the wood artefacts. With its advantages over almost all other current sterilization techniques, low energy X-ray is known to be very effective in the disinfection of wooden artefacts and X-ray sterilization is the future sterilization technique

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Chapter 5

Conclusions and future perspective

5.1 Summary

Cultural heritage inherited from past generations is an integral part of each society and country, and it should be preserved well to maintain for the future generation. Unfortunately, this cultural heritage is often deteriorated by various factors. Microorganism, especially fungi, are a significant factor for severe damage and degradation of cultural artifacts. Some of the fungi alter the aesthetics and cultural value of items and affect human health through infections (mycoses), allergic reactions, and toxic effects (mycotoxicoses). The elimination of fungi infecting cultural items is an essential goal in preserving cultural heritage. Chemical fumigation has been performed to prevent fungus infection of the heritage. However, such fumigations are now banned in some countries because of their carcinogenicity of the residual chemicals within the products. In such a situation, as a new alternative method, irradiation of ionizing radiations, such as gamma rays, X-rays are emerging as a new alternative method useful in that they penetrate well in-depth to the products without residues. Thus environmentally friendly radiation technology has convinced professional conservationists and managers of archives.

In Japan, rescuing books and the old documents of the archives from natural disasters, such as floods, typhoons, and tsunamis, has been a severe problem. Many documents in a storehouse were extensively damaged by floodwater in Hyogo, Japan, in 2004 and a large volume of historical archives was extensively damaged by floodwater, following Typhoon Hagibis in Fukushima, Japan, in October 2019, for example. Preservation of the archives has been urgently required (with Japan) because the deterioration or loss of cultural properties would quickly occur, affecting the keeping of the unique local traditions and cultures.

In Vietnam, woodblock, a vital cultural heritage, is the first candidate for fungal decontamination. Lime water and gas fumigation have been used as a conventional method for preserving this wooden artifact. No study using ionizing radiation for disinfection has been conducted because of the high cost, and the damage risk may occur during transporting to the radiation facilities. Portable irradiation, such as low energy X-rays irradiator, may overcome those drawbacks to essential to treat such archives with a museum.

In considering such a situation, we employed gamma rays for decontamination of archives and low energy X-rays for disinfection woodblock, a most important cultural heritage in Vietnam.

This thesis aimed to explore the effect of gamma radiation on fungal growth, particularly the radiation sensitivities of conidium-, germinating conidium, and mycelium-contaminated wet and dry paper, and the mechanical properties of the traditional Japanese paper (Kohzo-gami) using its replica paper to evaluate the feasibility of the irradiation sterilization. The additional purpose of the thesis is to demonstrate the practical application of irradiation in fungal-damaged paper documents rescued from a flood in Japan by using industrial gamma radiation service. Moreover, the research also intends to investigate the effect of X-rays on the fungal contaminated wooden artifacts in Vietnam to find an effective way to disinfect the harmful fungal effect on the cultural heritage of Vietnam. The thesis contents are summarized as follows:

Chapter 1 presents the general information of fungi-contamination of historical documents and archives and the decontamination methods for preserving cultural heritage. This chapter also introduces the advantages of radiation technology, explaining why radiation technology is useful for the fungal

sterilization of the cultural heritage as the most promising method. The impact of the radiation on microorganisms and the materials composed of cultural heritage is also shown.

Chapter 2 describes the effect of gamma irradiation on fungal growth and mechanical properties of the traditional Japanese paper, Kohzo-gami, infected by mesophilic fungi such as *Aspergillus sydowii*, *Penicillium chrysogenum*, and *Cladosporium cladosporioides*. The radiation sensitivities of conidium-, germinating conidium-, and mycelium-contaminated wet and dry paper were also determined. A radiation dose capable of inactivating 50% of a 30-sample population was used for comparison. Our results showed that the 50% inactivation dose did not significantly differ between wet and dry conidia. However, the survival percentage of dry conidia was higher than that of wet conidia at a high radiation dose. In contrast, the 50% inactivation doses for dry germinating conidia and dry mycelia were significantly lower than those for wet germinating conidia and wet mycelia. These results indicate that drought stress increased the radiation sensitivity of germinating conidia and mycelia. We also investigated the mechanical properties of Kohzo-gami irradiated at different doses. The order of the tensile strength of Kohzo-gami relative to that of control samples was as follows: 10 kGy > 30 kGy > 40 kGy. This result suggests that even a 10 kGy radiation dose can affect the mechanical properties of paper. The level of the color change of Kohzo-gami increased significantly at all doses; however, the National Bureau of Standards rating showed only “slight change” at all doses. The radiation doses required for fungal disinfection varied considerably depending on the fungal species and the total number of fungal cells on contaminated paper. Therefore, it was difficult to determine the standard exposure dose for treatment. However,

paper sterilization might not be required, as a combination of low-dose radiation and dryness can effectively kill fungal mycelia on contaminated paper.

Chapter 3 is a report of the successful practical use of irradiation in fungal-damaged paper documents using industrial gamma radiation service. A large volume of historical archives was extensively damaged by floodwater, following Typhoon Hagibis in Fukushima, Japan, in October 2019. They were rescued several months later; however, the prolonged exposure of paper documents to water caused severe biodegradation by fungal growth. The paper documents were exposed to gamma radiations emitted by a source of Cobalt 60 by the industrial irradiation service to disinfect fungi. Depending on the container's position, the absorbed dose, which was estimated using alanine dosimeters, varied from 11.9 to 16.5 kGy. The wet paper documents were mainly contaminated with hydrophilic and cellulolytic fungi, including *Trichoderma*, *Stachybotrys*, and *Fusarium*; no fungi grew after irradiation. These results indicated that the average absorbed dosage from 13.1 kGy to 16.1 kGy was sufficient to disinfect paper documents that were heavily contaminated with fungi.

Chapter 4 presents the potential for using low-energy X-rays to disinfection cultural heritage. In this study, The low-energy X-rays irradiation effect was investigated as an intervention strategy for the disinfection of fungi-contaminated woodblocks. Fungi were isolated from woodblocks of the Nguyen dynasty of Vietnam, and *Cladosporium* sp. was selected as the most radiation-resistant strain in woodblock. The dose rates of F1 (1-mm aluminum filtered) X-rays and F0 (non-filtered) X-rays at the surface of woodblock were 1.14 and 4.64 kGy/h, respectively. At the middle position (8.5mm thickness from the surface) of woodblock, the doses of F1 and F0 X-rays decreased to 76% and 20% of surface doses, respectively. F1 is useful to irradiate inside of woodblock, and the fungi at

the middle position were decreased more than 4 log fraction at 6.2 kGy and eliminated at 8.3 kGy of surface dose. The results suggest that contaminated fungi in woodblock are disinfected by both side irradiation of X-rays (F1) at 10 kGy with a dose uniformity 1.04.

In conclusion, this study has made significant contributions to the application of ionizing radiation on cultural heritage, opening new perspectives for the preservation of such precious materials not only in Japan but also in Eastern Asian countries, including Vietnam.

Firstly, we confirmed that the drought-rescued old Japanese paper documents could be sterilized by gamma irradiation and demonstrated that the average absorbed dosage from 13 kGy to 16 kGy is sufficient to disinfect paper documents heavily contaminated with fungi utilizing commercial irradiation company. These successes will provide efficient procedures for fungal decontamination on books and documents damaged by floodwater in the East Asian region with a possibility to facilitate the decontamination utilizing the radiation sensitivity of fungal germinating conidia and mycelia after long storage at the wet condition in combination with the conventional freeze-drying process.

Finally, we show that the low-energy X-rays through 1mm aluminum filters (F1) has the same fungal disinfection effect as gamma rays (Chapter 4). So, we suggest the dose of X-rays (F1) at 10 kGy with a dose uniformity of 1.04 can be used to disinfect the contaminated fungi in the woodblock of Nguyen's Dynasty of Vietnam. This result shows the feasibility of laboratory-based X-ray irradiators for research purposes. The design of the mobile, compact, reliable, and lower cost system is recommended for the irradiation of cultural heritage.

5.2 Future perspective

Although radiation technology has been successfully utilized in recent years with museums and libraries' participation for the preservation and consolidation of cultural heritage artifacts, the acceptance to using ionizing radiation in Japan to preserve the cultural heritage is limited. More data from the practical application of gamma irradiation may scientifically be convincing the users and conservationists that irradiation does not lead to unacceptable changes in the functional or decorative properties of artifacts. Further research into the application of our findings to practical use should be done.

Chapter 3 of this thesis shows the low-energy X-rays combined with 1mm aluminum filters (F1) has the same fungal disinfection effect as gamma rays. However, the dose distribution in the thinner paper sample is not analyzed in this experiment. Further study is necessary to clarify the effect of low energy parts of X-rays by using different energy and different cut filters. It is important to design the mobile system for the irradiation of cultural heritage based on the merit of low shielding requirements of low-energy X-rays.

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List of publications

No	Title of paper	Authors	Journal	Corresponding chapter
1	Effect of gamma radiation on fungal growth stages and mechanical properties of traditional Japanese paper	Linh, N. T. T Kumeda, Y Matsushita, M Amano, M Sakai, K Yoshikawa, K Fujita, K Uchida, T Furuta, M	Journal of Cultural Heritage (In press)	Chapter 2
2	Application of gamma radiation for disinfection of fungi in a large volume of historical archives damaged by flood following Typhoon Hagibis 2019, Japan: A case report	Linh, N. T. T Kumeda, Y Matsushita, M Hironiwa, T Furuta, M	Bioscience Control (Submitted)	Chapter 3
3	Disinfection of the Nguyen Dynasty's Woodblock of Viet Nam by Low Energy X-rays	Linh, N. T. T Furuta, M Kume, T	RADIOISOTOPES (Submitted)	Chapter 4