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論　文　名	「Application of gamma radiations and X-rays for disinfection of fungi in historical archives （歴史的資料のカビ殺菌へのガンマ線と X 線の応用）」
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論文要旨

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 local archives has been urgently required in Japan and East Asia because the deterioration or loss of cultural properties would quickly occur, affecting the keeping of the unique local traditions and cultures in each country.

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 essential to treat such archives within a museum.

In considering such a situation, we employed gamma rays for decontamination of archives and low energy X-rays for disinfection woodblocks used in the Nguyen Dynasty from the middle of the 17th century to the beginning of the 20th century, 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 irradiation 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.

審査結果の要旨

貴重な歴史的記録である古文書や木製版木などのカビ汚染は文化財の劣化の原因となり、文化財の修復作業時における作業者の中毒やアレルギー疾患などのリスク要因ともなる。本論文においては文化財の保全に必要とされる殺菌処理への放射線処理の適用を目指し、洪水、台風、津波などの自然災害により汚損された江戸時代期の古文書とユネスコ世界遺産に登録されているベトナムグエン王朝時代の（17 世紀半ばから 20 世紀初頭）の木製の版木を材料として以下の成果を得ている。

(1)台風による洪水で汚損した江戸時代期の古文書からは主な分離株として *Apergillus sydowii, Penicillium chrysogenum, Cladosporium cladosporioides* が同定され、江戸時代の製紙方法を再現した和紙に添加し、^{60}Co ガンマ線照射による殺菌効果を調べた。最も抵抗性を示した *Cladosporium cladosporioides* を指標として発芽胞子、菌糸体においては放射線照射時に乾燥することにより、より効果的に殺菌できることを示した。また、和紙の引張強度や色度変化による検討結果から文化財に対して提唱されている滅菌線量（～8kGy）が十分適用できることが示された。

(2) 2019 年 10 月に福島県で発生した台風 19 号により被災し深刻な劣化を受けた紙資料からカビ類を分離同定し、上記（1）と同等のカビ汚染であることを確認した。これらの殺菌を滅菌用照射サービス会社に依頼した。その結果、13.1kGy から 16.1kGy の平均吸収線量で ^{60}Co ガンマ線を照射した文書から分離したカビ類の生育は認められず、真菌類に汚染された紙文書については上記の線量で十分可能であることが実証された。

(3) ベトナムの博物館に保存されているグエン王朝時代の版木の汚染カビについて、可搬型低エネルギーＸ線装置の適用生を検討した。版木から分離されたカビの中で、最も放射線抵抗性の株は *Cladosporium* sp. であり、版木中の汚染カビはＸ線(F1:1㎜ アルミニウムフィルター装着)を用いた両面照射により、線量 10 kGy、線量均一度 1.04 で効果的な殺菌が可能であることが示された。

以上の研究結果は水害を受けた書籍や古文書類の除染をはじめ、広く文化財の殺菌や保存への放射線利用の有効性と博物館などの保存修復の現場における放射線利用の適用性が実証するものであり、今後の大きな発展を期待させるものである。これらの成果は申請者が自立して研究活動を行うに必要な能力と学識を有することを証したものである。学位論文審査委員会は、本論文の審査並びに最終試験の結果から、申請者に博士（工学）の学位を授与することを適当と認める。