Many historical public archives suffered from extensive flood caused by Typhoon Hagibis in Fukushima, Japan, in October 2019. They were rescued and moved to a temporary storage place within a week, however, severe biodegradation by fungal growth occurred because they were left in an undried condition for 5 months since a large refrigeration equipment could not be found soon. These damaged paper documents will, in future, be cleaned by hand with purified water, frozen for temporary storage, and subsequently freeze-dried for long-term preservation. Before carrying out this task, disinfection of fungi that has grown on the documents is desirable, 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.

Several methods have been developed to disinfect fungi-contaminated heritage objects. Chemical methods such as methyl bromide were banned in a number of countries because of potentially ozone layer depleting (Barry et al., 2012). The other chemicals, ethylene oxide or propylene oxide are now recommended as fumigants in Japan, however, it cannot use for water damaged materials because they react chemically with water (Kigawa et al., 2012). 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; 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 a commercial gamma-irradiation facility.

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. 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 (Fig. 1). On the most wet samples, fungi produced various color pigments and unusual odors. All samples were exposed to gamma radiations emitted by a source of Cobalt 60 at Koga Isotope, Ltd. (Shiga, Japan). Before irradiation, 9 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. 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 Co. Ltd. (Kyoto, Japan).

Results can be found in Table 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). 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).

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). 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. 4). 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 13.1 kGy to 16.1 kGy (Table 2).

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, however, the erosion and the spread of contaminated fungi on the samples stopped after irradiation. Within a

### TABLE 1. Representative fungal species isolated from paper documents damaged by floods

| Color change of the paper | Condition of the paper | Conidia production | Hyphae | Species                        | Identity (%) |
|---------------------------|------------------------|--------------------|--------|-------------------------------|--------------|
| yellow                    | dry                    | + + a              | +      | Penicillium commune           | 99%          |
| blue                      | wet                    | + +                | +      | Trichoderma harzianum         | 99%          |
| yellow                    | dry                    | +++                | +      | Penicillium commune           | 99%          |
| black                     | wet                    | +++                | +      | Stachybotrys chartarum        | 99%          |
| blue                      | wet                    | ++                 | +      | Trichoderma atroviride        | 100%         |
| blue                      | wet                    | +                  | ++     | Trichoderma harzianum         | 99%          |
| purple                    | wet                    | -                  | ++     | Fusarium sp.                  | 99%          |
| black                     | wet                    | ++                 | +      | Stachybotrys chartarum        | 99%          |
| black                     | wet                    | +++                | +      | Stachybotrys chartarum        | 99%          |

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

**FIG. 3.** Cross section of the assembly of the totes during ⁶⁰Co-gamma irradiation at the Koga Isotope irradiation facility.

**FIG. 4.** Gamma-ray dose distribution and position of alanine dosimeter within one tote. The positions of the dosimeter are indicated as X within the figure.
month, the samples got wet cleaning and freezing, and subsequently freeze-drying.

The effective dose to disinfect fungi was considerably different from the previously reported dosage from 5 Gy to 20 kGy (Tomazello & 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 (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 the mechanical properties of the paper, including the tensile strength and the color change showed only “slight change” at 10 kGy to 40 kGy (Linh et al., 2020). 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). 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. On the other hand, some reports indicated that gamma radiation increased the deterioration of the physical-chemical property of the paper over time (Bicchieri et al., 2016; Drábková et al., 2018). Therefore, a long-term observation analysis is necessary to evaluate the effect of gamma radiation.

In this work, we demonstrated the successful practical use of irradiation in a large volume of paper documents severely attacked by fungi, using a commercial gamma-irradiation facility. We expect that this case report will provide useful information about the restoration of heritage materials damaged by fungal growth in future natural disasters.

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