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^{137}Cs GAMMA RADIATION EFFECT ON FUNGAL STRAINS ON AN ARTWORK BY CANDIDO PORTINARI

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Abstract

Gamma radiation is an effective technique for the conservation of art collections, reducing microbial loads and can be obtained by the emission of a radioactive isotope, such as Cesium 137. A Portinari's artwork (National Museum, Brazil) was analysed, and the fungi contained therein were isolated and treated with gamma radiation for decontamination. Radiation doses used were 16, 19 and 22kGy. Results indicated 11 genera and 17 species of fungi isolated. Penicillium and Cladosporium were isolated from air, the artwork, and its support. The genera Penicillium, Cladosporium, Nigrospora and Curvularia showed high resistance to radiation (16kGy) being the most resistant species Cladosporium, with no growth just under 22kGy. The results outlined indicate that the rates of DNA damage and repair were critical, depending on chronic or acute doses. The biochemical mechanism acting on fungal cells under irradiation was basically the inactivation of specific enzymes and, probably, DNA damage, particularly stimulating double-strand breaks.

Keywords*: Gamma radiation; Fungi; Biodeterioration; Artwork; Portinari*

Introduction

One of the main problems faced by museum restaurateurs for the preservation of paper collections and artworks is the damage caused by the action of microorganisms [1-4]. Fungi play a key role in the biodegradation of papers and artworks in museums and archives. Due to their ability to form hyphae, they can penetrate into the materials, resulting in losses due to acid corrosion, enzymatic degradation, and mechanical attack. The most important genera of fungi 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 Rhodotorula*. For many years, in order to treat art collections, they were treated with fumigation techniques. New alternatives have emerged, such as the use of modified anoxic atmosphere and deep freezing. However, because they do not have a long-term effect, presenting rapid recontamination. Moreover, these procedures are not efficient in eliminating anaerobic fungi that may be infesting artworks [5, 6].

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Studies have been developed to formulate effective conservation strategies in order to prevent the biodeterioration of cultural heritage. The understanding of the biodeterioration process, colonization mechanisms developed by microorganisms, techniques used in the assessment of biodeterioration and strategies that seek to preserve the integrity of the heritage were outlined as relevant objectives in the work of *A. Negi and I.P. Sarethy* [7]. Therefore, the improvement of methods that detect and characterize microorganisms is being also studied as in the work of *S. Sanmartín et al.* [8], where traditional and modern methods were reviewed under three different approaches – molecular, sensory, and morphological and biological control methods – suggesting the use of an integrated approach regarding the use of such methods in the identification, monitoring, and control of microorganisms.

Gamma radiation is a safe and effective technique for treating artwork collections as it is used with high efficiency in reducing microbial contaminations without residual radioactivity [9-11]. Radiation processes were not yet widely accepted by museum restaurateurs because of the degradation of cellulose caused by radiation. To establish a safe dose of radiation that can cause a reduction in microbial load without damaging the paper structure has been the object of study by *I.V. Moise et al.* [12] and *H.R. Carvalho et al.* [13]. In the work of *A.A. Sakr et al.* [14], *46 Streptomyces* strains were isolated from paintings in the thumbs of Tell Basta and Tanis and exposed to low and high gamma radiation doses (5 to 25kGy). The authors concluded that gypsum, pigments, and Arabic gum were not damaged in the range of gamma radiation studied, indicating a potential technique for microbial decontamination.

For the disinfection of fungi and bacteria, radiation doses causing lethality start from 10kGy, usually used as a safe dose for disinfecting fungi and bacteria present in food ingredients. This dose has also been used in studies performed on the treatment of paintings contaminated by microorganisms and has been shown to be effective [9]. *M. Silva et al.* [15] inactivated fungi from paper materials working in the range from 14.5 to 25kGy using ^{60}Co, emphasizing the presence of resistant fungi inactivated only under 16kGy. In 2018, we started a study for the decontamination of an artwork (charcoal on paper), by Candido Portinari, and part of the collection of the National Museum (Rio de Janeiro, Brazil), unfortunately, destroyed by a big fire, in September of the same year. The artwork was analysed, and the fungi contained therein were collected and isolated, so that it could be properly treated and returned to an exhibition to the public. The study using gamma radiation for decontamination was then performed. The search for this technique came due to the structural nature of the artwork, charcoal on paper, which would prevent any possibility of chemical or aqueous treatment that could destroy its integrity. The choice for Candido Portinari's "Índios" (1937), was due to its importance as an object of study because it is one piece in a series of other artworks painted in the same period (1937-1938), all with the same type of paper and the same technique (coal), thus, serving as a pilot for the preservation of all other artworks with similar characteristics [16]. The work selected as a pilot for the present study belonged to the National Museum's collection in Brazil, which is an autonomous institution, member of the Science and Culture Forum of the Federal University of Rio de Janeiro.

A.M. Adamo et al. [17] focused their study on the reaction of the biological population to radiation treatment and that on the eventual negative effects on the paper substratum. On the other hand, *F.J. Butterfield* [18] studied long-term effect of gamma irradiation on different types of paper subjected to gamma irradiation, accelerated aging and radiation followed by aging. The author observed a synergistic effect when both irradiation and aging were performed resulting in a decrease in fold endurance and tear resistance. *M. Bicchieri et al.* [19] reported that the use of gamma radiation causes depolimerization and degradation of paper substrate; however, they indicate that the application of this technique can be envisaged in some conditions for cultural heritage documents and books. They also state that mechanical tests do not reflect the chemical modification induced in the cellulosic support.

Thus, the main objective of this work was to irradiate fungi isolated from Portinari's artwork "Índios" in order to evaluate the technique as a non-destructive treatment of works of art with similar characteristics contaminated with fungi. The resistance of fungal species to high levels of radiation was also investigated by classical and molecular biology techniques.

Experimental

Material

The charcoal on paper artwork "Índios" shows three Indian heads and another design of the head, sketched in the background (Fig. 1). The drawings occupy almost the entire area of the artwork. Its size was 99.5cm height by 119cm width.

Fig. 1. "Índios" from Candido Portinari, artwork from 1937

The work was produced on Kraft paper with charcoal, which excludes any attempt to use aqueous treatment to remove fungi or damage resulting from the biodeterioration or deterioration in the pulp structure of the paper. It is important to emphasize under what conditions the work was safeguarded. The ambient temperature was around 30°C with no control over the incidence of light or humidity.

Methods

Samplings

The microbiological characterization was performed in the air of the room where the artwork was placed, made by sedimentation on Petri dishes containing sterile Sabouraud Dextrose Agar supplemented with chloramphenicol solution. The samples were collected every cubic meter of the space, one meter above the floor, for 2 hours. Three replicate Petri dishes were placed per cubic meter. Some parts of the artwork were directly monitored, particularly those where fungal contamination was clearly present. On selected parts on the surface of the charcoal on paper, sterile swabs were used to collect the biological material (Fig. 2). The used swabs were placed within 9.0mL of saline water 0.9% ($m \cdot v^{-1}$).

Culture and isolation of filamentous and yeast like fungi

After serial dilutions, 0.1mL of each sample collected in Petri dishes were inoculated. The Petri dishes were then placed in a chamber at 25°C for 14 days. After growth, the fungi were isolated in agar media, and incubated for 7 – 14 days at 25°C. The fungi were then

isolated with the aid of sterile swabs in glass tubes containing Agar Sabouraud Dextrose culture medium and stored in mineral oil [20, 21].

Fig. 2. Sample collection with serile swabs

Macroscopic and microscopic identification of fungi

After isolation of the colonies, Petri dishes were photographed and macroscopic characteristics such as colony size, colouring, and texture were observed. From the previous cultures, slides were made for microcultivation of the filamentous fungi in Agar Malt Extract culture media for 5 – 14 days. After this period, the staining of the microcultivation slides with L-actophenol blue solution was performed. Microscopic observations of the morphological structures for identification at the genus level.

DNA Extraction

After growth in a specific culture medium, the fungi were removed by scraping the Petri dishes and transferred to a 15mL Falcon tube. The tubes were placed, for 5min, in a cylinder containing liquid nitrogen, heated in a water bath at 60ºC for 10min, and macerated with the aid of a pistil. This procedure was repeated three times. Subsequently, DNA extraction was performed with Kit Ultra Clean Soil Isolation (MO BIO Laboratories). According to the instructions provided by the supplier, the DNA was eluted in 30mL of the solution provided in the kit and quantified by reading on NanoDrop ND-1000 spectrophotometer.

Amplification of ITS region by PCR

A fragment of 600 pairs of DNA bases corresponding to the ITS gene was amplified from the genomic DNA using the ITS5 initiators (senso, 5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (antisense, 5'-TCCTCCGCTTATTGATATGC-3') in the PCR System 9700 ThermoCycler (Applied Biosystems, USA). Each reaction included 25µL of Top Taq Master Mix Kit (PCR Master Mix, Qiagen, Holland), 0.5µM of each initiator and 5µL of extracted DNA added to water, in a total volume of 50 µL. A first denaturation was performed at 94ºC for 4min followed by 30 cycles under the following conditions: 94°C for 30s, 50ºC for 30s and 72ºC for 30s. The experiment was finished at 72ºC for 10min. The amplified fragments were purified using the Kit Wizard® SV Gel and PCR Clean-Up System (Promega, USA), for the removal of nucleotides and

initiators not incorporated, followed by proper sequencing [22]. The amplification product was detected through an electrophoretic run in 1% agarose gel in TE 1X buffer.

Sequencing

The DNA fragments of the isolated fungi were submitted to sequencing using the Big Dye Terminator v. 3.1 (Applied Biosystems, USA) kit in an ABI 3130 automatic sequencer with 4 capillaries of 50cm. ITS-5 initiators (sense) and ITS-4 (antisense) were used to sequence the region ± 600Pb and the initiators Sadir (sense) and S17 (antisense) were used to sequence the region 1500Pb. The concentration of primers used was 3.2pmol. The chromatograms obtained from sequencing were submitted to the Chromas Lite, version 2.01 and Bioedit programs to analyse the quality of the sequences. The sequences validated by the programs were paired to those deposited in the Genbank's DNA database. To validate the sequences, NCBI BLAST (Basic Alignment Search Tool) tool was performed, to confirm the sequences obtained. Only fragments with similarities above 98% were considered "reliable" and duly annotated.

The spectrometer used was a Cora Family 5500 equipped with 785nm excitation laser, covering the 100 to 2300cm^{-1} range with resolution of 12cm^{-1} and laser power of 450mW. Vibrational spectra, FTIR and Raman, were compared with IRUG (Infrared and Raman Users Group) database.

Irradiation of samples with ^{137}Cs for selection of resistant fungal species

The irradiation time in the ^{137}Cs chamber was increased to establish the radiation intensities capable of eliminating each fungus individually. The irradiations were applied after 14 days of incubation of the isolated fungi at the Institute of Research and Development of the Technological Center of the Army (IPD/CTEx) in Rio de Janeiro. Plates with fungi grown separately were grouped in the irradiator, in order to occupy the central space of the chamber, according to the pre-established maximum height of 7cm, in order to decrease the uncertainty of the procedure. Then, the irradiation chamber was closed so that the samples were subjected to irradiation with a source of ^{137}Cs at the different times previously calculated. Irradiation time was defined by a program based on recent dosimetric mapping of the irradiator. The average uncertainty in the doses, estimated on the dimensions of the samples and in the dose distribution within the irradiator, was equal to ±5%. Immediately after irradiation (exposure time directly proportional to the desired dose), samples were replaced in boxes and returned to the laboratory, for analysis of fungal viability, according to the isolation procedure previously described. Thus, irradiations were performed for the time required to reach the doses proposed (14h under16kGy, 16h under 19kGy and 19h under 22kGy). Only doses much higher than those described in the literature were selected since the main objective of the study was to select fungi that were highly resistant to gamma irradiation.

Gamma Irradiation

The irradiation chamber of the Chemical, Biological, Radiological and Nuclear Institute of the Brazilian Army, located in the Army Technological Center (CTEx), is composed by an irradiator of cavity weighing 19 tons. Currently, its ^{137}Cs sources with 43.2kCi activity provide a maximum dose rate of 1.45kGy·h^{-1} in two rectangular irradiation chambers 68cm wide, 137cm depth, and 20cm height positioned above and 188 below the gamma source. The gamma source consists of 28 spaced parallel plates, doubly encapsulated containing cesium chloride. In addition, a pneumatic system allows not only the access the port to be moved, but also to move the sources through a control panel. The dosimetric mapping of the chamber, indicated a homogeneous distribution of the dose rate with a variation around ± 3%.

Post Irradiation Fungal Viability

Procedures for post-irradiation fungal viability analyses were performed 24h after the irradiations. Fungal species irradiated with ^{137}Cs at 16, 19 and 22kGy, were inoculated in sterile Petri dishes, containing the culture medium Agar Sabouraud Dextrose, in order to confirm the viability of isolated microorganisms, indicating high resistance to gamma radiation. After

inoculation, Petri dishes were placed in a germination chamber for 7 days at 25ºC. After this period, the observation of fungal growth was performed. In cases where no post-irradiation growth was observed, it was concluded that the irradiation was high enough to inactivate the cells.

Phylogenetic Analysis

Phylogenetic analyses of the amplified ITS5 and ITS4 sequences were inferred using the maximum parsimony method and MEGA11 software. The bootstrap analysis was used to evaluate the tree topology per 1000 resampling.

Results

Isolation and macroscopic identification of fungi allowed the identification of 17 different species present in the atmosphere of the room where the artwork is stored, on the artwork, and on the fabrics support of the piece. Table 1 presents the 17 identified species, which belong to 11 different genera of fungi.

Table 1. Fungal genera and species isolated from the atmosphere, artwork and support of the artwork "Índios" from Candido Portinari

Atmosphere	Artwork	Support (canvas) of the artwork
Aspergillus aculeatus	*Cladosporium xanthochromaticum*	*Cladosporium perangustum*
Rhizophus *sp.*	*Cladosporium cladosporioides*	*Penicillium* sp.
Fusarium equiseti	*Penicillium* sp.	*Cladosporium halotolerans*
Penicillium *sp.*	*Pestalotiopsis* sp.	*Curvularia lunata*
Cladosporium *sp.*	*Arthrinium marii*	*Cladosporium tenuissimum*
	Daldinia eschscholtzii	*Cladosporium xanthochromaticum*
	Nigrospora sp.	*Rhizopus* sp.
	Curvularia luneta	*Periconia macrospinosa*
	Cladosporium sp.	*Penicillium raistrickii*
		Cladosporium sp.

After obtaining the genomic DNA of the fungal isolates and subsequent sequencing of the ITS5 and ITS4 region, 11 fungal isolates were identified by similarity analysis with the NR database (Table 2). The presented results describe 9 different species, with similarity indexes equal or close to 100% (Table 2). In addition, the Accession column shows the access number of the hit that presented the highest similarity index.

Table 2. Results of the analysis of similarities between the sequences obtained from the isolates in this study with the related fungi

Isolate	Description of best hit	Cover	% similarity	NCBI Accession
F679	*C. xanthochromaticum*	100%	100%	MF473319.1
F680	*C. xanthochromaticum*	100%	100%	MT258647.1
F681	*C. cladosporioides*	99%	100%	JQ768317.1
F683	*C. xanthochromaticum*	100%	100%	MF473319.1
F693	*Pestalotiopsis sp.*	100%	100%	KX960814.1
F699	*C. pseudocladosporioides*	100%	100%	MT582794.1
F700	*Arhrinium marii*	100%	99.84%	NR_166043.1
F701	*Daldinia eschscholtzii*	100%	100%	MN341731.
F702	*Nigrospora sp.*	100%	99%	KX022499.1
F703	*Curvularia luneta*	100%	99.82%	OM267783.1
F704	*C. subuliforme*	100%	100%	LN850753.1

The sequences obtained in this study were used for the construction of phylogenetic trees with sequences of similar organisms deposited in the NR database, available at the NCBI website (Fig. 3). In Figure 3 it is possible to identify the proximity relationships between the

sequences of the fungi isolated in this study with organisms that showed similarities (Table 2 and Fig. 3).

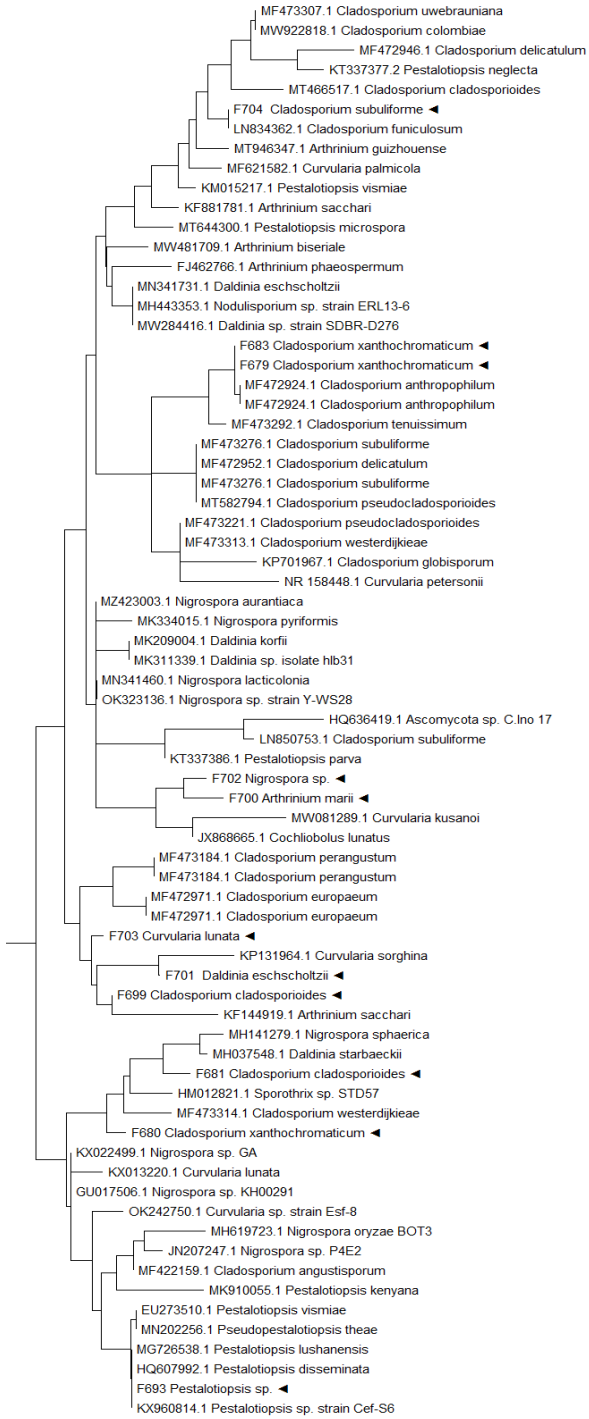


Fig. 3. Phylogenetic tree of ITS5/ITS4 sequences.
The tree was constructed based on the neighbour-joining method

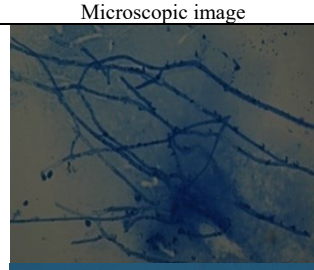
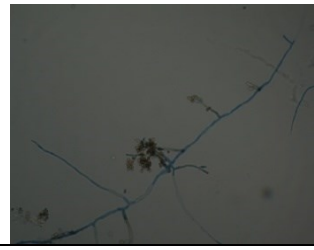
The fungal isolates identified in this study (Table 1 and Fig. 3) were widely detected on the surfaces, artwork, and support of the piece. Thus, with the purpose of eliminating the presence of fungi, different doses of gamma radiation were tested. The choice of gamma radiation is due to its ability to destroy the DNA structure of fungi, in addition to completely inhibiting their growth. Although the inhibition of its growth by gamma radiation results in only minor damage to the cells, its use proved to be effective in controlling the propagation of the isolates on surfaces. Most microorganisms did not present viability from the first radiation dose to which they were submitted, that is, 1kGy, being classified, then, as sensitive to gamma radiation. This viability was verified by the existence or not of microbial growth after irradiation. Of all the isolated fungi that were subjected to gamma radiation, only those listed in Table 3 were resistant to 16kGy. Thus, higher radiation doses were tested, i.e., 19kGy and 22kGy (Table 3). Table 4 presents macroscopic and microscopic aspects of the species resistant to 16kGy.

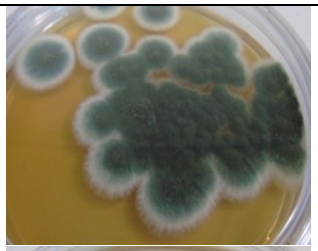
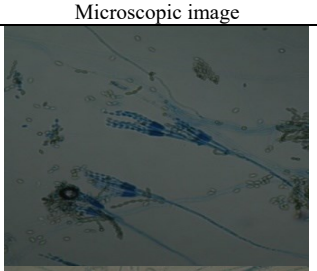
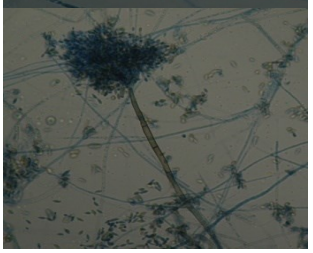
Table 3. Fungal viability after high doses of gamma irradiation

Fungus	16kGy	19kGy	22kGy
Nigrospora sp.	✓	X	X
Curvularia luneta	✓	X	X
Cladosporium halotolerans	✓	✓	X
Penicillium sp.	✓	X	X
Cladosporium sp.	✓	✓	X

✓ Active growth (viable); X: innactivated

Table 4. Macroscopic and microscopic aspects (400x magnification) of isolated fungi resistant to 16kGy

Petri dish	Microscopic image	Identified Fungus
		*Nigrospora* sp.
		*Curvularia Luneta*
		*Cladosporium Halotolerans*

Petri dish	Microscopic image	Identified Fungus
		*Penicillium* sp.
		*Cladosporium* sp.

Irradiation of fungal samples at three different doses of radiation, 16, 19 and 22kGy revealed different levels of tolerance between the microorganisms. Figures 4 to 6 show the growth on Petri dishes of radiation tolerant species. It can be observed that most of them are dark coloured. However, the most resistant of all was the genus *Cladosporium*, which only presented lack of growth in the dose of 22kGy (Fig. 5).

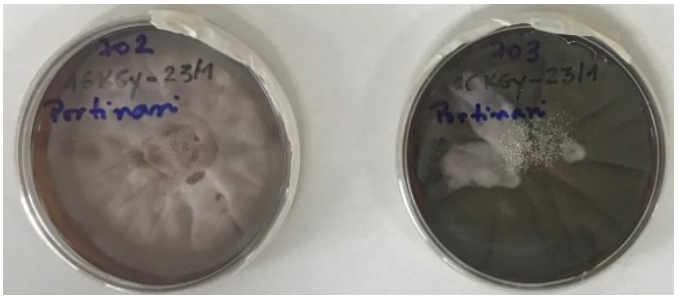


Fig. 4. Viability of the fungus *Nigrospora* sp. (left) and *Curvularia lunata* (right) after irradiation of 16kGy

Fig. 5. Viability of the fungus *Cladosporium halotolerans* after irradiation of 16kGy

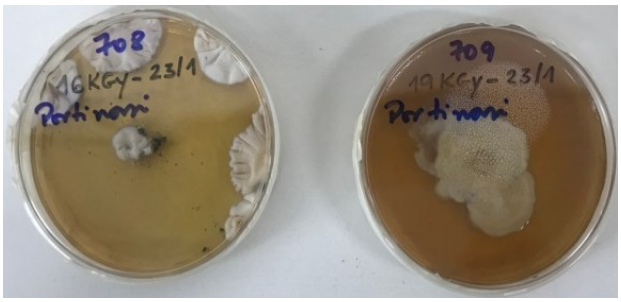


Fig. 6. Viability of the fungus *Penicillium* sp (left) and *Cladosporium* sp. (right) after irradiation of 16kGy and 19kGy, respectively

Discussion

Some genera and species found in the artwork of Portinari are common also in museums and libraries. Under moderate or humid climate conditions, fungal communities are dominated by *Alternaria*, *Cladosporium*, *Epicoccus*, *Aureobasidium* and *Phoma* species. *Penicillium*, *Aspergillus*, and *Fusarium*, among others, are also commonly found in books and paintings [23], although the presence of bacteria is also documented [24, 25]. The presence of certain species of contaminating fungi can represent, by itself, a threat to human health and to the conservation of cultural heritage, since many of them may present some degree of pathogenicity, in addition to being, mostly, potential cellulolytic agents. In these cases, fungi of the genera *Cladosporium* and *Penicilium* are included, both found in the present artwork and considered potential pathogenic agents [13]. However, based on the results obtained and presented in Table 1, we verified the presence of fungal species that are not so common in museum indoor environments, such as *Cladosporium halotolerans, Cladosporium xantocromaticum,* and *Daldinia eschscholtzzi*. However, all these species contribute to the biodeterioration processes. A similar observation was verified in the work by *R. Ortiz et al.* [26] who identified wood rot fungal species in eight historic churches in Chile and found several groups of fungi not previously reported in Chile, demonstrating the contribution of this microflora to biodeterioration.

The proliferation of fungi in museums is highly determined by the interior climate conditions and available nutrients, which is directly related to the number of impurities brought by the air or air quality in the external environment. The internal conditions, as indicated by temperature and relative humidity is the most important factor responsible for fungal growth. In spaces where humidity is higher than 70% for many weeks or months, a large fungal diversity can be expected. Thus, climate control should be adjusted to values below 55% since water availability seems to be suitable for uncontrolled proliferation. Museums, galleries, libraries, and archives are strongly advised to control temperature and humidity conditions in order to maintain their collections free from mechanical damage and biodeterioration [23].

Regarding the present study with "Índios", official records for the city of Rio de Janeiro indicate that the average relative humidity of the air at the date of sample collection was around 64%. However, inside the museum there was no control of relative humidity, temperature, or light intensity. Such conditions may have worsened the contamination and fungal proliferation. As reported, even when the relative humidity is maintained around 60%, the works of art or collections of books are not free from contamination and fungal proliferation. The condensation of water occurs on cold surfaces even when they are under low relative humidity, and this represents an opportunity for fungal growth, since they are not very demanding for specific substrates, being able to grow in a wide variety of substrates. Due to the great diversity of exoenzymes produced by fungi and bacteria – cellulases, glucanases, lacases, phenolases,

keratinases, monooxygenases, among others – and its remarkable ability to grow in low water activity values, the preservation of museum objects is inevitably connected with the prevention, monitoring, and treatment of the occurrence of fungi in contaminated pieces [23].

In the present work, the isolation of several species of *Penicillium* and *Cladosporium* corroborated the predominance these fungi usually associated to proliferation in works of art. Studies show that the genus *Penicillium*, among most biodeteriorating agents, is a ubiquitous organism responsible for modifications on paper support. As a cellulolytic fungus, it attacks the support of artworks when living under a favourable environment, that is, with favourable temperature and humidity conditions and availability of other essential nutritional elements. Such conditions are common in libraries and collections as reported by *G. Magaudda* [27]. Because they produce acidic pigments, fungi end up generating particular local conditions that modify the physicochemical properties of materials, as seen in the work and *L. Lavin et al.* [28]. They verified that the biofilm formation by *Scopularriopsis* sp. and *Fusarium* sp., isolated from paper documents, produced reddish-brown stains, attacked the paper structure, and produced a pH reduction by one unit, accelerating the paper's biodeterioration processes.

The present work also revealed high contamination levels inside the room due to the presence of some species of fungi. The danger of this contamination lies in the fact that spores suspended in the air represent a risk for both human health and the constitution of the materials. Its biodeterioration potential causes irreversible damage to artworks and other objects of cultural heritage. It has been reported by other authors that air contamination is the main vehicle for spore's dissemination. In addition, the co-occurrence of the same fungal species in works of art and in air samples is by itself a demonstration of this cross-contamination [13].

Other factors may be involved in the viability of fungi or tolerance to gamma radiation. Multicellular or bicellular spores are more tolerant to gamma radiation than unicellular spores. In addition, the number or density of mycelia in the inoculum exposed to radiation may affect the radiation dose required for the inactivation of the microorganism. Generally, a high density of mycelium requires an elevation of the radiation dose [29]. *I. Shuryak et al.* [30] also studied fungi and yeasts resistant to chronic and acute radiation up to 36kGy. They concluded that resistance levels were different among organisms from the same order and even species sharing a large core of genes. Basidiomycetes and Ascomycetes constitute a relatively resistant group with marked differences in acute and chronic radiation doses. Levels of DNA damage production and repair are critical to chronic resistance in replicating cells. On the other hand, DNA damage (particularly double-strand breaks) prevent survival during acute irradiation, as observed in the present work. As previously mentioned, three radiation doses were adopted: 16, 19, and 22kGy. These values were based on previous studies already conducted and published in the literature that reveal that some species of fungi may be resistant to gamma radiation or may develop some mechanisms of tolerance. The results showed that the fungi of the genus *Penicillium*, *Cladosporium*, *Nigrospora*, and *Curvularia* were resistant up to 16kGy, since, after irradiated, they still presented growth. The results obtained in this study agree with those already presented by other authors who conducted studies with *C. cladosporioides*. According to *D. Boniek et al.* [31], this was the only species resistant to exposure of gamma radiation and this characteristic may be related to the fact that some strains have the metabolic capacity to produce a dark-coloured pigment (the biopolymer melanin) that accumulates within the mycelium and protects against UV rays and ionizing radiation. Other published studies have shown that radiotrophic fungal species use melanin to convert beta and gamma radiation to chemical energy for growth. In fact, according to the results presented in the present work, it was found that the tolerant fungi exhibited a dark colour, evidencing the presence of the melanin pigment that may be associated with this resistance mechanism.

The resistance of some fungi common to gamma radiation was also studied by *Y.G. Saleh et al.* [32]. Ten species of fungi representing the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium* and *Penicillium* were examined for their resistance to

gamma radiation from a source of ^{137}Cs. In this study, it was found that fungi with melanin hyphae such as *Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia lunata* and *Curvularia geniculata* survived after high doses of gamma radiation. The macroconidia of *Curvularia* and *Alternaria* sp., which are of a thick wall and contain the pigment melanin, showed resistance and, these two characteristics can probably contribute to the increasing resistance of these species. In another study, it was observed that among all fungi isolated from documents with parchment as support, the most frequent (and more resistant to gamma radiation) genera were *Penicillium* and *Aspergillus* [33]. These results corroborate those already presented in this study, where a high resistance to radiation of the genus *Penicillium* was observed.

Microbial resistance to gamma radiation was investigated by *V. Múčka et al.* [34] and Neuzilová et al. [35], using ^{60}Co in order to determine DNA stability and effects on cell membranes. Other authors also studied the effect of ^{60}Co, confirming that the negative effect of the radiation was not linearly dependent on the radiation dose [36]. It is important to emphasize that there is a concern about the consequences of high radiation doses used in relation to the integrity of the support of artworks. There is a common-sense about this matter that only very high doses could affect the integrity of the support. In our work, this was not a concern as the isolated fungi were irradiated on Petri dishes. The literature reports that gamma radiation has already been used in other opportunities to decontaminate paper, showing that doses between 3 and 10kGy were effective in fungal decontamination, without causing significant damage to the materials. The authors also identified that high doses (around 15kGy) were tested for disinfection of paper, without any injury to the support. Even so, there is almost nothing in the literature about the effects of gamma radiation on parchment documents. The authors suggested 5kGy as a minimum dose to be used for decontamination of parchment documents when the main objective is to decontaminate instead of sterilizing [34]. A work was carried out by *M.M. Rizzo et al.* [37] and *M.G.C. Tomazello* [38] in the restoration of 17$^{th.}$ Century paintings considered that the appropriate radiation dose to eliminate microorganisms is 6kGy, being 25kGy the standard dose for sterilization. In other studies, it was found that the maximum dose already used on parchment support was 30kGy since there was no damage to the support. However, the study was not conclusive, as there are many types of supports that need to be tested [33]. *C. Negut et al.* [39] conducted a study on the defects induced by gamma radiation with a ^{60}Co source in historical pigments. The results revealed the radiation-induced changes in 22 historical pigments. Even after three months after irradiation, changes were not significant either because there was in fact no pigment colour change or because the changes were reversible. Thus, it was concluded that gamma radiation presented itself as a reliable decontamination treatment. Decontamination techniques using other radiation sources have been studied in search of decontamination techniques that have less harmful effects on the object of cultural heritage to be disinfected.

It was concluded that cosmopolitan fungi, isolated from the air, charcoal on paper artwork, and fabrics of the artwork can be highly resistant to gamma radiation. The same cosmopolitan fungi that proved to be resistant to radiation are worldwide spread in museums, libraries, and archives: *Nigrospora*, *Curvularia*, *Cladosporium* and *Penicillium*. Even though, the literature reports lower levels of radiation, some species are only eliminated under radiation up to 22kGy. The mechanism involved in the elimination of fungi is probably associated to DNA damages due to acute irradiation. The results here observed are useful for similar Portinari's artworks exposed in museums all over the world if a decision for a non-destructive decontamination technique is taken.

There is no doubt that the technique used has fundamental applicability for the nature of the work in question, but it still needs to be studied in conjunction with other control techniques as there is a possibility of some damage to the support (paper). Of course, any analysis of intervention in a work of art deserves to go through a cost/benefit analysis and the technique

used obviously took this into account. Unfortunately, the work was accidentally lost in a fire, but future studies can and should be conducted in other works of the same nature to improve the use of this disinfection technique in works with the same characteristics. In the present work, it is important to emphasize that fungi were inactivated under ideal growth conditions (rich culture medium and proper incubation conditions); if the radiation is directly applied on the artwork, biochemical effects developed by fungal strains against the radiation would be probably minimized, due to the poor growth conditions on the cellulosic substrate. Under this condition, a much lower irradiation dose will probably lead to the same result here obtained.

Chemical composition of artist paints commercialized in Brazil varies greatly. Therefore, a multi-component characterization requires multi-technique analyses [40-42]. Advantages of each technique (FTIR, Raman, Py-GC/MS and SEM-EDS) [43-46] need to be pointed out to present a broader knowledge of the paints used by Brazilian contemporary artists.

Conclusions

Biodeterioration processes are closely related to the presence of bacteria and fungi in cellulosic materials. Microorganisms detected in the present work, are closely related to the type of environment they were found, since fungal genera found are very common in museums. However, they pose a threat to health and heritage conservation. *Penicillium* and *Cladosporium* species were predominant in contamination and fungal proliferation.

Fungi spores suspended in the air were the main vehicle for dissemination and the co-occurrence of the same species in the work of art and in the air reveals the existence of cross-contamination.

The use of gamma radiation proved to be a suitable decontamination technique for the artwork studied, a drawing made with charcoal, where no other treatment technique could be used. The radiation dose tested was effective in eliminating resistant fungal isolates. Although there is no standard dose recommended.

The Portinari Institute still have some pieces with the same structural characteristics (support, design printing medium, storage conditions, natural aging process), to be studied and that can be submitted to gamma radiation, as disinfection treatment, in order to confirm the effectiveness of the technique as proposed.

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