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Gamma Radiation (^{137}Cs) for the Treatment Against Resistant Fungi in Two Brazilian Libraries

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***Keywords*— Gamma irradiation, Fungi, Cesium, Molecular Biology, Libraries.**

***Abstract*— The present work showed that a wide diversity of fungi was found in the environment where the Collection Academia Brasileira de Ciências is stored, in the Henrique Morize Library, being this diversity quite common in spaces storing this kind of cellulosic material. Fungal genera such as Aspergillus, Cladosporium, Rhizopus and Trichoderma were identified through modern Molecular Biology techniques. The presence of these fungal genera is probably associated to climatization and humidity control, which prevent the occurrence of a wider diversity of microbes. However, two fungal genera, Trichoderma and Rhizopus, resisted 137Cs irradiation doses with up to 19 kGy, considered high for the elimination of fungi. On the other hand, in the National Library, particularly in some sectors, an even higher diversity of fungal species/genera was observed, a fact that seems to be compatible with the size of the library, with a much higher circulation of people. This higher microbial diversity indicated the occurrence of fungal species absolutely uncommon for in libraries and archives, such as: Diaphorte, Trametes, Arxotrichum, Grammothele, Pessiophora, Phebia and Talaromyces. Just successive samplings in the same areas will allow a confirmation if these fungal genera are permanent of occasional, due to some oscillation in the operation of circulation systems or a possible transport of these species from the outside to the library, which allows those species to remain latent in relation to growth. All microbial samples from the National Library, survived irradiation up to 16kGy.*

I. INTRODUCTION

The problem of microbial contamination in air-conditioned artificially controlled spaces is being focused by several researchers [1-4].

Fungal diversity in these spaces becomes critical due to the possibility of recirculation of suspended particles into the interior of those spaces. Climatization equipments are usually associated to relative humidity control systems, which, may contribute to the concentration of microbial species from the air, if not properly operated.

This problem becomes even more severe, if climatized spaces constitute spaces for the safeguard of cellulosic materials, that may contribute for the proliferation of fungi and bacterial cells.

Libraries and archives constitute suitable spaces for these types of microbial proliferation, a fact that can compromise the quality of atmospheric air for users and workers, as well as a decrease in the permanence index of books, historical documents, personal archives, photographs, parchments, all of them from lignocellulosic nature.

What is usually observed is the search for local solutions, almost never reproducible for other spaces, even with similar characteristics, due to local specificities, distinction in the occurrence of fungal species and availability of techniques for microbiological monitoring and technological solutions.

Microbiological monitoring of climatized environments has become a common practice all over the world, in particular when it is related to deteriorating processes of specific substrates. It is known that microorganisms of the most diverse types are present in environments, often associated with suspended particles resulting from inadequate preventive maintenance of air circulation devices or air humidity controllers in bad operating conditions. Literature reports several methodologies for monitoring air-conditioned environments, with regard to the quantification of microorganisms. These are adapted or targeted to the environmental characteristics of the monitored area, and the likely occurrence of microbial genera.

Pasanena et al. [5] studied fungal growth and viability in building materials under controlled humidity conditions. The materials were submitted to various environmental conditions with variable water absorption and relative air humidity, and, after appropriate treatments, fungi and actinomycetes were cultivated after two weeks of incubation. The results showed that when water was absorbed by capillary action, fungal growth started more quickly in wood-based materials, under humidity in the order of 20% (m/v). Condensation under variable conditions of relative humidity and temperature caused differentiated growth of fungal populations, followed by rapid growth, particularly at high relative humidity. It is noteworthy that the fungal species were particularly tolerant to fluctuations in temperature and relative humidity conditions, with very few effects that compromised fungal viability. In a recent literature review, Pinheiro and Sequeira [6] wrote an extensive review focusing on the action of fungi in cultural heritage collections, including paper-based documents, photographs, films, presenting current techniques for prevention, treatment and more appropriate strategies for studies in the area of biodeterioration. Shirakawaa et al. [7] verified the susceptibility of fungal attack to phosphogypsum. Procedures described by ASTM were used, which suggest a combination of three fungal species for biodegradation studies. Furthermore, the authors chose to use a strain of *Cladosporium* sp. isolated from the material itself. It was verified in the study that the species introduced in the tests did not present growth on phosphogypsum, a fact that was not observed for the genus *Cladosporium*. In order to inhibit fungal growth, the authors tested phosphogypsum heated to 600 ºC as a substrate for development of *Cladosporium*, *Aspergillus niger* and *Trichoderma* strains, previously isolated from the environment and stored for two years. All showed development in media containing phosphogypsum as a substrate, although *Fusarium* and *Rhizopus* strains showed different behavior, not causing discoloration of the phosphogypsum contained in the media on Petri dishes. Nielsena et al. [8] studied the influence of relative humidity and temperature on the growth and metabolism of fungal species in various types of building materials. The authors, in order to evaluate the metabolic diversity, incubated several samples of building materials based on wood, starch and composite materials, at temperatures ranging from 5 to 25 ºC, under conditions of relative humidity of 69 to 95% and for a period of up to seven months. From these tests, authors were able to conclude about the diversity of species acting on the materials, most of them belonging to the genera *Penicillium*, *Aspergillus* and *Eurotium*, all of them able to produce secondary metabolites and mycotoxins.

Giannantonio et al. [9] observed the formation of surface incrustations on concrete, under controlled conditions as well as with the supplementation of compounds to the concrete. Authors verified that fungal strains of *Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Mucor*, *Penicillium*, *Pestalotiopsis* and *Trichoderma* colonized directly on the concrete.

Hoang et al. [10] evaluated the susceptibility of green building materials to biodeterioration by *Aspergillus niger*, as a reference fungus. The detection of spores and the presence of nutrients contributed to the growth of *Aspergillus niger* on plastic-based walls and ceilings. The authors observed a strong correlation between the content of the mixture and the organic materials, observing the time in which the coating of 50% of the surface area by fungi took place. The results suggest that the presence of organic matter in a given material seems to be a key factor for the diagnosis of fungal susceptibility with consequent biodeterioration. Not only the materials are responsible for

the spread of fungal and bacterial spores, but also the climatic conditions of the environment.

Picco and Rodolfi [11] studied the dissemination of fungal species in the Milan metro, identifying species typical of the external environment and their correlation with the internal environment. They identified the presence of *Cladosporium*, *Penicillium*, *Epicoccum* and *Alternaria* in the external environment, and, internally, the possibilities of spore diffusion of these same species, led to the development of the same genera.

Milanesia et al. [12] identified signs of deterioration in three regions of an 18th-century fresco in the Santissima Annunziata Church in Siena, Italy. In addition to identifying the composition of the fragments of the fresco by scanning electron microscopy, part of the fragments was incubated in suitable culture media for the growth of heterotrophic aerobic microorganisms, identifying, after incubation, strains of *Kocuria erythromyxa* and *Sphingomonas echinoides*, by sequencing of DNA. These microorganisms grew rapidly in a mineral medium free from a carbon source, with visible biofilm formation of a deteriorating nature.

Portugal et al. [13] used molecular biology techniques to elucidate fungal morphology in order to assess the infection of historical documents. The researchers identified a wide diversity of fungi on all types of papers. Fourteen fungal genera were identified, the most frequent being *Cladosporium*, *Penicillium* and *Aspergillus*, and the least abundant of the genera *Alternaria*, *Botrytis*, *Chaetomium*, *Chromelosporium*, *Epicoccum*, *Phlebiopsys* and *Toxicocladosporium*. The authors emphasize that, among the genera found, in all types of papers there was the presence of *Cladosporium cladosporioides* and *Penicillium chrysogenum* as the most representative.

Abe [14] identified the occurrence of fungal contamination in materials stored in an art museum, which was monitored using a biological index related to climatic parameters, which gives an indication of the environmental capacity of the fungus to proliferate in that region. In order to determine this index, fungal spores were encapsulated at the site and spore germination, measured by the extent of fungal hyphae, was measured. The predominant *Aspergillus penicillioides* and *Eurotium herbariorum*, are most likely species in that environment.

The main modes of action of microbial species typical of paper collections can be found in Tables 1 and 2.

A series of other microbial populations are reported in the published literature, and in each of them specific microbial groups are identified that are related to the characteristics of the materials where populations grow, as well as to environmental factors that regulate the proliferation of these populations.. All these works allow us to conclude that microorganisms that colonize building materials or are found in aerial microenvironments are the same that colonize surfaces, including those made up of ligno-cellulosic materials that are part of documents in collections. Thus, for transferring files and book collections between areas under different climate conditions, attention must be paid to the fact that the maintenance of microbiological activity must be carefully evaluated.

Table 1 – Bacteria in archives and libraries

Bacteria	Source	Enzyme/Product	Effect
Acinetobacter	Paper Air	Protease Amilase	Support degradation
Bacillus	Organic material Air	Cellulase Organic acids	Acidification Fiber degradation
Cellvibrio	Paper Textiles	Cellulase Acetic acid	Acidification
Lactobacillus	Organic material	Cellulase Latic acid	Acidification
Micrococcus	Organic material Air	Cellulase Organic acids	Acidification Decoloration
Pseudomonas	Organic material	Protease Organic acids	Decoloration
Staphylococcus	Paper Textiles	Latic and acetic acids	Acidification
Streptococcus	Paper Textiles	Latic and acetic acids	Acidification

Based on these information, the present article aims to identify fungal populations present in two different libraries, evaluating atmospheric contamination and also in selected pieces from special collections. Once the

populations present in both cases are known, the use of the gamma irradiation will be used, aiming to evaluate the minimum concentration of radiation capable of preventing the proliferation of fungi in each case.

Table 2 – Fungi in archives and libraries

Fungus	Source	Enzyme/Product	Effect
Alternaria	Organic material Air	Protease Amilase	Decoloration
Aspergillus	Organic material Air	Organic acids	Acidification
Chaetomium	Papel, Cartão	Celullase Organic acids	Decoloration
Cladosporium	Organic material Air	Protease Latic acid	Decoloration Acidification
Fusarium	Organic material Air	Celullase Organic acids	Fiber damage
Mucor	Organic material Air	Protease Organic acids	Decoloration Acidification
Penicillium	Organic material Air	Enzymes Organic acids	Decoloration Acidification
Rhizopus	Organic material Air	Enzymes Organic acids	Decoloration Acidification
Sporotrichum	Paper Air	Cellulase Ligninase	Decoloration Acidification
Trichoderma	Paper Wood	Celulase e ác. Orgânicos	Decoloration Acidification
Verticillium	Paper Textiles	Celulase e ác. Orgânicos	Decoloration Acidification

II. MATERIALS AND METHODS

2.1 Samples collection

The locations selected were: (1) Henrique Morize Library of the Museum of Astronomy and Related Sciences (MAST) and the National Library (BN), both located in the city of Rio de Janeiro, Brazil.

2.2 Culture media

The culture medium used for the growth of total fungi was Sabouraud Dextrose with chloramphenicol, an agar recommended for the cultivation of fungi.

The culture medium, as specified above, is a preferential medium for the isolation of fungi and yeasts, since it has satisfactory sources of nitrogen, in addition to a mixture of amino acids due to the presence of meat peptones and casein. The high concentration of dextrose and the acidic pH resulting from the composition of the medium mean that this medium tends to inhibit bacterial growth, in addition to the inhibitory effect chloramphenicol.

The culture medium was dissolved in distilled water at a concentration of 65.0 g/L, autoclaved at 121°C for 20 minutes in a vertical autoclave, and distributed in sterile Petri dishes, in a laminar flow chamber, waiting for solidification after cooling, around 30 minutes. The medium thus prepared and distributed in Petri dishes was stored for a maximum time of 48 hours, proceeding to the collection of samples to evaluate the fungal growth.

2.3 Environmental and surface procedures

This phase of the work describes the procedures used to collect environmental and selected books/documents from the two libraries. Two strategies were adopted: (1) Environmental collections: In this case, Petri dishes containing Sabouraud medium were opened and placed in several spaces and the particles were allowed to settle for 1 hour. In this case, it is expected to have an estimate of

aerial fungal populations, potentially contaminating the books; (2) Surface collections (Figure 1). In this case, selected works (in the same spaces where environmental collections were carried out), were separated and subjected to a brief surface "scraping", to evaluate the possible contamination on the surface of the work(s) resulting from environmental deposition. In this case, after swabbing, the swabs were again swabbed on the surface of Petri dishes containing Sabouraud medium.

Fig. 1: Procedure for surface collection

Samples collected, both in the environment and on the surfaces, were incubated for 7 days at 23 ºC. After this period, a photographic record of each plate was carried out, for further identification by Molecular Biology.

2.3.1 Henrique Morize Library (MAST)

The collection at the Henrique Morize Library was carried out at the end of 2018, comprising a total of 120 Petri dishes containing Sabouraud medium, distributed as follows: 60 for the general environment, including the Library's sliding shelves, 25 for evaluation of the books in the Collection from the Brazilian Academy of Sciences processed by the MAST team and 35 for books from the same collection not processed by the MAST team.

The purpose of this distribution may indicate the effectiveness of the cleaning process of the works (processing) and also, to verify if samples from the environment have somehow contaminated the works of the collection. Collection points were determined jointly with the Henrique Morize Library team and based mainly on the recent acquisition of the collection of the Brazilian Academy of Sciences by MAST. This collection showed particular interest in the development of the present work, due to the fact that it is a large collection, with a wide documental diversity and because it contains processed (sanitized) and unprocessed books/documents, which could indicate interesting results about the possible fungal contamination. Simultaneously, documents/books from the ABC Collection were selected, to assess if the documents/books of the Collection could bring some external contamination to the MAST collections, since there was no precise information about its previous condition of storage. Thus, we sought to have a wide range of documents and books so that we could have an accurate diagnosis of previous contamination (Table 3). Table 3 therefore indicates the number and diversity of books and documents that were individually evaluated by surface rubbing with sterile swabs, as previously described.

Table 3: Documents/books selected for surface collection of samples from the ABC Collection

Number of pieces	Type
5 (B1 a B5)*	Reference books
5 (B6 a B10)	Leaflets
5 (B11 a B15)	Thesis
5 (B16 a B20)	History books
5 (B21 a B25)	Regular books
5 (B26 a B30)	Books – Good conservation state
5 (B31 a B35)	Books – Bad conservation state
5 (B36 a B40)	Common books – Good conservation state
5 (B41 a B45)	Common books – Bad conservation state
5 (B46 a B50)	Books – Unconventional languages
5 (B51 a B55)	Periodicals
5 (B56 a B60)	Annals of Congresses

* Shadowed cells indicate processed (cleaned) books/documents.

2.3.2. National Library

Similarly, we tried to use the same methods carried out in the Henrique Morize Library, collecting samples in the environment and in specific works of the National Library. Although the characteristics of location, size, light incidence, among other factors, do not allow a direct comparison between the two libraries, we tried to adopt the same methodology.

The collection at the National Library, carried out in early 2020, is specified in Tables 4 and 5.

Environmental collections were carried out in the area where Common Books and Periodicals were located and also in copies selected by the staff of the National Library. The location of each work is specified and can be easily located in the National Library.

Similarly, samples collected were incubated for fungal growth, isolated and identified.

Table 4: Sampling – Common Books

Common Books – 6th. Floor	
Environment	
1	Bookcase 453 – 1st. Shelf
2	Bookcase 450 - 1st. Shelf
3	Bookcase 446 - 1st. Shelf
4	Bookcase 443 - 1st. Shelf
5	Bookcase 441 – Bookcase (Low)
6	Bookcase 472 - 1st. Bookcase (Up)
7	Bookcase 221 – 2nd. Shelf
8	Bookcase 240 – 3rd. Shelf
9	Bookcase 246 – 3rd. Shelf
10	Bookcase 265 – Bookcase (Low)
11	Bookcase 269 – 2nd. Shelf
12	Bookcase 289 – 1st. Shelf
13	Bookcase 289 – 5th. Shelf
14	Bookcase 302 – 2nd. Shelf
15	Bookcase 297 – 3rd. Shelf
16	Bookcase 313 – 7th. Shelf
17	Bookcases 305 and 316 – Floor
18	Cabinet VI
19	Bookcase 409 – 4th. Shelf
Surface of books/documents	
A	Loc: VI - 289, 5, 17 / Low
B	Loc: VI - 289, 1, 15 / Low
C	Loc: VI - 289, 3, 36 / Up
D	Loc: VI - 302, 2, 85 / Back
E	Loc: VI - 302, 2, 82 / Low

2.3.3. Molecular Biology

The extraction of DNA from isolated colonies was done with Quick DNA Fungal/Bacterial Miniprep kit, from ZymoResearch. The polymerase chain reaction was performed to amplify the specific region of DNA (ITS), which is unique to fungi. PCR (polymerase chain reaction) results were obtained by gel electrophoresis and the samples were then evaluated for their sequences. From the results it was possible to identify most of the fungi.

This procedure was adopted for all species isolated in both libraries. Photographic records (macroscopic and microscopic) were only possible for the fungi isolated in the Henrique Morize Library, due to operational reasons. However, this did not prevent the complete identification of fungi from both libraries.

Table 5: Sampling - Periodicals

Periodicals - 4th. Floor	
Environment	
1	Bookcase 19 – 2nd. Shelf
2	Bookcase 21 – 3rd. Shelf
3	Bookcase 48 - 2nd. Shelf
4	Bookcase 46 - 2nd.Shelf
5	Bookcase 98 - 2nd.Shelf
6	Bookcase 92 - 3rd.Shelf
7	Bookcase 150 – 5th. Shelf
8	Bookcase 145 - 3rd.Shelf
9	Bookcase 193 - 2nd.Shelf
10	Bookcase 198 - 3rd.Shelf
11	Bookcase 482 – 1st. Shelf
12	Bookcase 203 – 4th. Shelf
13	Bookcase 211 – 5th. Shelf
14	Bookcase 277 - 2nd.Shelf
15	Bookcase 270 – 4th. Shelf
16	Bookcase 300 - 3rd. Shelf
17	Bookcase 307 - 3rd. Shelf
18	Bookcase 334 - 2nd. Shelf
19	Bookcase 346 - 3rd.Shelf
20	Bookcase 359 – 4th. Shelf
Surface of books/documents	
A	Loc: 4, 019, 03, 14 / Cover – Front
B	Loc: 4, 019, 03, 05 / Cover – Front
C	Loc: 4, 020, 03, 01 / Cover - Back
D	Loc: 4, 020, 03, 15 / Cover – Front
E	Loc: 4, 020, 03, 05 / Cover - Back

2.3.4. Irradiation with ^{137}Cs

Fungi were irradiated in increasing doses, aiming to identify the ideal dose to eliminate the overgrown fungi. Thus, although the procedure was carried out directly on

the Petri dishes where the fungi grew, the same procedure could be applied to the fungi on the surface of the works.

The equipment used was a 19-ton cavity research irradiator (Figure 2). Currently, its sources of ^{137}Cs with activity of 43.2 KCi provide a maximum dose rate of 1.45 $KGy.h^{-1}$ inside two rectangular irradiation chambers 68 cm wide, 137 cm long and 20 cm high positioned above and below the plane of the gamma source. The gamma source consists of 28 parallel spaced, double-encapsulated plates containing cesium-137 (Figure 3). A pneumatic system allows not only the access door to be moved, but also the sources, through a control panel. IDQBRN adopts gamma irradiation as a multipurpose technique, with a qualified team dedicated to the application of ionizing radiation in industrial processes, as well as in environmental preservation.

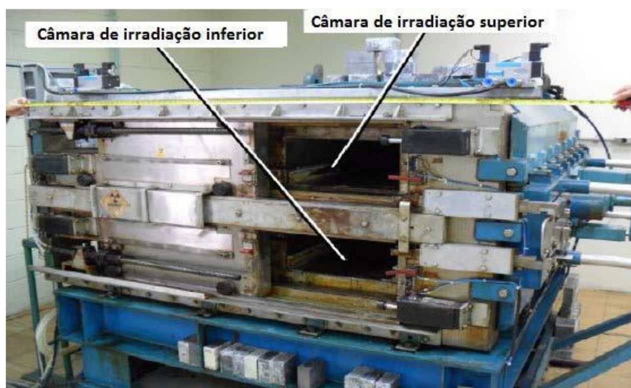

Fig 2: Front view of IDQBRN gamma irradiator

An ascending irradiation time was adopted in the assessment of the threshold concentration for each fungal species. The irradiations were carried out after an incubation period of 14 days and the tests were carried out at the Research and Development Institute of the Army Technological Center (IPD / CTEX), in Guaratiba, Rio de Janeiro.

Petri dishes were placed in groups in the irradiation chamber, occupying the central section of the shelf, with a predefined threshold height of 7 cm, in order to achieve the lowest dose uncertainty. Next, the samples were irradiated with a source of ^{137}Cs for specific periods of time, as shown in Figure 3.

In order to prevent uncertainty, exposure times were calculated using a computer software developed specifically for this purpose, based on the latest dosimetric charts from the irradiator. The dose range was: 1, 2, 3, 5, 6, 9, 12, 16, 19, 22 and 25 KGy, with an average uncertainty of ± 5% [15].

Fig. 3: Petri dishes inside the chambre of the irradiator

Immediately after the irradiation sessions, samples were moved to boxes and submitted to analytical tests in the laboratory, in order to monitor the post-radiation fungi viability. This means that after the irradiation procedure, the same samples in a Petri dish were taken back to the laboratory and new transfers were made to freshly prepared culture medium in order to verify the fungal viability after the procedure. This would allow to assess the fungal resistance to the treatment. The post-incubation methodology, after irradiation, was the same as presented previously.

III. RESULTS AND DISCUSSION

3.1 Fungal growth – Henrique Morize Library

All Petri dishes were placed in a controlled chamber at 25°C for 14 days. The number of Petri dishes with fungi in the collection and in bookcases were substantially large (Figure 4).

Figure 4 shows the most characteristic fungi isolated from environmental collections and selected pieces from the Henrique Morize Library. From Figure 4 it can be seen the occurrence of approximately ten morphological types, regardless of the collection location. This is in accordance to what was intended to be proved: the fact that potential fungal contamination could indicate a cross-contamination of the space on the ABC Collection or vice versa. This isolation also allowed the evaluation of fungal resistance to irradiation, showing a possible variability among the species found.

Fig. 4: Samples from Henrique Morize Library (fungi already isolated)

It is worth mentioning, in this phase of the work, that the selection and isolation of the species was made only by morphological characteristics of the types grown in Petri dishes. There was still no exact information about who these species would be.

Table 6 shows the fungal species identified after isolation and their post-growth macroscopic and microscopic images on Sabouraud Dextrose medium, now based on Molecular Biology techniques.

The identification procedure by Molecular Biology, was carried out in cooperation with the National Institute of Technology (INT) and the main fungi identified are described in Table 7.

Since the samples collected were grouped into morphological groups; the identification presented in Table 7 showed the average occurrence of 15 fungal species, restricted to 9 fungal genera (*Cladosporium*, *Pestalotiopsis*, *Hamigera*, *Pleospora*, *Aspergillus*, *Rhizopus*, *Pleosporales*, *Trichoderma* and *Hypocrea*). These are cosmopolitan fungi, originating from the atmosphere and common in libraries and archives.

Bensch et al. [16] carried out a large study showing the universal occurrence of fungi of the genus *Cladosporium* in climate-controlled environments, showing that in samples collected in Europe, North America, South Africa, New Zealand and China, the *Cladosporium* genus presents worldwide dissemination. The authors showed the occurrence of 46 species belonging to this genus, all of them documented, 16 of which are new species. The study, however, does not present data about the occurrence of this genus in samples collected in South America, although it is known that its occurrence is also common, for example, in Brazil.

Table 6: Fungi identified in the ABC Collection (MAST)

Classification	Microscopy	Macroscopy
Cladosporium cladosporioides		
Pestalotiopsis sp.		
Hamigera paravellanea		
Rhizopus oryzae		
Aspergillus niger		
Periconia sp.		
Trichoderma longibrachiatum		
Trichoderma viride		
Hypocrea viride		
Hypocrea lixii		

Table 7: Fungi from Henrique Morize Library

Sample	Classification
1	*Cladosporium cladosporioides*
2	*Pestalotiopsis sp.*
3	*Hamigera paravellanea*
4	*Pleospora sp.*
5	*Aspergillus niger*
6	*Rhizopus oryzae*
7	*Aspergillus sp.*
8	*Pleosporales sp.*
9	*Aspergillus flavus*
10	*Trichoderma viride*
11	*Hypocrea viride*
13	*Hypocrea lixii*
14	*Periconia sp.*
15	*Trichoderma longibrachiatum*

Hassan et al. [17] also attest the occurrence of *Cladosporium* in public libraries, comparing the results with the microbial occurrence in internal and external environments of libraries. The authors conclude that the external environment becomes the main source of contamination in libraries, with bacteria resulting from human activities, usually inappropriate handling of pieces. Fungi of the genera *Penicillium*, *Cladosporium* and *Aspergillus* are, respectively, the major contaminants. It is observed that two of the three genera mentioned by the authors were found in the Henrique Morize Library (or in selected pieces from the ABC Collection), confirming the authors' indication.

The *Pestalotipsis* fungus is normally associated with the degradation of petroleum, and there are no references that indicate its relationship with the degradation of cellulose or its usual atmospheric occurrence. However, recently, Vieto et al. [18] showed its occurrence and cellulolytic activity in a 19th century work of art, thus showing the importance of further studies that can understand the combined effect of fungal consortia in biodeterioration processes.

The same applies to the genera *Hamigera*, *Pleospora* and *Pleosporales* where no references were found that could explain their occurrence in libraries or archives. It should be noted that it is not always possible to explain or correlate the occurrence of microbial species in certain environments, which may be due to external factors that cannot always be explained or detected.

Zyska [19] proved the wide occurrence of microorganisms in library spaces, exemplifying the wide diversity found. The author reports the presence of 84 fungal genera isolated in a period of 60 years from materials from libraries and archives (books, articles, papyrus, glues, paints, magnetic tapes, wood, etc.). The author reports that 43 genera of fungi were isolated from the air, and each fungus occurred at least 3 times. It should be noted that the scope of the research included surveys carried out for more than 60 years. In the limited space of time for the development of this present work, it was not possible to collect samples to confirm the occurrence of the species, which may be momentarily in the environment, not being characteristic species of that space.

Literature presents several articles that correlate the occurrence of the *Rhizopus* genus with the biodegradation of cultural heritage, particularly those made of cellulosic materials. Cappitelli et al. [20] carried out an extensive study on the biodeterioration of synthetic polymers by microorganisms of the genus *Rhizopus*, concluding that most synthetic polymers are susceptible to fungal attack, alerting to the need to fill a gap in the study of this type of biodeterioration. Pinheiro and Sequeira [21] also studied biodeterioration of cultural heritage by microorganisms of this fungal genus.

Beata [22] also reports the occurrence of the *Rhizopus* related to the biodeterioration of cultural heritage, particularly due to its cellulolytic characteristic, with emphasis on advanced proteomics techniques for identification.

The same observation was made for *Trichoderma* genus, a fungus with wide occurrence, particularly in archives and libraries. Jia et al. [23] suggest antifungal substances for the preservation of cellulosic materials against the action of fungi of the genus *Trichoderma*. Zhang et al. [24] report the biodegradation of leather (eg, book covers) and papyrus by microorganisms of this genus.

Finally, the genus *Hypocrea* is reported in the literature as being responsible for the degradation of photographic materials and also as producers of pigments during colonization on papers [25-26].

Considering now the results from the ABC Collection books, the Henrique Morize library (MAST) showed the presence of fungal growth until 16 kGy for the genera *Rhizopus*, *Periconia* and *Trichoderma* , thus showing that these three genera are the most resistant to treatment with gamma radiation. Additional fungal growth in the same samples was not observed at 19 kGy , and radiation-induced fungal inactivation was therefore assumed. The

irradiation curve for the samples most resistant to gamma radiation treatment can be found in Figure 5.

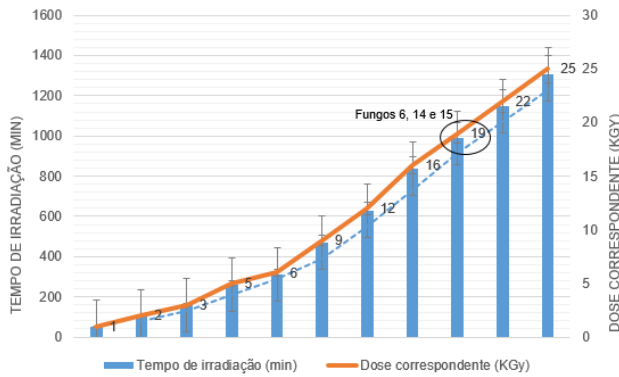

Fig. 5: Irradiation up to 16 kGy

In the work by Kalawate and Mehetre [27], the authors studied fungal resistance to gamma radiation on wood. Doses up to 10 kGy were efficient in eliminating fungi, although they studied doses up to 50 kGy. An important fact reported by the authors is that dosages up to 10 kGy did not compromise the cellulosic structure, a fact confirmed by scanning electron microscopy.

Maity et al. [28] studied the effect of gamma radiation on fungi of *Trichoderma* and *Aspergillus* genera, both also found in the present study. They found that small doses of Cobalt inhibited fungal germination, with the elimination of species at 2.5 kGy. The authors, however, worked with fungi present in seeds, a matrix quite different from the one studied in the present work.

Linh et al. [29] studied the effect of gamma radiation on fungi that colonize Japanese paper. The objective of that work was to evaluate the effect of gamma radiation on fungi of the genera *Aspergillus*, *Penicillium* and *Cladosporium*, also evaluating the effect of radiation on the mechanical structure of the paper. In order to achieve this goal, Japanese paper samples were moistened and contaminated with the aforementioned fungi. The effects of gamma treatment were measured at different stages of fungal growth. It was observed that doses around 10 kGy already affected the structure of the paper, as well as its color.

3.2 Fungal growth – National Library

Observing the results obtained from the samples from the National Library, with the morphological types found in the Henrique Morize Library, it can be predicted that, at least macroscopically, the morphological types seem distinct (Figure 6). This is a relevant fact, since it is to be expected that, due to the characteristics of location, ventilation, temperature control and relative air humidity, among others, different morphological types can occur in different spaces.

Fig. 6: Samples from National Library

Culturable fungi isolated were identified using Molecular Biology techniques and the list of fungi is shown below (Table 8).

Since these fungi were isolated from another space, from another library, it is possible to observe the variability of genera/species in relation to those previously identified in the Henrique Morize Library. The classic environmental contaminants, present almost universally, were also found here: *Penicillium*, *Aspergillus* and *Fusarium*.

What draws attention in Table 8 is the presence of unusual species, probably due to factors such as: incidence of light, chemical contamination due to the central location of the library and intense movement of personnel that can contribute to the movement of exogenous organisms to the internal space. For example, about the species *Diaphorte paranensis*, no mention was found in the published literature. Another example is the fungus *Trametes,* associated with white rot.

Table 8: Culturable fungi from National Library

Classification
Penicillium citrinum
Simplicillium obclavatum
Aspergillus versicolor
Fusarium lichenicola
Diaphorte paranensis

Trametes vilosa
Arxotrichum sp.
Aspergillus calidoutus
Eutypella scoparia
Grammothele subargentea
Peniophora albobadia
Phebia floridensis
Talaromyces amestokiae
Penicillium resedanum

Fungi of the *Arxotrichum* genus are usually endophytic, associated with the inner parts of certain plants [30]. Fungi of the genus *Eutypella* are usually associated with sediments and in high depth environments [31].

Unusual occurrences, such as *Grammothele*, *Peniophora*, *Phebia* and *Talaromyces* were found. This fact does not invalidate the procedures adopted in the present work, since this diversity of species can indicate the need for a continuous monitoring, since a seasonal change can alter the environmental microflora.

Table 9 presents the distribution of fungi. Given the presence of so many isolated species, some repeated at various points, the use of gamma radiation was used to eliminate them.

All fungi showed viability up to 16 kGy, a result different from that observed for the Henrique Morize Library, where 3 fungal species were resistant up to 19 kGy radiation.

It is known that gamma radiation destroys the DNA structure of cells inhibiting the growth of fungi completely once they lose their functions. Incomplete inhibition can cause only minor damage to cells. High-energy irradiation directly impacts the DNA of living organisms, inducing cross-links and other changes that render the organism unable to grow or reproduce. When these rays interact with water molecules in an organism, they generate transient free radicals that can cause DNA damage.

These results corroborate what was previously reported: the variability of species, their origins, growth cycles and environmental conditions can contribute to a large amount of fungi capable of resisting even to high doses of radiation.

Table 9: Fungal viability after gamma irradiation

Fungi[1]	1 to 16kGy	19kGy	22kGy	25kGy
Penicillium citrinum	√	**X**	**X**	**X**
Simplicillium obclavatum	√	**X**	**X**	**X**
Aspergillus versicolor	√	**X**	**X**	**X**
Fusarium lichenicola	√	**X**	**X**	**X**
Diaphorte paranensis	√	**X**	**X**	**X**
Trametes vilosa	√	**X**	**X**	**X**
Arxotrichum sp.	√	**X**	**X**	**X**
Aspergillus calidoutus	√	**X**	**X**	**X**
Eutypella scoparia	√	**X**	**X**	**X**
Grammothele subargentea	√	**X**	**X**	**X**
Peniophora albobadia	√	**X**	**X**	**X**
Phebia floridensis	√	**X**	**X**	**X**
Talaromyces amestokiae	√	**X**	**X**	**X**
Penicillium resedanum	√	**X**	**X**	**X**

✓ Viable cells; X: Innactivated cells

IV. CONCLUSIONS

- Procedures adopted made it possible to identify the fungi present in the environment and in selected works from the Collection of the Brazilian Academy of Sciences. A diversity of fungi was observed, apparently compatible with the size of the library, with the occurrence of cosmopolitan fungi.

- In the National Library, where the Common Books and Periodicals are located, the fungal diversity was much greater than that found in the Henrique Morize Library, showing consistency with the size of the spaces.

- Many identified species are not common in archives and libraries, requiring periodic sampling in the same spaces in order to verify the prevalence of the species.

- It cannot be said that one library is more contaminated than the other; it can be said that the levels of fungal occurrence are compatible with the dimensions of the spaces and with the existence of air conditioning and relative humidity control systems present in the spaces.

- Molecular Biology techniques employed allowed the identification of unusual fungal species, which may not have relevance in the processes of microbiological degradation of cellulosic materials.

- Three fungal species isolated from the Henrique Morize Library (ABC Collection) were resistant to treatment with ^{137}Cs up to a dose of 19 kGy, a value that, according to the literature, already compromises the cellulose structure. Only doses above 19kGy were effective.

- Despite the greater diversity of species found in the spaces of the National Library, all were sensitive to treatment with ^{137}Cs up to a dose of 16 kGy.

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