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Mycoflora and DNA sequencing analysis of radioresistant fungi isolated from irradiated vehicular air conditioning filters collected in São Paulo, Brazil

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ABSTRACT

Fungal bioburdens in filters from car air conditioning systems represent a potential risk of respiratory or pulmonary diseases to both passengers and drivers. The present study demonstrated high fungal contamination in air conditioning filters collected from fifty-seven vehicles from the city of São Paulo, Brazil. The filters samples were irradiated with 10, 15 and 20 kGy gamma ray doses with a cobalt-60 source. The presence of *Aspergillus flavus, Penicillium glabrum* and *Fusarium incarnatum-equiseti* remaining after the ionizing treatment with 10 and 15 kGy. The radioresistant pathogenic species was confirmed through genetic sequencing of the ITS, β-tubulin and calmodulin gene rDNA regions. The 20 kGy dose was efficient in inhibiting pathogenic fungi growth in all samples but promoted the fungal decontamination in 79% of the samples. These results and other efforts will enable ionizing radiation to become an important tool in contributing to the recycling of automotive vehicle air filters and in ensuring indoor air quality for both drivers and passengers.

Keywords: Fungal contamination, indoor air quality, vehicle air filters, ionizing radiation.

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INTRODUCTION

Human activities have been discovered to be the leading causes of poor Indoor Air Quality (IAQ). Occupants may experience exposure to various pollutants that have been generated and accumulated in the indoor environment (Nitmetawong et al., 2019). Air-conditioning equipment can be contaminated by particles and dust, and their filters may be colonized by different microorganisms, such as bacteria and fungi, which are able to survive dry environments for relatively long periods of time. Furthermore, air conditioners can also act as microbial propagators (bioburden), since they usually only recirculate air already present in a closed environment (Andrade et al., 2015).

Fungi are eukaryotic, filamentous, and mostly spore-bearing organisms, which exist as saprotrophs and as animal or plant parasites. They are ubiquitous, and estimates indicate that the actual range of species is 2.2 to 3.8 million in the environment (Hawksworth and Lücking, 2017). They are noteworthy due to their ability to contaminate food and water, deteriorate collections, wood, and a variety of other materials, and may also produce mycotoxins, which can be lethal to humans. Fungi dispersed by atmospheric air are called airborne fungi or anemophilous fungi, and variations in the fungal microbiota of different cities or regions may occur (Kurup et al., 2000).

Qualitative and quantitative knowledge concerning these fungi in a certain region is important, as they can cause several respiratory diseases when inhaled, such as asthma and rhinitis. Respiratory system infection develops as a result of airborne fungal spore inhalation (Mezzari et al., 2002). However, the small internal space

of most vehicles may result in concentrations of various chemicals and organic compounds as much as two or three-fold higher than in other closed environments. Fungal bioburdens in filters from car air conditioning systems represent a possible source of respiratory diseases to both passengers and drivers and display potential impacts as bioaccumulators concerning indoor air quality (Aquino et al., 2018).

These indoor vehicle conditions are favorable to anemophilous fungi, whose spores are spread by air. In air-conditioning (AC) systems, the ventilation duct, filter, heat exchanger and fan coil are susceptible locations to the microorganisms that attach to these components can grow rapidly, which might reach the trachea, bronchi and alveoli, and contribute to adverse symptoms in the respiratory system. Such results could indicate the existence of a serious potential risk of exposure to particles of respirable sizes, possibly causing fungal epidemic infection (Bragoszewska, 2019). According to Gołofit-Szymczak et al. (2019), the automobile AC systems or cabin filters may be contaminated with microorganisms, and their extended use may result in an increase in microbial concentrations inside the car. The same authors identified a total number of 31 fungal species from 18 genera in the air of car cabins and, a qualitative analysis demonstrated that the most prevalent fungal species in all the cars studied were those from *Penicillium, Aspergillus* and *Cladosporium* genera (Gołofit-Szymczak et al., 2019).

Fungi control by ionizing radiation is well known in industrial food control, agriculture, medical supply sterilization and in the preservation of old books and artistic paintings. A study performed by Gumus et al. (2008) evaluated the effect of gamma radiation (^{60}Co source) on two heat-resistant fungi in food, by applying absorbed doses of 1, 3, 5 and 7 kGy for 52, 156, 260 and 364 min exposure times, respectively. The D_{10} value ranged from 1.08 to 0.59 kGy for filamentous fungi. However, Aquino et al. (2010) observed the presence of radioresistant fungi in medicinal herbs using a 5 kGy dose.

The costs (environmental and financial) of buying new air filters for each single use are worrying. The recycling of air filters is a viable alternative, as a way of successful recycling (Hall, 2009). According to Ted Magee (CEO of Delta M enterprise), the air filtration is essential for air-conditioning systems to function properly, providing thermal comfort and ensuring high indoor air quality (Magee, 2018). Typically, these filters are thrown away after just one use. Air filters must be replaced every few months, creating large amounts of waste sent to landfills that take several decades to decompose. The filters can be recyclable and reusable, meaning they prevent solid waste generation while delivering improvements to indoor air quality. Using a circular ‘product as service’ business model, once filters could be used, they are collected for cleaning and replaced with fresh filters. The filters can be reused several times without compromising on quality and performance. Delta's CEO estimates that cleaning filters generate less CO_2 than the manufacture of a new cardboard filter. In addition, recycling a filter 8-10 times consumes less water than an average load of laundry (Global Opportunity Explorer, 2018).

No studies applying gamma radiation in the decontamination of air filters used in vehicle air conditioning systems for the purpose of recycling the filter material are available. In this context, the aim of the present study was to analyze the effects of gamma radiation (10, 15 and 20 kGy) in the control of fungal air vehicle conditioning filter contamination and assess their recycling potential. In order to confirm the presence of radioresistant pathogenic fungi, the conidia were collected from irradiated samples and analyzed by DNA sequencing.

MATERIALS AND METHODS

Sample preparation

A total of 57 air-conditioning filters were collected from different vehicle models from October 2018 to January 2020, in the city of São Paulo, Brazil, from five different São Paulo metropolitan region districts. The samples were collected individually in polyethylene bags and wrapped in Kraft paper for microbial analyses and irradiation.

Sample fungi isolation procedures were performed by swabs directly in Sabouraud Dextrose Agar (SDA) in accordance to good laboratory practices. The material was manipulated in a laminar flow hood prior to the ionizing treatment, according to the Pitt and Hocking (2009) laboratory guide (Figure 1a, b, c and d).

Fungal isolation and enumeration

A standard plating regimen was used for the initial examination of all isolates in Petri dish SDA, which were incubated for 7 days and stored in a standard Biochemical Oxygen Demand (BOD) incubator at 25°C (± 2°C), for fungal culture growth. A mycological survey performed, including macro- and microscopic feature analyzes of and differential fungi genera counting, expressed as colony-forming units (CFU). The macroscopic taxonomic method examination of all fungal isolates and the microscopic identification procedures were carried out according to Pitt and Hocking (2009). The irradiated samples were grouped and numbered from 1 to 19 (10 kGy), 20 to 38 (15 kGy) and 39 to 57 (20 kGy), with their respective control group.

A qualitative statistical analysis was used to compare fungal control gamma radiation treatment (10, 15 and 20 kGy) counts. According to Ostertagová and Ostertag (2013), the aim of an analysis of variance (ANOVA) is to test for significant differences between the class means, by analyzing their variances. The PAST version 4.01 software was applied for data analysis using ANOVA and Tukey tests. An alpha of 0.05 was used as the cutoff for significance.

Gamma radiation treatment

After the microbiological control group analysis, the samples were individually packed in kraft paper and kept in a box during

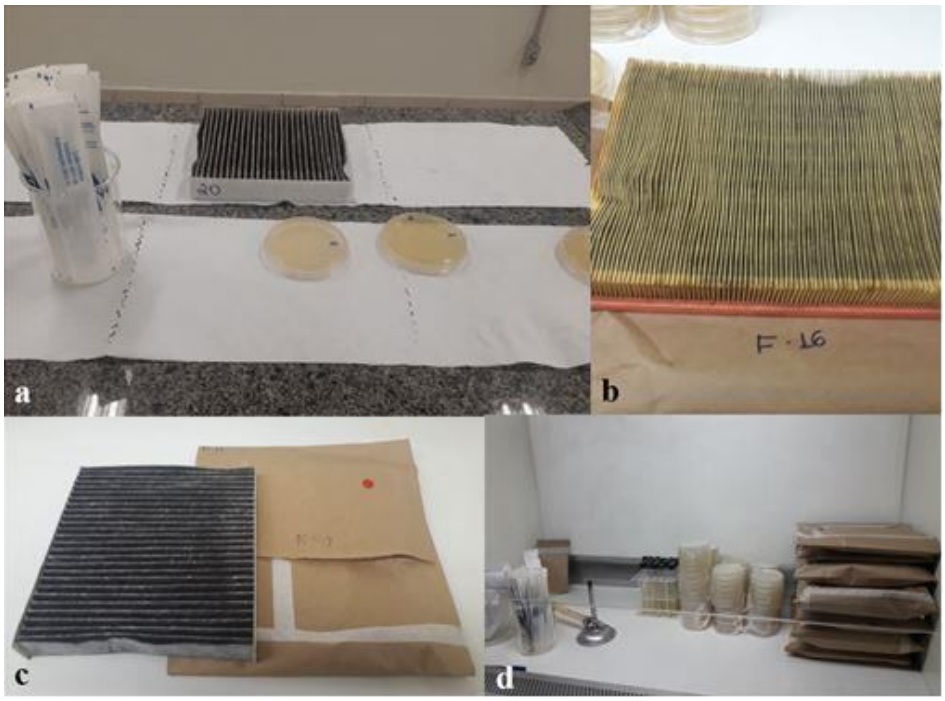

Figure 1. Filter sample laboratory analysis and gamma radiation treatment preparation (a, b, c and d).

irradiation. The gamma radiation treatments were performed using a ^{60}Co gamma-ray source, exposing the samples to 10, 15 and 20 kGy doses at room temperature (25 ± 2°C), at a dose rate of 5.5 kGy/h. The multipurpose irradiator is located at the Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN), in São Paulo, Brazil. Dosimetry was carried out using a poly-methylmethacrylate (PMMA) Harwell Red Perpex® dosimeter.

DNA extraction, amplification and sequencing

Radioresistant mycotoxigenic fungi (n=8) were confirmed by sequencing the ITS, β-tubulin and calmodulin gene rDNA regions of samples irradiated at 10 and 15 kGy. Fungal taxonomy discrimination is possible after molecular tests, which are indeed most appropriate and suitable for taxa characterization, since the classic identification of some species is difficult due to variations and overlapping of morphological and biochemical isolate characteristics. Sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) is, thus, recommended (Carbone and Kohn, 1999). In addition to the ITS region, the β-tubulin and calmodulin genes have also been applied for fungi sequencing. In this regard, DNA sequence assessments may provide important information for species definition and appropriate identification (Samson et al., 2006; Glass and Donaldson, 1995; Hinrikson et al., 2005).

DNA was extracted and directly purified from the fungal colonies grown on yeast extract sucrose agar at 25°C, in the dark, for three days using the PrepMan Ultra® kit (Applied Biosystems, Carlsbad, CA, USA). DNA was quantified using the GeneQuant pro Calculator (Amersham Pharmacia Biotech, Cambridge, UK). A fragment of the ITS region was amplified with the ITS1 (5′ TCCGTAGGTGAACCTGCG 3′) and ITS4 (5′ TCCGCTTATTGATAT 3′) primer pairs. Part of the β-tubulin gene was amplified using the T22 (5′ TCTGGATGTTGTTGGGAATCC 3′) and TUB-F (5′ CTGTCCAACCCCTCTTAGGGCGACT 3′) primers. Part of the calmodulin gene was amplified using the CMD 42 (5′ GGCCTTCTCCCTATTCGTAA 3′) and CMD 637 (5′ CTCGCGGATCATCTCATC 3′) primers.

The PCR mixture contained 12.5 µL 2× PCR Master Mix (Promega, San Luis Obispo, CA, USA), 6.5 µl Milli-Q water, 2 µL DNA (40 ng), and 2 µl (20 pmol) of each primer (Prodimol Biotecnologia, Minas Gerais, Brazil). The amplification program included an initial denaturation at 94°C for 3 min, followed by 35 denaturation cycles at 94°C for 1 min, annealing at 57°C (ITS), 49°C (β-tubulin), or 54°C (camodulin) for 1 min, and extension at 72°C for 1 min. A final extension step at 72°C for 5 min was included at the end of the amplification (Reis et al., 2012; White et al., 1990).

After the PCR, the products were purified using the QIAquick PCR Purification kit (Qiagen, Hilden, Germany) and stored at −20°C until sequencing. The PCR products were sequenced using the same primers as those employed for amplification, using the Big Dye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). The reactions were run on a 3100 DNA sequencer (Applied Biosystems). Consensus sequences were obtained using the AutoAssembler program (Perkin Elmer-Applied Biosystems) and SeqMan software (Lasergene, Madison, WI, USA). The sequences were used in BLASTn searches (www.ncbi.nlm.nih.gov) in order to confirm preliminary identifications.

RESULTS

Control samples

Concerning the mycobiota control group (n = 57), 95% of the filter samples were contaminated with different fungal genera (Figure 2). The total fungal count is presented in

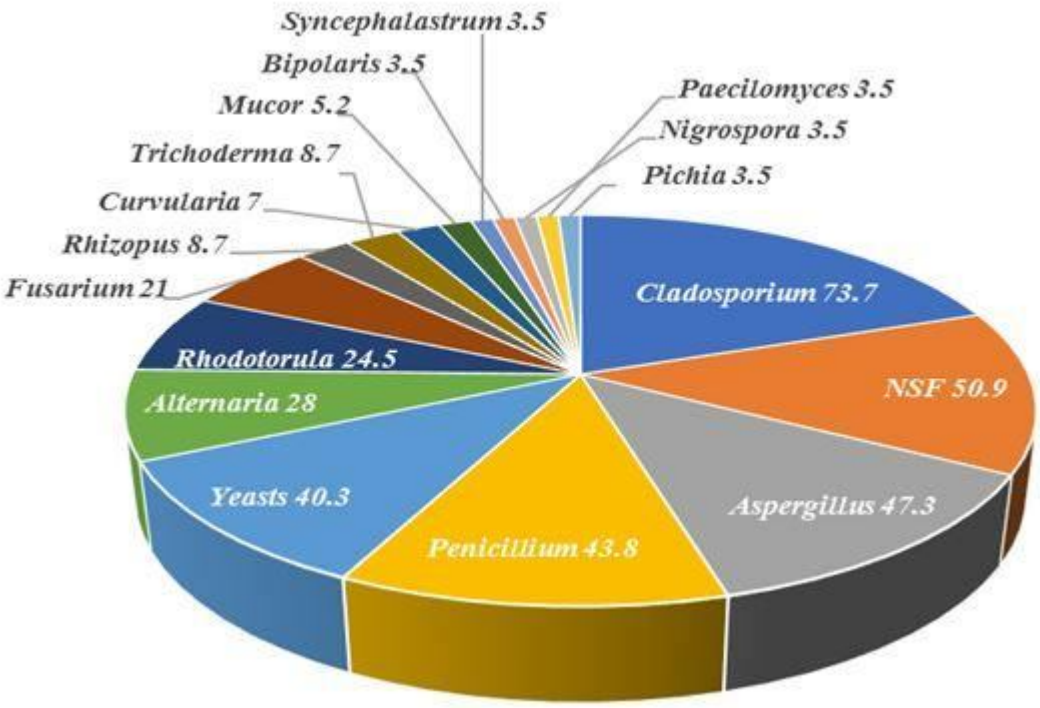

Figure 2. Fungal Frequency (%) in non-irradiated samples (control group).

Table 1.

Irradiated samples

No fungal growth in 15.8 and 42% of the filters irradiated at 10 kGy and 15 kGy was detected, respectively, while the 20 kGy dose reduced 79% of fungal contamination (Table 1). All treatments indicated a significant difference ($p < 0.05$) regarding CFU mean values in relation to the control group (0 kGy) (Table 2).

The results of present study indicate that 10 kGy resulted in fungal growth in 84.2% of samples. The dose was not efficient to avoid the presence of *Aspergillus* spp., *Cladosporium* spp., *Rhodotorula* spp., non-sporulated fungus (NSF) and yeasts (Table 1). Fungi are a group of highly radiation-resistant eukaryotes. In order to understand acute and chronic ionizing radiation (CIR) effects in fungi in association to other stressors, Shuryak et al. (2019) assessed the diverse response of *Ascomycota* and *Basidiomycota* to CIR (36 Gy/h) and acute ionizing radiation (10 kGy/h) and reported D_{10} values for *Rhodotorula* CIR as ranging from 0.9 to 2.5 kGy. *Rhodotorula* (Basidiomycota phylum) is a common environmental yeast found in air, soil, lakes, plants, ocean water, milk, CO_2 and fruit juice, and is able to colonize humans and other mammals.

In present study, gamma irradiation completely eliminated fungal contamination in 42% of all samples at 15 kGy (Table 2). This dose did not, however, control the fungal burden of 10 samples contaminated by *Aspergillus* spp*.*, *Penicillium* spp*.*, *Fusarium* spp*.* and yeasts, such as *Rhodotorula* spp. The filamentous fungi known as mycotoxigenic fungi (pathogenic group) surviving the effect of gamma radiation at 10 and 15 kGy belonged to the *Aspergillus, Penicillium* and *Fusarium* genera (Figure 3).

A 20 kGy dose led to the decontamination of 79% of the samples compared to the control group. Some yeasts, *Rhodotorula* spp. and NSF colonies were observed, and no mycotoxigenic fungi growth was noted in this group.

DNA sequencing analysis of radioresistant fungi

DNA sequencing can improve the detection of fungal pathogenic species. Mycotoxins are toxic secondary metabolites produced by various filamentous fungi, mainly the *Fusarium, Aspergillus*, and *Penicillium* genera (Pitt and Hocking, 2009; Greeff-Laubscher et al., 2019). Therefore, the three filamentous fungi genera known as mycotoxigenic that survived the gamma radiation treatments at 10 and 15 kGy were sequenced (Table 3).

According to Saraswathy and Ramalingam (2001), contig is a series of overlapping DNA sequences used to create a physical map that reconstructs the original DNA sequence of a chromosome or a chromosome region. The results of the contigs obtained from the fungal DNA sequencing of radioresistant filamentous fungi are presented in Figure 4.

Table 1. Total fungal count in control and irradiated filter samples (10, 15 and 20 kGy).

No.	Control group (0 kGy)		Dose 10 kGy	
	Fungi	**Total CFU**	**Fungi**	**Total CFU**
1	*Cladosporium* spp.	250	*Cladosporium* spp.	5
2	*Aspergillus* spp. *Cladosporium* spp. *Fusarium solani* Yeasts	20	Yeasts	4
3	*Aspergillus* spp. *Cladosporium* spp. *Mucor* spp. NSF *Penicillium* spp.	241	*Cladosporium* spp. NSF	11
4	*Alternaria alternata* *Aspergillus fumigatus* *Cladosporium* spp. *Fusarium* spp. NSF *Penicillium* spp. *Rhizopus* spp. Yeasts	235	*Cladosporium* spp. NSF	15
5	*Cladosporium* spp Yeasts	6	No growth	0
6	*Aspergillus niger* *Cladosporium* spp. NSF Yeasts	15	*Aspergillus* spp. *Cladosporium* spp. NSF	7
7	*Aspergillus* spp. *Cladosporium* spp NSF *Rhodotorula* spp.	67	*Rhodotorula* spp.	4
8	Yeasts *Fusarium* spp	5	No growth	0
9	*Aspergillus niger* *Fusarium* spp. NSF *Penicillium* spp. *Rhodotorula* spp. *Trichoderma* spp.	83	*Rhodotorula* spp.	12
10	*Aspergillus* spp. *Penicillium* spp. Yeasts	7	*Aspergillus* spp.	1

Table 1. Continues.

11	*Aspergillus niger* *Cladosporium* spp. *Penicillium* spp. *Rhodotorula* spp.	172	*Cladosporium* spp.	13
12	*Cladosporium* spp.	200	*Cladosporium* spp.	1
13	*Aspergillus niger* *Bipolaris* spp. *Cladosporium* spp. NSF *Paecilomyces* spp. *Penicillium* spp.	22	No growth	0
14	*Aspergillus* spp. *Cladosporium* spp. *Curvularia* spp. NSF *Penicillium* spp. *Syncephalastrum* spp.	17	*Aspergillus* spp.	2
15	*Alternaria alternata* *Aspergillus* spp. *Fusarium solani* *Mucor* spp. *Rhizopus* spp. *Rhodotorula* spp. Yeasts	49	Yeasts	2
16	*Cladosporium* spp. *Fusarium* spp. Yeasts *Phoma* spp.	70	NSF	2
17	*Alternaria alternata* *Aspergillus niger* *Cladosporium* spp. *Penicillium* spp. *Pichia* spp. *Rhodotorula* spp. *Trichoderma* spp. Yeasts	81	*Aspergillus* spp. *Rhodotorula* spp. Yeasts	21
18	*Aspergillus niger* *Cladosporium* spp. *Penicillium* spp. *Rhodotorula* spp.	59	NSF *Rhodotorula* spp.	13
19	*Alternaria alternata* *Cladosporium* spp. *Penicillium* spp.	129	*Cladosporium* spp.	12

Table 1. Continues.

No.	Control group (0 kGy)		Dose 15 kGy	
	Fungi	**Total CFU**	**Fungi**	**Total CFU**
20	*Alternaria alternata* *Cladosporium* spp.	129	No growth	0
21	*Cladosporium* spp. *Penicillium* spp. *Trichoderma* spp. NSF	43	No growth	0
22	*Aspergillus* spp. *Cladosporium* spp. *Fusarium solani* Yeasts	31	Yeasts *Aspergillus* spp.	6
23	NSF	1	No growth	0
24	NSF	8	No growth	0
25	*Aspergillus* spp. *Cladosporium* spp. *Rhodotorula* spp.	67	No growth	0
26	*Aspergillus* spp. NSF Yeasts	21	NSF *Aspergillus* spp.	3
27	*Cladosporium* spp. NSF *Rhodotorula* spp.	9	*Rhodotorula* spp.	2
28	Yeasts	5	No growth	0
29	*Aspergillus* spp. *Fusarium* spp. NSF *Penicillium* spp. *Rhodotorula* spp. *Trichoderma* spp.	98	*Aspergillus* spp. *Fusarium* spp. *Rhodotorula* spp.	6
30	NSF *Penicillium* spp. Yeasts *Phoma* spp.	7	*Penicillium* spp.	1
31	*Aspergillus* spp. *Cladosporium* spp. *Penicillium* spp. *Rhodotorula* spp.	172	*Aspergillus* spp. *Cladosporium* spp.	4
32	*Cladosporium* spp. Yeasts	204	No growth	0

Table 1. Continues.

33	*Aspergillus niger* *Bipolaris* spp. *Cladosporium* spp. NSF *Paecilomyces* spp. *Penicillium* spp.	22	No growth	0
34	*Cladosporium* spp. *Curvularia* spp. NSF *Penicillium* spp. *Rhodotorula* spp. *Syncephalastrum* spp.	27	NSF	2
35	*Alternaria alternata* *Aspergillus flavus* *Fusarium* spp. *Mucor* spp. NSF *Rhizopus* spp. Yeasts	33	*Fusarium* spp.	2
36	NSF *Penicillium* spp. Yeasts	78	NSF	3
37	Yeasts *Rhodotorula* spp. *Pichia* spp. *Aspergillus* spp. *Alternaria* spp.	144	*Rhodotorula* spp. Yeasts *Aspergillus* spp.	19
38	*Cladosporium* spp. *Penicillium* spp. *Rhodotorula* spp	57	*Rhodotorula* spp.	8

No.	Control group (0 kGy)		Dose 20 kGy	
	Fungi	**Total CFU**	**Fungi**	**Total CFU**
39	*Cladosporium* spp. Yeasts	28	Yeasts	2
40	*Curvularia* spp. NSF	15	No growth	0
41	*Cladosporium* spp.	18	No growth	0
42	*Cladosporium* NSF Yeasts	35	No growth	0
43	*Cladosporium* spp. NSF	26	No growth	0

Table 1. Continues.

44	*Alternaria alternata* *Cladosporium* spp. NSF *Penicillium* spp.	77	1 NSF	1
45	*Aspergillus niger* *Cladosporium* spp. NSF	75	No growth	0
46	*Cladosporium* spp. *Curvularia* spp. NSF *Penicillium* spp. Yeasts	48	No growth	0
47	*Alternaria alternata* *Cladosporium* spp. NSF	260	No growth	0
48	*Alternaria alternata* *Cladosporium* spp. NSF *Penicillium* spp. *Trichoderma* spp. Yeasts	324	NSF	1
49	*Aspergillus niger* *Cladosporium* spp. *Fusarium* spp. NSF *Rhodotorula* spp.	26	*Rhodotorula* spp.	1
50	*Alternaria alternata* *Cladosporium* spp. NSF *Penicillium* spp.	16	No growth	0
51	*Aspergillus niger* *Cladosporium* spp.	24	No growth	0
52	*Alternaria alternata* *Aspergillus niger* *Fusarium* spp *Penicillium* spp. *Rhizopus* spp.	20	No growth	0
53	*Cladosporium* spp. *Nigrospora* spp. *Penicillium* spp.	8	No growth	0

Table 1. Continues.

54	*Alternaria alternata* *Aspergillus* spp. *Cladosporium* spp. *Nigrospora* spp. *Penicillium* spp. Yeasts	55	No growth	0
55	*Cladosporium Alternaria alternata* NSF	16	No growth	0
56	*Alternaria alternata* *Aspergillus ochraceus* *Cladosporium* spp. *Rhizopus* spp. Yeasts	286	No growth	0
57	*Alternaria alternata* *Cladosporium* spp. *Fusarium solani*	17	No growth	0

Note: CFU - colony-forming unit; NSF - non-sporulating fungus.

Table 2. CFU mean comparisons by the Tukey test for all car filter samples.

Dose (kGy)	N	Sum	Mean	P value	Statistical significance
0	57	4258	74.6842	>0.05	Not significant
10	19	125	6.57895	<0.05	Significant
15	19	56	2.94737	<0.05	Significant
20	19	5	0.26316	<0.05	Significant

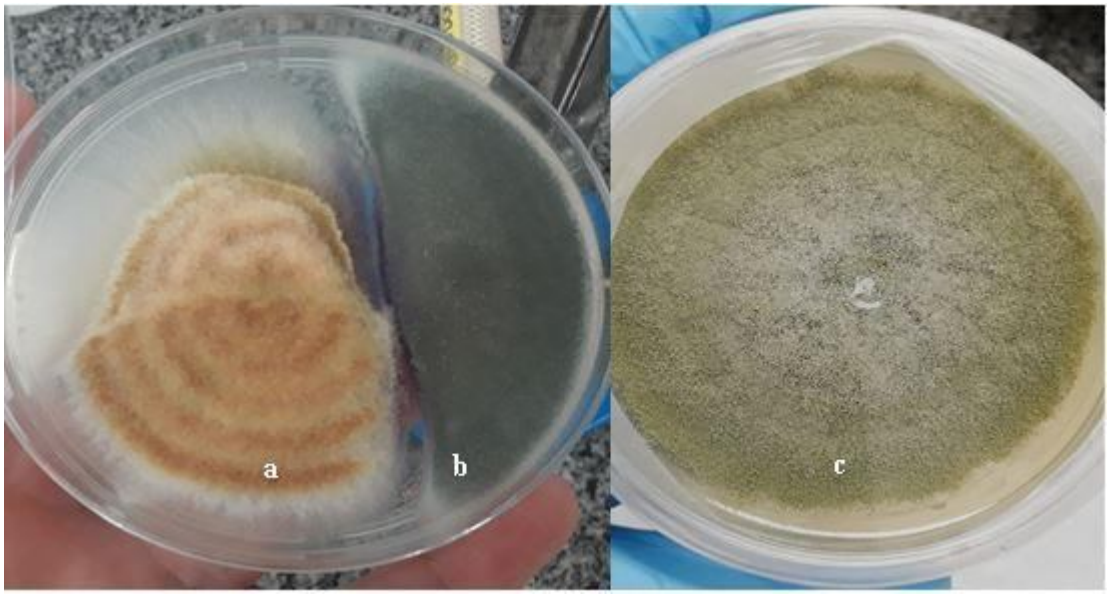

Figure 3. Macromorphology of radioresistant fungi: *Fusarium* spp. (a); *Penicillium* spp. (b) and *Aspergillus* (c).

Table 3. Sequencing results of radioresistant pathogenic fungi isolate from car filter samples.

Sample (Nº)	Fungi	Dose (kGy)
6	*Aspergillus flavus*	10
10	*Aspergillus flavus*	10
14	*Aspergillus flavus*	10
17	*Aspergillus flavus*	10
37	*Aspergillus flavus*	15
30	*Penicillium glabrum* species complex	15
29	*Fusarium incarnatum-equiseti* species complex	15
35	*Fusarium incarnatum-equiseti* species complex	15

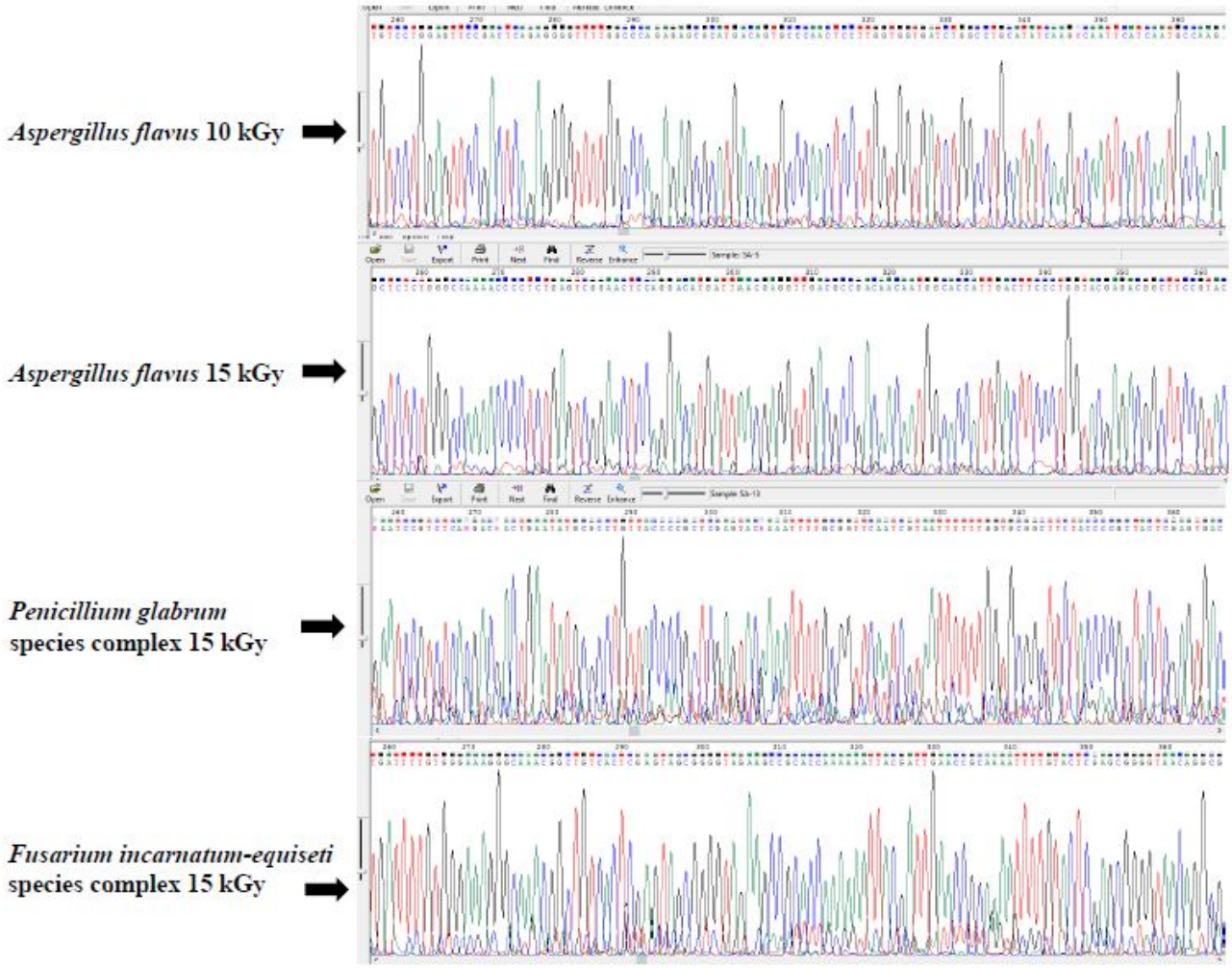

Figure 4. Contigs of DNA sequencing of radioresistant filamentous fungi.

DISCUSSION

Heating, Ventilation, and Air Conditioning (HVAC) is the technology of indoor and vehicular environmental comfort. To prevent and reduce microbial contamination, it is essential to have an overall understanding of microbial characteristics in HVAC systems (Liu et al., 2018). Anemophilous fungi are spread globally and some genera, such as *Alternaria*, *Aspergillus, Cladosporium* and *Penicillium*, are a major source of inhalant fungal allergens, having been reported as the predominant organisms in warm, humid and dry climates (Kurup et al., 2000). Cartaxo et al. (2007) verified high fungi growth of *Penicillium* spp., *Paecilomyces* spp., *Cladosporium* spp.,

Rhizopus spp., *Mucor* spp., *Aspergillus* spp. and *Rhodotorula* spp. in air conditioner filters collected from households in Manaus, Brazil.

The Portuguese Indoor Air Quality (IAQ) legislation proposes a specific threshold of less than 12 CFU.m^{-3} for *Aspergillus* sections *Versicolor, Flavi, Circumdati, Terrei* and *Fumigati* in air samples for all indoor environments to be verified only when the quantitative cut-off (indoor/outdoor ratio higher than 1) is surpassed (Ordinance n° 353-A 2013). Viegas et al. (2019) investigated the prevalence of *Aspergillus* species in the clinical environment, focusing on ten Primary Health Care Centers (PHCC), in Lisbon, Portugal, using Real Time PCR, determining *Aspergillus* section *Flavi* as a contaminant in a heating, ventilation, and air conditioning (HVAC) filter sample.

Li et al. (2013) sampled dust samples from the filters of 30 cars from four different geographical locations in China, and reported significant amounts of biological agents, including diverse fungi, i.s. *Alternaria alternata, Aspergillus* spp., *Cladosporium cladosporioides, Penicillium* spp., *Trichoderma viride, Curvularia lunata*, *Phoma* spp. and yeasts. However, a lack of studies concerning air contamination is noted, especially in relation to automotive vehicles, and no Brazilian standards or guidelines established for the inspection of vehicle air filters or for the quality of the air conditioning circulating inside cars have been established (Aquino et al., 2018).

The predominance of fungi in indoor air may result in high infection risk to drivers, as pointed in the present study, that detected the highest genera frequencies comprised *Cladosporium* (73.7%), non-sporulated fungus - NSF (50.9%) and *Aspergillus* (47.3%) followed by a decreasing frequency of *Penicillium* (43.8%), Yeasts (40.3%), *Alternaria* (28%), *Rhodotorula* (24.5%) and *Fusarium* (21%). The findings of present study also demonstrated that *Rhodotorula* spp., *Cladosporium* spp., *Penicillium* spp., *Fusarium* spp. and *Aspergillus* spp. were resistant to irradiation doses.

Many studies have reported that fungi tolerate high irradiation doses under laboratory conditions, while also surviving in environments heavily contaminated by radioisotopes. The radio-resistance is probably an evolutionary mechanism concerning other properties, e.g. resistance to desiccation and/or to other genotoxic agents, which are important for survival in natural environments (Byrne et al., 2014; Dadachova and Casadevall, 2008). Radioresistant organisms can survive high ionizing radiation doses (hundreds or thousands of Gy) without losing reproductive potential. Different organisms may evolve radiation resistance through different mechanisms, and even seemingly subtle ancient evolutionarily pathway modulations (e.g. DNA repair machinery) may substantially enhance radio-resistance (Daly, 2009).

Rhodotorula infection cases in humans were described in special conditions, such as immunosuppressed and hospitalized patients (Wirth and Goldani, 2012; Miceli et al., 2011). These yeasts have been recognized as emerging pathogens in humans in the last two decades (Wirth and Goldani, 2012). Sharma et al. (2017) reported that a feature by yeasts shown to possess resistance to high radiation doses is their great reactive oxygen species (ROS) scavenging capacity. Fungi also accumulate high concentrations of Mn antioxidants and are highly resistant to oxidative stress. It was recently reported that polyextremotolerant fungi accumulate high concentrations of Mn^{2+} metabolite antioxidant complexes (Mn antioxidants), which very efficiently scavenge IR-induced ROS. Tkavc et al. (2018) studied the characterization of 27 diverse environmental yeasts for their resistance to ionizing radiation (chronic and acute), heavy metals, pH minima, temperature maxima and optima, and their ability to form biofilms. Many radioresistant yeasts are resistant to heavy metals, excrete carboxylic acids and are exceptionally tolerant to low pH. A special focus was placed on *Rhodotorula taiwanensis* MD1149, which was the most resistant to acid and gamma radiation, being capable of growing under 66 Gy/h at pH 2.3 and in the presence of high concentrations of mercury and chromium compounds and forming biofilms under high-level chronic radiation and low pH.

Melanized microorganisms are often found in environments with very high background radiation levels, such as in nuclear reactor cooling pools and the destroyed Chernobyl reactor. The resistance of melanized fungi to ionizing radiation suggests a role for this pigment in radioprotection, due to its chemical composition, free radical quenching ability and spherical spatial arrangement (Dadachova et al., 2008). Vember et al. (1999) investigated the role of melanin in the cell wall of *Cladosporium cladosporioides* (Fres.) de Vries strains isolated from habitats presenting different radionuclide contamination degrees. The lipid peroxidation and antioxidant glutathione-dependent system activities (catalase, glutathione-transferase) explain the radio-tropism property in *Cladosporium* dark-pigmented strains (Dadachova et al., 2008).

Barreto et al. (2011) grouped the *Penicillium* Glabra series according to their partial β-tubulin gene and calmodulin gene sequences in a study on cork contamination, in Portugal, and reported that the *Penicillium glabrum* cork isolates displayed high intraspecific variability. The macro- and micro-morphologies, extrolite profiles and sequencing results of partial β-tubulin and calmodulin gene regions support this variability.

Gumus et al. (2008) investigated the effects of gamma radiation on two heat-resistant moulds, clearly demonstrating a significant decrease in *Aspergillus fumigatus* and *Penicillium variotii* counts with increasing irradiation doses. A 7 kGy dose was required for complete *A. fumigatus* spore inactivation, while a 5 kGy irradiation dose was necessary for *P. variotii* spores. In

aqueous solutions, the D_{10} value for *Aspergillus niger* was reported as 0.715 kGy, increasing to 0.909 kGy in inoculated products (Secer and Íc 2003). Aquino et al. (2010) demonstrated that the fungus *A. flavus* was radioresistant after and ionizing treatment at 5 kGy in packed herbs stored after 30 days of treatment. The authors also observed that the irradiated *A. flavus* did not lose its ability to produce aflatoxins after the irradiation process, even when a 5 kGy dose decreased the amount of inoculum, while a 10 kGy dose was found to be effective for fungal decontamination.

Penicillium is one of the most widespread fungal genera isolated from food products and, due to its ability to disperse a high number of spores in the environment, *P. glabrum* is very frequently encountered in the food manufacturing industry (Karpenko et al., 2006; Adam and Attaby, 2001). In addition to the economic losses caused by this contaminant, many *Penicillium* species can also produce mycotoxins, representing potential health risks for both humans and animals (Pitt and Hocking, 2009; Samson et al., 2006). A study performed by Karpenko et al. (2006) on 24 genera of microscopic fungi isolated from radioactive soil and other radioactive substrates of a 10-km alienation zone from the Chernobyl Nuclear Power Plant demonstrated that 19% of all fungi displayed positive radiotropism towards the ionizing irradiation source. Strains displaying radiotropism and photostimulation demonstrated an adaptive nature, and were probably a strain-related feature, including *P. glabrum*.

Adam and Attaby (2001) described that radioresistant *Fusarium verticillioides* accumulated ten amino acids (cystine, glutamic acid, serine, methionine, histidine, proline, arginine, alanine, glycine and, therionine), also demonstrated by Ferreira-Castro et al. (2007) when the authors irradiated *F. verticillioides* in maize. The explanation of *Fusarium* radioresistance is proportionally associated to total lipid content in the cells and wall cells of fungi contain appreciable lipid fractions (over 20%), as in the case of certain *Aspergillus* species (Harwood and Russell, 1984). *Fusarium incarnatum* is extremely widespread and common in the tropics and subtropics, but also found in the Mediterranean and, occasionally, in temperate regions (Leslie and Summerell, 2006). No previous study has demonstrated gamma radiation effects on the *Fusarium incarnatum-equiseti* species complex (FIESC). *F. equiseti* is a cosmopolitan soil fungus isolated from roots and plant tissues worldwide (O'Donnell et al., 2000). Molecular phylogenetic studies have led to important advances in the recognition of morphological species among FIESC members, due to high levels enigmatic speciation and extreme morphological homoplasia (O'Donnell et al., 2000; O'Donnell et al., 2004; O'Donnell et al., 2008).

The current *F. equiseti* description includes at least two varieties presenting different morphological types (Samson et al., 2004). The FIESC is also commonly detected in Brazilian rice, but knowledge regarding species limits and toxigenic potential is lacking, although Avila and colleagues (Avila et al., 2019) demonstrated that 16 strains from 46 FIESC isolates produced detectable mycotoxin levels *in vitro.*

CONCLUSION

The control group have demonstrated that vehicle air-conditioning filters contribute to the bioaccumulation of several yeasts and filamentous fungi, including mycotoxigenic moulds such as *Aspergillus, Penicillium* and *Fusarium* genera. No previous study has applied genetic sequencing to confirm *P. glabrum* and *F. incarnatum-equiseti* (FIESC) as radioresistant strains submitted to 10 and 15 kGy doses.

Filamentous fungi that survived gamma radiation effects, such as *A. flavus*, *P. glabrum* and FIESC*,* also belong to the mycotoxigenic fungi (pathogenic group). However, no mycotoxigenic fungi were detected in samples irradiated with 20 kGy, which eliminated fungal contamination in 79% of filters. This demonstrated that gamma radiation is an important physical method for fungal control.

The 20 kGy dose was proven efficient in decontaminating samples with uncountable fungal loads, reducing the number of colonies to low levels. Gamma radiation was proven efficient as a fungi control method for air filters, able to control mycotoxigenic or pathogenic fungi, leading to filter recycling and indoor vehicle air quality maintenance against allergenic fungi.

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