BJRS

BRAZILIAN JOURNAL
OF
RADIATION SCIENCES
09-01A (2021) 01-14

Bioburden proliferation in vehicle air filters waste: the use of gamma radiation on fungal decontamination

Aquino[a] S., Lima[b] J.E.A., Borrely[a] S.I.

[a] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)/Centro de Tecnologia das Radiações (CTR), Postal code 05508-000, São Paulo, SP, Brazil
b Universidade Nove de Julho (UNINOVE)/Departamento de Saúde II, Postal code 05001-900, São Paulo, SP, Brazil
e-mail:siaq06@hotmail.com

ABSTRACT

This study aimed to analyze the fungal contamination of air-conditioning filters waste (n=20) as an indicator of Quality Air Indoor from different car models, that were collected from 10 exchange stations located in the South, North, West, Downtown and East, of the city of São Paulo in São Paulo State, Brazil, during the period from October 2017 to November 2018. Sampling of filter particles (33 fragments of 10 × 10-mm size) were plated onto solidified Potato Dextrose agar in Petri dishes. The samples were incubated for 7 days at 25 °C and were stored in a standard Biochemical Oxygen Demand incubator, for growth of fungal cultures. After incubation, the fungal culture in the plates was evaluated, and the total counting of infected fragments was expressed as a percentage. The fungi were examined by Lactophenol blue solution staining for microscopy. All samples were contaminated with various fungal genera, including *Aspergillus, Alternaria, Cladosporium*, and *Penicillium*. The study also aimed to evaluate the fungal enumeration in the samples that were irradiated with dose of 10 kGy to fungal decontamination of air-conditioning filters waste. Of total samples, 50% were completed decontaminated, but some genera such as *Aspergillus, Penicillium, Rhizopus, Cladosporium* and yeasts demonstrated radioresistance at the dose of 10 kGy. The only yeast called *Rhodotorula* showed an increase in growth after the irradiation process.

Keywords: air, car, fungi, gamma.

ISSN: 2319-0612
Accepted: 2021-02-28

1. INTRODUCTION

During many years, little attention has been paid to Indoor Air Quality (IAQ). Fungi, bacteria, and carbon dioxide in indoor air-conditioned environments are relevant health aspects which have involved many issues nowadays [1]. According to Aquino *et al.* [2] to protect occupants, air-conditioning filters of vehicles are intended to retain aerial bioburden (microorganisms in sprinkler or bioaerosols). However, under favorable conditions, biofilm proliferation in air filters and following airflow release into the vehicle enclosure represents a potential source of exposure to bioaerosols, mainly if it contains respirable fragments (<1.1 μm). Once those particles or microorganisms happen to reach the cabin of the car, a possibility for allergic, toxic or irritant reactions (e.g., of the respiratory tract) for passengers exists in principle [3].

The fungal contamination in vehicle air filters and their impact as bio-accumulator on IAQ was carried in São Paulo, confirming the presence of many genera, including toxigenic *Aspergillus* species [2]. Li *et al.* [4] collected filters in four different geographical locations in China, filter dust samples from 30 automobiles and reported that under high humidity levels, an automobile filter could be a hotbed for incubating many pathogens and presenting a critical source of respiratory allergies or infections.

Li and colleagues [4] showed that automobile air-conditioning filters harbored significant amounts of biological agents, including diverse bacteria and fungi and, high levels of endotoxin. Some of these agents could reproduce under high humidity conditions. In modern societies, people spend 90% of their time indoors or inside the home, and it is not surprising that factors contributing to poor IAQ are receiving significant attention from researchers, government and public in general [5].

On the other hand, the amounts of solids residues are increasing in excessive magnitude. The treatment of car's filters by gamma radiation can reduce the residues of filters accumulated in exchange stations. Among the possibilities to reuse/recycle materials, we may have ionizing radiation as an essential tool for microbial control in a different type of residues [6]. The aim of the study was the evaluation of different type of matrices and the fungal contamination of automobile

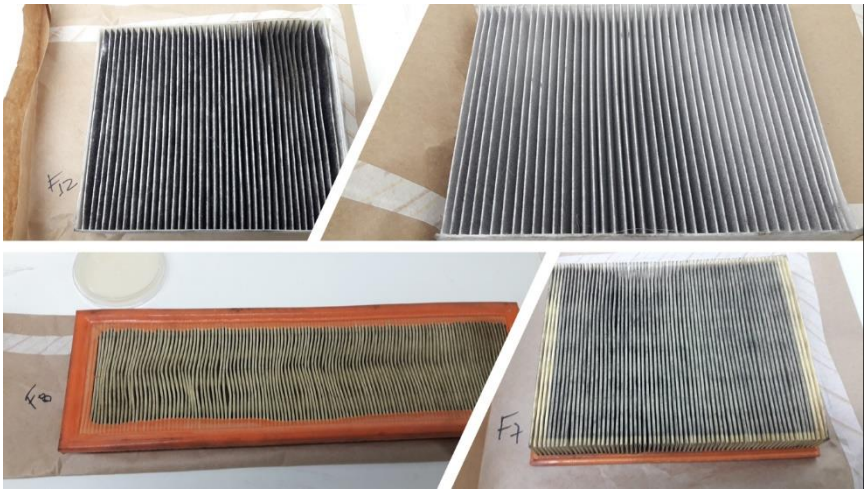
air-conditioning filters. The evaluation of fungal decontamination effect by the gamma radiation process (10 kGy) was carried out in 20 samples of filters collected in São Paulo city, Brazil.

2. MATERIALS AND METHODS

2.1. Sampling and fungi isolation

The replaced pieces of air-conditioning filters were selected for the superficial and internal sampling of fungi. A total of 20 air-conditioning filters of different car models (Figure 1) were collected from 10 exchange stations located in the South, North, West, Downtown and East, of the city of São Paulo (Latitude 23° 32′ 51″ S and Longitude 46° 38′ 10″ W) in São Paulo State, Brazil, from October 2017 to November 2018.

Figure 1: *Filters of car air-conditioning collected in São Paulo.*

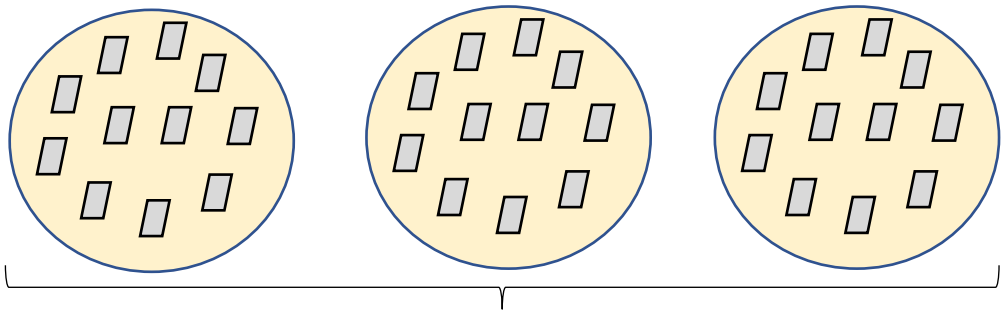


Source: author's figure

A standard plating regimen has been used for the initial examination of all isolates, so those identification procedures were carried out without foreknowledge of genus or even their subkingdom. Cultural characters, which can be broadly defined as the application of microbiological techniques to mycology, have been used throughout. The use of cultural characters has long been implicit in the study of fungi in pure culture on artificial substrates [7]. All technique was conducted in accordance with good laboratory practice, and the material was manipulated in a

laminar flow cabinet, according to the laboratory guide for the routine isolation and identification of common fungi designed by Pitt and Hocking [7]. In this study, a filter sample was divided into 33 fragments of 10 × 10-mm size (cut in sterile conditions) and distributed into three Petri dishes, with 11 fragments in each plate containing Potato Dextrose agar (PDA) as demonstrated in Figure 2.

Figure 2. *Distribution of sample filter divided into 33 fragments in Petri dishes.*



Source: author's figure

The Petri dishes were incubated for 7 days at 25 °C and were stored in a standard Biochemical Oxygen Demand (BOD) incubator, for growth of fungal cultures (Figure 3).

Figure 3: *Fungi incubation onto PDA at 25ºC for 7 days.*

Source: author's figure

After incubation, the examination of fungal culture in the plates was visual, and the total counting of infected fragments was expressed in results as a percentage (%). For each sample, the

counting was based on the fragments with fungal growth comparing with the total of 33 filter fragments (100%), according to the technique described by Berjak [8].

2.2. Irradiaton at cobalt source

The samples were individually protected by a Kraft paper envelope, and materials were maintained onto plastic box during irradiation at 5 kGy/h at Multipurpose irradiator of IPEN/CNEN (Figure 4). The search of fungal contamination was carried out at the control group of air-conditioner filters samples and irradiated samples treated with 10 kGy (to fungal decontamination) using Co^{60} source by gamma rays.

Figure 4: *Sample package and multipurpose irradiator, IPEN/CTR.*

Source: author's figure

3. RESULTS AND DISCUSSION

The present study showed that control samples were contaminated with a diversity of fungal genera, including non-sporulating fungi (NSF). The fungal counting of samples of the control group (0 kGy) and 10 kGy were summarized at Table 1.

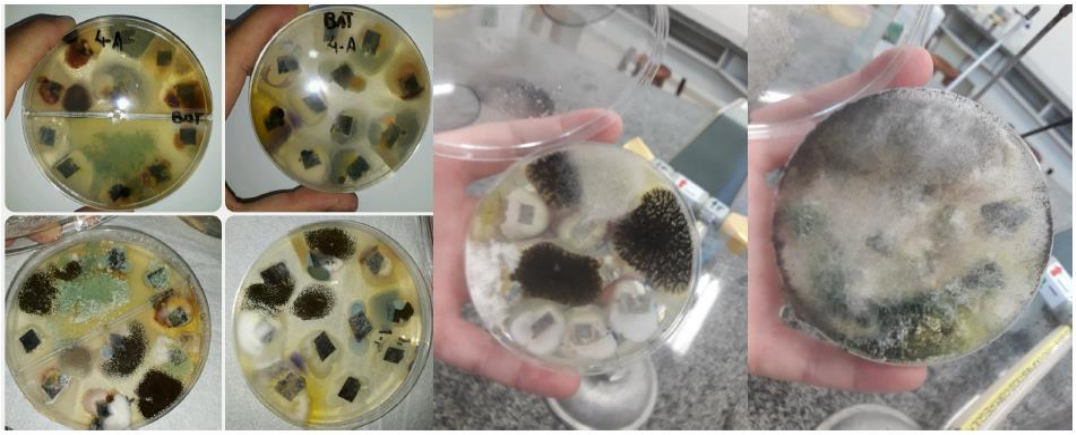
Fungi genera	**Sample number (%)**

	Control (0 kGy)	**10 kGy**
Alternaria	2(5%); 5(33%); 6(24%); 8(45%); 9(3%)	No fungal growth
Aspergillus	1(36%); 3(6%); 4(60%); 5(12%); 7(3%); 8(20%); 9(21%); 10 (15%); 13(2%); 14(3%); 16(15%); 19 (12%); 20(4%).	3(6%); 5(12%); 7(3%); 13(2%)
Bipolaris	12(3%)	No fungal growth
Chaetomium	12(3%); 13(8%); 16(7%); 17(1,5%); 18(4,5%)	No fungal growth
Cladosporium	5(33%); 6(13%); 7(72%); 10(54%); 11(10%); 12(36%); 14(63%); 15(18%); 17(18%);	5(3%); 7(2%); 10(4%); 17(1%)
Curvularia	17(3%); 18(19%)	No fungal growth
Fusarium	6(6%)	No fungal growth
Non-Sporulating Fungi - NSF	1(3%); 2(24%); 5(9%); 6(36%); 7(54%); 8(33%); 9(45%); 11(4.5%); 13(7%); 14(3%); 15(9%); 16(18%); 17(15%); 18(30%)	13(7%); 15(3%); 16(8%)
Nigrospora	9(27%); 12(7%); 18(30%)	No fungal growth
Paecilomyces	13(8.5%);	No fungal growth
Penicillium	2(3%); 4(40%); 5(7%); 12(2%); 13(12%); 15(3%); 16(33%); 18(1,5%); 20(10%)	6(3%); 18(1%)
Phoma	7(3%); 13(1.5%)	No fungal growth
Rhizopus	1(61%); 2(58%); 3(21%); 5(3%); 6(33%); 9(20%); 16(9%); 19(4%)	1(1%); 5(3%); 6(6%); 17(1%)
Rhodotorula	1(12%); 3(48%)	3(2%); 5(8%); 6(5%); 7(5%); 10(7%); 18(2%)

Scytalidium	13(7%)	No fungal growth
Syncephalastrum	1(3%);	No fungal growth
Trichoderma	2(6%); 3(21%); 5(3%); 6(45%); 8(5%); 9(3%); 10(10%); 19(5%)	5(1%); 13(4%); 20(1%)
Ulocladium	8(6%)	No fungal growth
Yeasts	1(15%); 4(34%); 5(12%); 6(3%); 7(12%); 8(3%); 10(21%); 12(46%); 13(53%); 17(60%); 18(20%)	5(15%); 13(40%); 15(80%); 18(30%); 20(45%)

Previous studies found fungi in indoor conditions [9-13]. Chen et al. [13] showed the percentages of the three dominant airborne groups in Terra-Cotta Museum, including *Penicillium* (45%), *Alternaria* (31%), and *Aspergillus* (16%). The study performed by Simmons et al. [14] showed that automotive air-conditioning system was contaminated by various fungi genera, including *Acremonium, Aspergillus, Alternaria, Aureobasidium, Cladosporium* and *Penicillium*, and the evaporator was also colonized by odor producing fungi such as *Penicillium viridicatum. Alternaria alternata, Aspergillus* spp., *Cladosporium cladosporioides, Penicillium* spp., *Trichoderma viride, Curvularia lunata*, and *Phoma* spp. declared the highly possible that yeasts were also present in dust samples found in the vehicle filters in China. The Figure 5 shows the genera of fungi isolated from fragments in Potato Dextrose agar (PDA) of control samples.

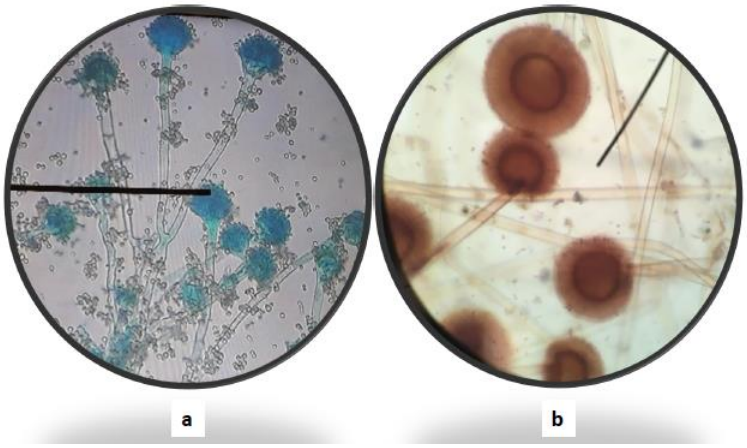
Figure 5: *Samples of fragments in agar Potato Dextrose of control samples.*



Source: author’s figure

The present study demonstrated the presence of pathogenic *Aspergillus* species such as *Aspergillus* section Flavi and *Aspergillus* section Nigri (*A. niger*) isolates were found, as well as *Aspergillus fumigatus, Aspergillus clavatus* and *Aspergillus ochraceus.* Figure 6 shows *Aspergillus fumigatus* and *A. niger* in a microscopy image (400X).

Figure 6: *Microscopy of A. fumigatus(a) and A. niger (b) isolated from air-conditioning filters.*



Source: author's figure

Schoenlein-Crusius *et al.* [15] performed a study to search airborne fungi in the city of Cubatão, in São Paulo State (Brazil), where the number of genera found corresponding to 19, such as *Aspergillus, Cladosporium, Penicillium,* and *Trichoderma*, including the NSF. Domination of this species in the indoor air may be tantamount to a high risk of infection.

For the *Aspergillus* type, the obvious portal of entry is the respiratory system, as well as skin with lesions, e.g. a burn or damaged cornea. Infection within the respiratory system develops as a result of inhalation of the fungal spores present in the air [16].

In a study performed by Li *et al.* [4] in three Chinese cities (Beijing, Guangzhou, and Haikou), the dust from air-conditioning filters in vehicles demonstrated the presence of *Aspergillus* genera, such as *A. niger, A. fumigatus, A. ustus, A. oryzae, A. ochraceus, A. terreus, A. restrictus, A. versicolor, A. sydowii* and *A. amstalodami*, corroborating the results of the present study. Studies on mycological cleanness of hospital rooms' indoor air revealed that the *Aspergillus* species amounted to 20–38% of all of the molds isolated, and the most frequently identified species was *A. fumigatus* [16].

Some fungal genera were not observed after gamma radiation treatment, as shown in Table 1. In contrast, the results of the irradiated samples showed fungal growth in ten samples (50%), although the count was reduced for *Aspergillus, Cladosporium*, NSF, *Penicillium, Rhizopus, Trichoderma* and yeasts. However, the fungal radiation resistance was observed in contaminated samples with *Rhodotorula* spp., with the increase of counting.

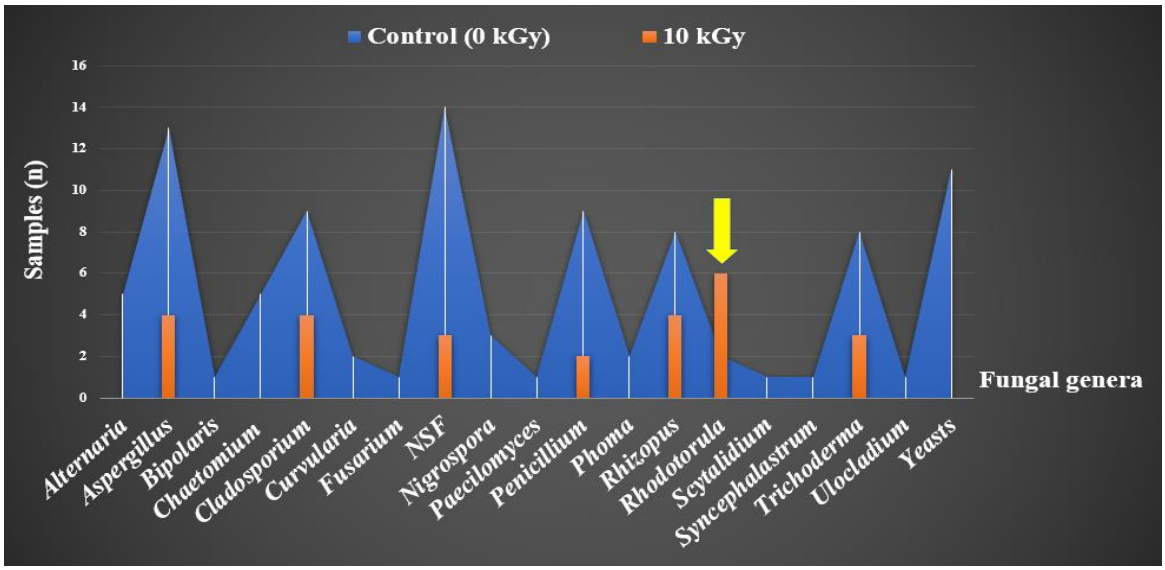
Many studies have already reported the resistance of microorganisms to radiation [17-20]. In eubacteria, *Deinococcus radiodurans*, which is ubiquitously found in soil, is a known radiation-resistant bacterium, which can survive high doses of gamma radiation. The radiation dose yielding 10% survival (D10) of *D. radiodurans* is 12 kGy [18].

Concerning the fungal radiation resistance, Jung *et al.* [21] reported that the basidiomycetous fungus *Cryptococcus neoformans* (yeasts) is highly radiation resistant and it has been found in fatal radioactive environments such as the damaged nuclear reactor at Chernobyl. In eukaryotes cells, the DNA repair systems of the phytopathogenic fungus *Ustilago maydis* (D10, 3.6 kGy) have been studied to explain resistance theirs to radiation [22].

Yeasts (unicellular and anaerobic fungi) are more resistant than filamentous fungi (aerobic and multicellular fungi). *Rhodotorula* is a red-pigmented, unicellular, non-sporulating, ovoidal, budding yeast.

A study of Tkavc et al. [23] about the role of fungi in bioremediation of acidic radioactive waste sites demonstrated that *Rhodotorula taiwanensis* MD1149 was the most resistant strain to acid and gamma radiation. According to the authors, the *R. taiwanensis* MD1149 is 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. The D10 of MD1149 is 2.5 kGy and ranks among the most radiation-resistant yeasts identified both for acute and chronic exposures. In the present study, it was observed that *Rhodotorula* yeasts showed an increase in growth in samples after radiation exposure to 10 kGy, as shown in the yellow arrow in Figure 7.

Figure 7: *Presence of fungal genera in the filter samples before and after irradiation.*



Source: author's figure

The variation in gamma radiation resistance in filamentous fungus strains can be explained by multiple factors [24, 25]. Aziz *et al.* [25] showed that there is a correlation between radioresistance of *A. flavus* spores and the percentage of total lipid in mycelia. The variation in radiation resistance of fungi is also an inherent characteristic connected with mycelial water content and natural radioprotector chemicals. The cell walls of some fungi contain significant fractions of lipids (up to 20%) as in the case of some *Aspergillus* species. Some authors also reported intracellular constituents such as sulfhydryl compounds, pigments, amino acids, proteins, and fatty acids [26, 27].

There are indications about the radioresistance of microorganisms that are related of melanization of their cells [28]. Melanized fungal (black fungi) genera were also found to dominate other fungi in soil communities at the site of Nuclear Chernobyl Accident, and it explains how the pigments have a protective effect in fungal cells. Melanized fungi *Cryptococcus neoformans* and *Histoplasma capsulatum* are more resistant to gamma radiation than non-melanized fungal cells [29].

4. CONCLUSION

High amounts of the fungal genus in control samples demonstrated that the Air Quality Indoor in vehicles is a potential health risk to cause respiratory diseases to drivers, in São Paulo city. The present study demonstrated that dose of 10 kGy of gamma radiation was not enough for total fungal decontamination of packed filters collected in São Paulo city.

More experiments with the sequential and increased doses such as 15, 20, and 25 kGy are necessary to understand the fungal control in air conditioner filters of vehicles in São Paulo (Brazil). In order to establish an efficient method for the total control of contaminated fungi, complementary studies using combined methods, such as the association of gamma radiation and disinfectant chemicals should be developed as new treatments to be applied in the fungal decontamination of vehicle air filters. In addition, ionizing radiation can reduce solid waste, such as contaminated filters that could be reused in fleets of vehicles such as buses, ambulances and taxis.

ACKNOWLEDGMENT

The authors would like to thank Pablo Antonio Vasquez Salvatore and Paulo de Souza Santos for the prompt irradiations at IPEN and the International Atomic Energy Agency, for the financial support IAEA RC nº 23033.

REFERENCES

[1] VIEGAS, C.; MONTEIRO, A.; SANTOS, M.; FARIA, T.; CAETANO, L.A.; CAROLINO, E. Filters from taxis air conditioning system: a tool to characterize driver's occupational exposure to bioburden? **Environ Res**, v. 164, p. 522–529, 2018.

[2] AQUINO, S.; LIMA, J.E.A.; NASCIMENTO, A.P.B.; REIS, F.C. Analysis of fungal contamination in vehicle air filters and their impact as a bioaccumulator on indoor air quality. **Air Qual Atmos Health**, v. 11, p. 1143–1153, 2018.

[3] OPPLIGER; A. Advancing the science of bioaerosol exposure assessment. **Ann Occup Hyg**, v. 58, p. 661–663, 2014.

[4] LI, J. ; LI, M.; SHEN, F.; ZOU, Z.; YAO, M.; WU, C. Characterization of biological aerosol exposure risks from automobile air conditioning system. **Environ Sci Technol**, v. 47, p. 10660–10666, 2013.

[5] CERQUEIRA, P.E.S.; GUIMARÃES, A.B.F. Indoor air quality in a petrochemical industry. **Cientefico,** v. 17, p. 1–18, 2017.

[6] COTE, C.K.; BUHR, T.; BERNHARDS, C.B.; GIBBINS, H.S. et al. A Standard Method to Inactivate *Bacillus anthracis* Spores to Sterility Using γ-Irradiation. **Appl Environ Microb**, v. 84, p. e00106-18, 2018.

[7] PITT, J.I. ; HOCKING, A.D. **Fungi and food spoilage**, 3th ed., London: Springer Science & Business Media, 2009.

[8] BERJAK, P. Report of seed storage group working group on the effects of storage fungi on seed viability. **Seed Sci Technol**, v. 12, p. 233–253, 1984.

[9] OWEN, M.K.; ENSOR, D.S.; SPARKS, L.E. Airborne particle sizes and sources found in indoor air. **Atmos Environ**, v. 26, p. 2149–2162, 1992.

[10] HONG, J.B.; CHUNG, Y.H.; CHANG, Y.H. Distribution of hospital airborne microorganisms in Seoul, Korea. **Korean J Environ Health**, v. 29, p. 1–7, 2003.

[11] CAO, J.J.; RONG, B.; LEE, S.C.; CHOW, J.C.; HO, K.F.; LIU, S.X.; ZHU, C.S. Composition of indoor aerosols at Emperor Gin's Terra-Cotta Museum, Xi'an, China, during summer, 2004. **China Particuology**, v. 3, p. 170–175, 2005.

[12] LEE, J.; JO, W. Exposure to airborne fungi and bacteria while commuting in passenger cars and public buses. **Atmos Environ**, v. 39, p. 7342–7350, 2005.

[13] CHEN, Y.P.; CUI, Y.; DONG, J.G. Variation of airborne bacteria and fungi at Emperor Qin's Terra-Cotta Museum, Xi'an, China, during the "Oct. 1" Gold Week Period of 2006. **Environ Sci Pollut Res**, v. 17, p. 478–485, 2010.

[14] SIMMONS, R.B.; NOBLE, J.A.; ROSE, L.; PRICE, D.L.; CROW, S.A.; AHEARN, D.G. Fungal colonization of automobile air conditioning systems. **J Ind Microbiol Biot**, v. 19, p. 150–153, 1997.

[15] SCHOENLEIN-CRUSIUS, I.H.; TRUFEM, S.F.B.; GRANDI, R.A.P.; MILANEZ, A.I.M.; PIRES-ZOTTARELLI, C.L.A. Airborne fungi in the region of Cubatão, São Paulo State, Brazil. **Braz J Microbiol**, v. 32, p. 61-65, 2001.

[16] GNIADEK, A. Cytotoxicity of *Aspergillus* fungi as a potential infectious threat. In: ROY, P.R. **Insight and control of infectious disease in global scenario**. London: IntechOpen, 2012, p. 231–248.

[17] DALY, M.J. Death by protein damage in irradiated cells. **DNA Repair (Amst),** v. 11, p. 12-21, 2012.

[18] SLADE, D.; RADMAN, M. Oxidative stress resistance in *Deinococcus radiodurans.* **Microbiol Mol Biol R**, v. 75, p.133–191, 2011.

[19] WILLIAMS, E.; LOWE, T.M.; SAVAS, J.; DIRUGGIERO, J. Microarray analysis of the hyperthermophilic archaeon *Pyrococcus furiosus* exposed to gamma irradiation. **Extremophiles,** v. 11, p.19–29, 2007.

[20] LIU, Y.; ZHOU, J.; OMELCHENKO, M.V.; BELIAEV A.S.; VENKATESWARAN A. *et al.* Transcriptome dynamics of *Deinococcus radiodurans* recovering from ionizing radiation. **Natl Acad Sci USA**, v. 100, p. 4191–196, 2003.

[21] JUNG, K.W.; YANG, D.H.; KIM, M.K.; SEO, H.S.; LIM, S.; BAHN, Y. S. Unraveling fungal radiation resistance regulatory networks through the genome-wide transcriptome and genetic analyses of *Cryptococcus neoformans*. **mBio,** v. 7, p. e01483-16, 2016.

[22] HOLLOMAN, W.K.; SCHIRAWSKI, J.; HOLLIDAY, R. Towards understanding the extreme radiation resistance of Ustilago maydis. **Trends Microbiol,** v. 15, p. 525–529, 2007.

[23] TKAVC, R.; MATROSOVA, V.Y.; GRICHENKO, O.E. et al. Prospects for fungal bioremediation of acidic radioactive waste sites: characterization and genome sequence of *Rhodotorula taiwanensis* MD1149. **Front Microbiol**, v. 8, p. 1-21, 2018.

[24] AQUINO, S.; GONÇALEZ, E.; ROSSI, M.H., *et al.* Evaluation of Fungal Burden and Aflatoxin Presence in Packed Medicinal Plants Treated by Gamma Radiation. **J Food Protect**, v. 73, p. 932–937, 2010.

[25] AZIZ, N.H.; EL-FOULY, M. Z.; ABU-SHADY, M.R. ; MOUSSA, L.A.A. Effect of gamma radiation on the survival of fungal and actinomycetal florae contaminating medicinal plants. **Appl Radiat Isot**, v. 48, p. 71–76, 1997.

[26] LEWIS, N.F. ; MADHAVESH, D.A. ; QUMTA, U.S. Role of carotenoid pigments on radio-resistance on Micrococci. **Canadian J Microbiol,** v. 20, p. 455–459, 1974.

[27] LIN, M.T. ; DIANESE, J.C. A coconut agar medium for rapid detection of aflatoxin production by *Aspergillus* spp. **Phytopathology,** v. 66, p. 1466–1469, 1976.

[28] WORK, E. Amino acids of walls of Micrococcus radiodurans. **Nature**, v. 201, p. 107–109, 1984.

[29] DADACHOVA, E.; BRYAN, R.A.; HOWELL, R.C.; SCHWEITZER, A.D.; AISEN, P.; NOSANCHUK, J.D.; CASADEVALL, A. The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. **Pigm Cell Melanoma R,** v. 21, p.192–199, 2008.