BJRS

BRAZILIAN JOURNAL
OF
RADIATION SCIENCES
11-02 (2023) | 01-11 | e2244

Combined application of gamma radiation, cleaning and chemical sanitizers in decontamination of vehicle air conditioning filters

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 work aimed to analyze the fungal contamination of air-conditioning filter waste (n=15) as an indicator of Quality Air Indoor from different car models in São Paulo city in São Paulo State, Brazil, during the period from October 2018 to July 2019. Three different treatments were used for the decontamination of car air conditioning filters, such as mechanical vacuum cleaning (I), vacuum cleaning and use of sanitizing product (II), and sanitizing product associated with radiation treatment at a dose of 17 kGy (III). After the treatments, microbiological analyses were performed and samples were plated in Petri dishes containing Sabouraud agar transferred by Swabs, and incubated for 7 days at 25 °C. The Petri dishes were stored in a standard Biochemical Oxygen Demand incubator, for the growth of fungal cultures. After incubation, the fungal cultures were evaluated, and the fungal counting was expressed in unit-forming colonies (UFC) and frequency in samples (%). The fungi were examined by lactophenol blue solution staining for microscopy. All samples of treatment I and II were contaminated with various fungal genera and high bioburden, namely (treatment I) *Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium* spp., *Fusarium* spp., *Mucor* spp., *Nigrospora* spp., Not Sporulated Fungi (NSF), *Penicillium* spp., *Rhizopus* spp., *Rhodotorula* spp., *Trichoderma* spp. and yeasts. Treatment II showed *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, Cladosporium* spp., *Mucor* spp., NSF, *Penicillium* spp., *Phoma* spp., *Rhizopus* spp., *Rhodotorula* spp., *Trichoderma* spp., and yeasts. Treatment III presented NSF and yeasts, with 80% of material decontamination.

Keywords: **air, filter, car, fungi, gamma radiation.**

ISSN: 2319-0612
DOI: https://doi.org/10.15392/10.15392/2319-0612.2023.2244
Submitted: 2023-01-03
Accepted: 2023-04-26

1. INTRODUCTION

Indoor air quality is an indicator of environmental health that takes into account thermal comfort, factors that interfere in precarious air conditions, such as the presence of fungi, bacteria and carbon dioxide in indoor air-conditioned environments [1].

In a study performed by Zulauf *et al.* [2] that determined particulate matter (PM0.3 and PM2.5), fungi, and airborne bacteria concentrations. This search was carried out during a routine travel trip of 51 cars, in eastern Germany and rural areas. The mean bacterial count was 350 colony-forming units (CFU) m^{-3} and mean fungal count was 13 $CFUm^{-3}$. Barnes *et al.* [3] found a significant positive correlation (0.30, < 0.05) between bacterial counts and particulate matter (PM 2.5).

The lack of studies on air quality within automotive passenger vehicles in the downtown area of São Paulo, one of the most polluted cities in the world and with the largest fleet of vehicles in Brazil, was the objective of the study of Aquino *et al.* [1]. They aimed to analyze the fungal contamination in air-conditioning filters collected from twenty-one automotive vehicles and the results of the study showed seventeen fungal genera in all samples collected (100%), including toxigenic fungi such as *Penicillium*, *Fusarium* and *Aspergillus*. Such an indoor air quality may compromise the health of a portion of the population, such as professional drivers.

According to IAEA [4], the combinations of the processes and their applications are being pursued to meet the end objectives of improved decontamination, waste volume reduction, safety and overall cost-effectiveness in the irradiation treatment. According to Thakur and Singh [5], the use of combined processes has been found to inhibit the development of undesirable changes caused by irradiation into substrate material. Almost all standard production car filters are paper, but filter elements are also generally made from different materials: paper, cotton, or foam. Inlet air filtration systems in modern passenger car engines use single-stage filters with panel filters made of pleated filter paper, which are porous materials, characterized by particle separation efficiency (≥ 5 μm), filtration performance of 99.9%, and low thickness (0.4–0.8 mm). Filter paper retains dust particles at the fibers of a porous filter media as a result of different forces and separation mechanisms [6].

American Society of Mechanical Engineers (ASME), Nuclear Regulatory Commission (NRC) and American Society for Testing and Materials (ASTM) published documents with specifications that combine to basic physical, chemical, test, and performance standards for filter media and impregnated activated carbon as well as for assembled componentes. Aging mechanisms and effects are discussed in con**j**unction with stressors, the agents, or stimuli that can result degradation [7, 8].

DeAngelis and cols. [9] observed that filtration performance was degraded after 25 kGy in different models of N95 respirators (mask filter). Electrostatic potential was measured on each respirator layer and statistically significant changes were observed in layers with electret filter media.

One way to prevent these changes in constituents of different irradiated materials is to reduce the radiation doses and use combined irradiation with heating, chemicals, cryogenic temperature, and a modified atmosphere. The decreased cost of irradiation at lowered absorbed doses may offset the additional cost of any other applied process, depending upon the cost of the process relative to the cost of irradiation. In this study it is convenient to try previous cleaning and chemical treatment of 15 filters of vehicles before irradiation in order to use 17 kGy of dose of radiation. Soriani *et al*. [10] after using gamma radiation at the dose of 17.8 kGy showed that the content of the main chemical active principles of raw plant materials was not modified and controlled high microbial bioburden. Denatonium benzoate ($C_{28}H_{34}N_2O_3$) is a substance of chemical classes of nitrogen compounds that is used in cleaning car filters. denatonium benzoate appears to be safe when used at low concentrations and has been used as an alcohol denaturant [11, 12].

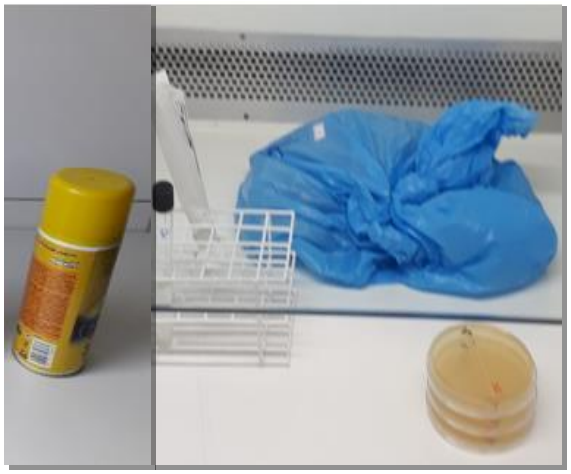
2. MATERIALS AND METHODS

2.1. Sampling and fungi isolation

Fungal isolation was entirely based on inoculation in Petri dishes with swabs directly in Sabouraud agar and the incubation of samples during 7 days at 25 ºC was under carefully standardized conditions, according to Pitt and Hocking methods [13] in a Biochemical Oxygen Demand (BOD) incubator.

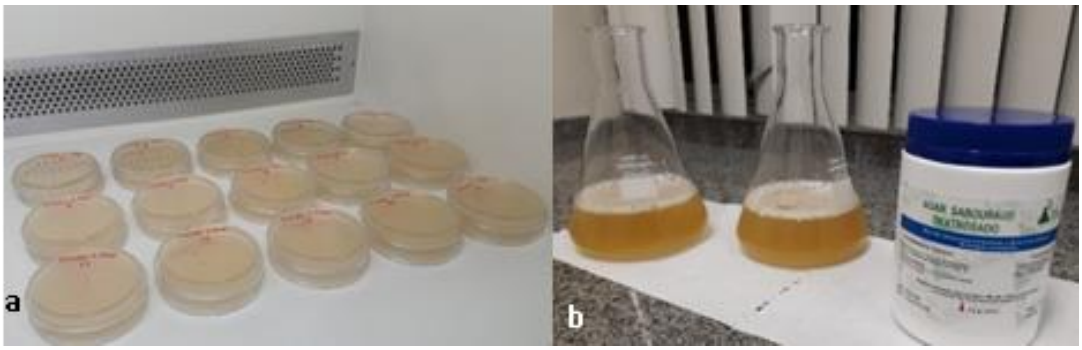
The fungal identification was performed using lactophenol cotton blue solution for microscopy, for staining molds, as described by Pitt and Hocking [13]. After the fungal analysis of control group, 15 filter samples were cleaning with a vacuum system and treated by sanitizer for vehicle air conditioning filters. The air conditioner sanitizer spray was used in the samples for 5 minutes, and they were composed by denatonium benzoate and were kept in bags (Figure 1) with an atmosphere filled with sanitizer spray and kept in cardboard boxes before the gamma radiation treatment.

Figure 1: Filter samples kept in bags with sanitizer spray of denatonium benzoate.



After all treatment procedures, the samples were analyzed in Petri dishes, in triplicate (Figure 2), to fungal counting, expressed as colony-forming units (CFU) and percentage (%) [13].

Figure 2: Platting onto Sabouraud agar in Petri dishes (a); Sabouraud agar (b).



.

2.2. Irradiaton at cobalt source

The filters samples were individually protected in plastic bags (Figure 4) and were maintained onto cardboard box during irradiation. The search of fungal contamination was carried out at the control group of air-conditioner filters samples and irradiated samples treated with 17 kGy (to fungal decontamination) using Co^{60} source by gamma at room temperature (25 ± 2°C), at a dose rate of 5.5 kGy/h, in a total of 185.4 minutes. 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.

Figure 3: Sample filters packages to treatment I, II and III.

3. RESULTS AND DISCUSSION

Modern automotive air conditioning (AC) installations become quite often an active source of harmful biological agents emission. The development of microorganisms is a result of surface contamination of the AC system, strongly supported by the increase in air humidity caused by the small diameter of air-conditioning cords, air cleaners, air refrigerators, etc. [14].

The control samples showed were contaminated with a diversity of 10 genera and Not Sporulated Fungi (NSF). The results of the control group (0 kGy) demonstrated the presence of *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Cladosporium* spp., *Fusarium* spp., yeasts, *Penicillium* spp., *Phoma* spp., *Rhizopus* spp., *Rhodotorula* spp. and *Trichoderma* spp.

The vacuum cleaning treatment (I) samples showed 11 fungal genera and NSF. The same 10 genera found in control samples, but with two more such as *Mucor* spp. and *Nigrospora* spp. The cleaning and chemical treatment samples (II) showed 10 fungal genera (with one UFC of NSF.). The treatment I resulted in more fungal contamination in 46.6 % of samples, comparing with control samples. However, the samples treated by vacuum cleaning and sanitizer spray (II) showed the increase of fungal counting in ten samples (66 %).

Two samples showed 2 CFU (colony-forming units) of NSF and one showed 1 CFU of yeasts in treatment III. Table 1 shows the decrease of fungal burden data (%) of treatment III in comparison with treatment I and II.

Table 1: Fungal genera isolated from control samples and the treatment I, II and III.

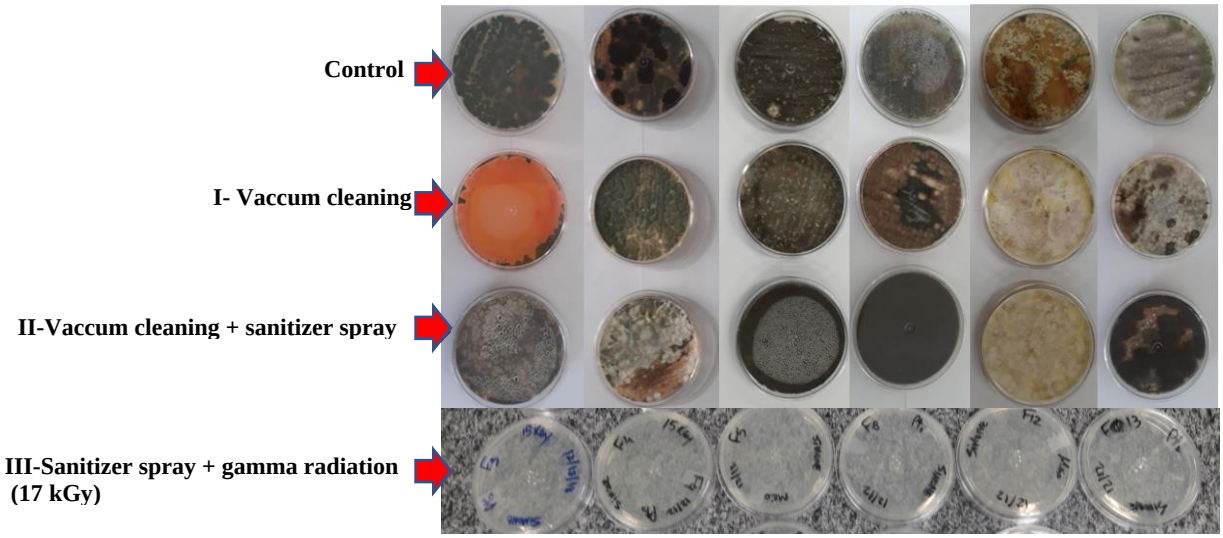
Control	**Nº of samples (%)**
Alternaria alternata	1(85%); 12 (100%)
Aspergillus flavus	4 (55%); 6 (60%)
Aspergillus fumigatus	2 (100%); 4 (75%); 5 (50%); 8 (8%)
Aspergillus niger	1 (35%); 2 (35%); 3(62%); 4 (35%); 5 (45%); 6 (100%); 8 (40%)
Aspergillus terreus	4 (38%)
Cladosporium spp.	3(30%); 7(100%); 11 (100%); 13 (100%)
Fusarium spp.	9(20%)
NSF	1(65%); 5(12%); 8(10%); 9 (40%); 10 (14%); 15(50%)
Penicillium spp.	10 (25%)
Phoma spp.	9 (23%); 10(48%)
Rhizopus spp.	3 (70%); 5 (20%)
Rhodotorula spp.	3 (50%); 5 (50%); 10 (50%)
Trichoderma spp.	1 (85%); 12 (100%)
Yeasts	1 (50%); 4 (48%); 8(50%); 9 (80%);14 (100%)
Treatment I	**Nº of samples (%)**
Alternaria alternata	8(50%); 15 (25%)
Aspergillus flavus	1 (48%); 15 (40%)
Aspergillus niger	1(40%); 3 (75%); 10(34%);
Cladosporium spp.	3(36%); 4 (100%); 5 (49%); 6 (38%); 7 (100%); 11(22%); 13 (58%); 14 (100%)
Fusarium spp.	11 (35%)
Mucor spp.	1(10%)
Nigrospora spp.	3(45%)
NSF	1(48%); 2 (50%); 3(68%); 4 (10%); 6 (22%); 7 (38%); 8 (50%); 9 (50%); 10 (55%); 11(100%); 13 (78%); 15 (50%)
Penicillium spp.	2 (32%); 5 (25%); 8 (55%); 10 (35%);
Rhizopus spp.	3(5%); 6 (50%); 10(15%)
Rhodotorula spp.	5(50%); 15 (50%)
Trichoderma spp.	12(100%)
Yeasts	3(80%); 5(16%); 6 (68%); 8 (2%); 9 (72%); 10 (48%); 13 (55%);
Treatment II	**Nº of samples (%)**
Alternaria alternata	1(40%)
Aspergillus flavus	2(30%); 3(5%); 6(35%); 8(92%); 9(42%); 11(50%)
Aspergillus fumigatus	1(7%); 4 (36%); 7(15%);9 (68%); 10 (80%); 13(32%)
Aspergillus niger	1(70%); 2(72%); 3(80%); 5(45%); 6(38%); 7(78%); 9(28%); 11(62%); 12 (5%); 15 (40%)
Aspergillus ochraceus	1(10%); 4 (19%);
Cladosporium spp.	8(22%); 14(82%); 15(35%)
Mucor spp.	1(5%)
NSF	1(8%); 3(40%); 4(25%); 5(50%); 6(30%); 8(12%); 10(22%); 12(38%); 13(25%); 14(35%)
Penicillium spp.	1(12%); 7 (50%); 13(62%); 14 (75%)

Phoma spp.	3(50%)
Rhizopus spp.	6(25%); 7(10%); 12 (25%)
Rhodotorula spp.	5 (52%); 6(25%); 9(3%)
Trichoderma spp.	6(50%); 9(50%); 12(38%); 15(100%)
Yeasts	2(50%); 4(80%); 5(50%); 7 (40%); 8(15%); 10(45%); 12(28%); 13(88%)
Treatment III	**Nº of samples (%)**
Yeasts	2 (1%)
NSF	6 (3%); 11 (5%)

The size and nature of fungal spores can contribute to their efficient long-distance dispersal in the air. Besides, fungal spores are hydrophobic in water, they are not easily wetted and tend to float on the water surface [15, 16].

The fungal growth was favored by high humidity conditions in samples that were sprayed with sanitizer, that did not inhibit the fungal contamination. According to Reponen and cols. [17], the relative humidity changes the spore size. The same authors reported that the highest change of the aerodynamic diameter was found in *Cladosporium cladosporioides* spores, that increased from 1.8 μm to 2.3 μm when the relative humidity increased from 30% to ~ 100%. The size increase corresponds to an approximate doubling of the particle volume [17].

Figure 4: Petri dishes with fungi in control samples, vacuum cleaning (I), vacuum cleaning and sanitizer spray (II) and sanitizer spray with gamma radiation treatment (III).

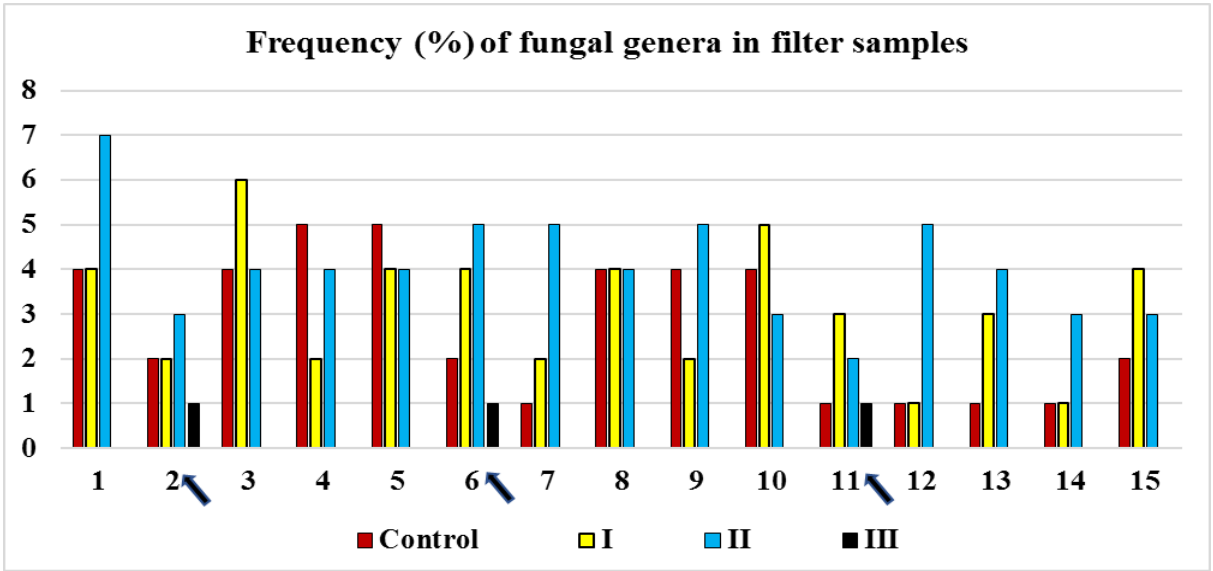


Udaya *et al.* [15] isolated 17 genera from the surface of bus seats. *Aspergillus* spp. was most frequently represented inside the vehicle. Viegas *et al.* [16] reported that *Cladosporium* spp. was the most prevalent fungal species from taxi filters in three cities of Portugal (Lisbon, Loures and Setúbal). In this present study in São Paulo city, the *Aspergillus* genera was predominant, after NSF and *Cladosporium* spp.

Figure 5: *Cladospororium* spp. in Petri dishes (yellow arrows).

On the other hand, treatment III, which used the denatonium benzoate (spray) in association with gamma radiation (17 kGy) showed complete decontamination in 80% of samples. No fungal growth was observed in 12 filter samples (1, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14, and 15). Only 3 samples (filters 2, 6, and 11) presented the lowest initial bioburden, as demonstrated in Figure 6, due to the fact that the filters were cleaner at the time of replacement.

Figure 6: Fungal genera (%) in control filter samples and combined treatments (I, II, and III).



The effect of water radiolysis plus the chemical effect of denatonium benzoate justified the reduction of bioburden in Treatment III because of higher humidity, with the use of the liquid spray in samples. The indirect effect of the ionizing radiation is associated with water presence in the substrate. The direct effect causing direct damage to biomolecules, such as double-strand breakage in DNA, and provoking genotoxic DNA alterations and cell damage [18]. The liquid spray of denatonium benzoate is associated with water radiolysis (indirect effect of radiation) and leads to the production of free radicals (e−aq, ·OH, H·, and HO_2·) and molecular products (H_2, H_2O_2), that are referred to as primary products of radiolysis [19].

They are usually classified into reducing (e−aq, H·) and oxidizing (·OH, HO_2·, H_2O_2) equivalents. With a longer lifetime, hydroxyl radical (·OH) is the most effective oxidant radical towards biological molecules: it has a high standard potential ($E° = 2.34$ V) with respect to Standard Hydrogen Electrode (SHE) at neutral pH [19]. The primary products of radiolysis are capable of damaging fungal cells causing growth inhibition.

4. CONCLUSION

The methods of vacuum cleaning and sanitizer spray with denatonium benzoate were not efficient to fungal control. The association with radiation, 17 kGy, showed that 80% of samples were completely decontaminated.

Two samples demonstrated only the growth of yeasts (not pathogenic fungi). No mycotoxigenic fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* genera were detected in irradiated samples using combined methods.

In order to establish a protocol with two combined methods for the control of fungi in air filters, the use of gamma radiation and sanitizer products showed that it is an efficient way to control mycotoxigenic or pathogenic fungi, using low doses to recycle the material against radiorresistant species.

ACKNOWLEDGMENT

The authors thanks IAEA (RC Project - 22644) and CNPq for financial support. We also thanks Pablo A.V. Salvador and Paulo Souza Santos for radiation processing.

REFERENCES

[1] 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.

[2] ZULAUF, N.; DRÖGE, J.; KLINGELHÖFER, D.; BRAUN, M.; OREMEK, G.M.; GRONEBERG, D.A. Indoor Air Pollution in Cars: An Update on Novel Insights. **Int J Environ Res Public Health**, v.16, n.13, p. 2441, 2019.

[3] BARNES, N.M.; NG, T.W.; MA, K.K.; LAI, K.M. In-Cabin Air Quality during Driving and Engine Idling in Air-Conditioned Private Vehicles in Hong Kong. **Int J Environ Res Public Health**,v.15, p. 611, 2018.

[4] INTERNATIONAL ATOMIC ENERGY AGENCY. Combined methods for liquid radioactive waste treatment Final report of a co-ordinated research project 1997–2001. **TECDOC,** nº.1336, 2003.

[5] THAKUR, B.T.; SINGH K. Combined processes in food irradiation. **Trends Food Sci Technol**., v. 6, p. 7-11, 1995.

[6] DZIUBAK, T. ; DZIUBAK, S.D. Experimental study of filtration materials used in the car air intake. **Materials**, v. 13, p. 3498, 2020.

[7] ASME STANDARD N509-1989. **Nuclear Power Plant Air-Cleaning Units and Components**, The American Society of Mechanical Engineers, New York, 1989.

[8] WINEGARDNER, W. K. **Aging Assessment of Nuclear Air-Treatment System HEPA Filters and Adsorbers Phase I**. Division of Engineering Office of Nuclear Regulatory Research U.S. Nuclear Regulatory Commission Washington, DC 20555--0001 NKC FIN B2911 Pacific Northwest Laboratory Richland, WA 99352, 1993.

[9] DEANGELIS, H.E.; GRILLET, A.M.; NEMER, M.B. *et al.* Gamma radiation sterilization of N95 respirators leads to decreased respirator performance. **PLoS One**, v. 16, n. 4, p. e0248859, 2021.

[10] SORIANI, R.R. ; SATOMI, L.C. ; PINTO, T.J.A. Effects of ionizing radiation in ginkgo and guarana. **Radiat Phys Chem**, v. 73, n. 4, p. 239-242, 2005.

[11] NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. **Denatonium benzoate**, 2022. Available at: <https://pubchem.ncbi.nlm.nih.gov/compound/Denatonium-benzoate>. Last accessed: 23 Dec. 2022.

[12] PULCE, C.; DESCOTES, J. Household products. **Hum Toxicol**, p.683-702, 1996.

[13] PITT, J.I.; HOCKING, A.D. **Fungi and Food Spoilage**, London & UK: Springer Science & Business Media, 2009.

[14] GOLOFIT-SZYMCZAK, M.; STOBNICKA-KUPIEC, A.; GORNY, R.L. Impact of air-conditioning system disinfection on microbial contamination of passenger cars. **Air Qual Atmos Health**, v 12, p.1127–1135, 2019.

[15] UDAYA, P.N.K.; BHUVANESWARI, S.; KUMAR, M.R.; RUPESH, S.L.K. A study on the prevalence of indoor mycoflora in air-conditioned buses. **Br Microbiol Res J**, v. 4, p. 282-292, 2014.

[16] VIEGAS, C.; MONTEIRO, A.; SANTOS, M. *et al.* Filters from taxis air conditioning system: a tool to characterize driver's occupational exposure to bioburden? **Environ Res,** v. 164, p.522–529, 2018.

[17] REPONEN, T.; WILLEK, K.; ULEVICIUS, V., REPONEN, A.; GRINSHPUN, S.A. Effect of relative humidity on the aerodynamic diameter and respiratory deposition of fungal spores. **Atmos Environ**, v. 30, p. 3967-3974, 1996.

[18] NASCIMENTO, F.J.A.; BRADSHAW, C. Direct and indirect effects of ionizing radiation on grazer–phytoplankton interactions. **J Environ Radioact**, v. 155-156, p. 63-70, 2016.

[19] BALDACCHINO, G.; BRUN, E.; DENDEN, I. *et al.* Importance of radiolytic reactions during high-LET irradiation modalities: LET effect, role of O_2 and radiosensitization by nanoparticles. **Cancer Nano**, v. 10, n. 3, 2019.