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BIOBURDEN PROLIFERATION IN VEHICLE AIR FILTERS WASTE: THE USE OF GAMMA RADIATION ON FUNGAL DECONTAMINATION

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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 August 2018 to December 2018. A non-selective fungal agar was used, as a commercially prepared media. Sampling consisted of filters particles (33 fragments of 10 × 10-mm size) were plated onto solidified agar in Petri dishes in triplicate. The samples were incubated for seven days at 25 °C and were stored in a standard Biochemical Oxygen Demand (BOD) 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 molds 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 a dose of 10 kGy to fungal decontamination of air-conditioning filters waste. Of total samples, 50% were completed decontaminated, but yeasts, *Cladosporium* spp., *Penicillium* spp., *Aspergillus niger* demonstrated radioresistance at the dose of 10 kGy.

Keywords: Air quality, fungi, contamination, filter, waste, gamma radiation.

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 (Viegas et al., 2018). According to Aquino (2018) 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 (Oppliger, 2014).

The fungal contamination in vehicle air filters and their impact as a bio-accumulator on IAQ was carried in São Paulo, confirming the presence of many genera, including toxigenic Aspergillus species (Aquino et al., 2018). Li et al. (2013) 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 (2013) 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 (Cerqueira and Guimarães, 2017).

On the other hand, the amounts of solids residues are increasing in excessive magnitude. The treatment of car's filters by gamma radiation can to 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 (Cote et al., 2018). 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 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 models (Fig. 1) were collected from different models of passenger vehicles in 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.

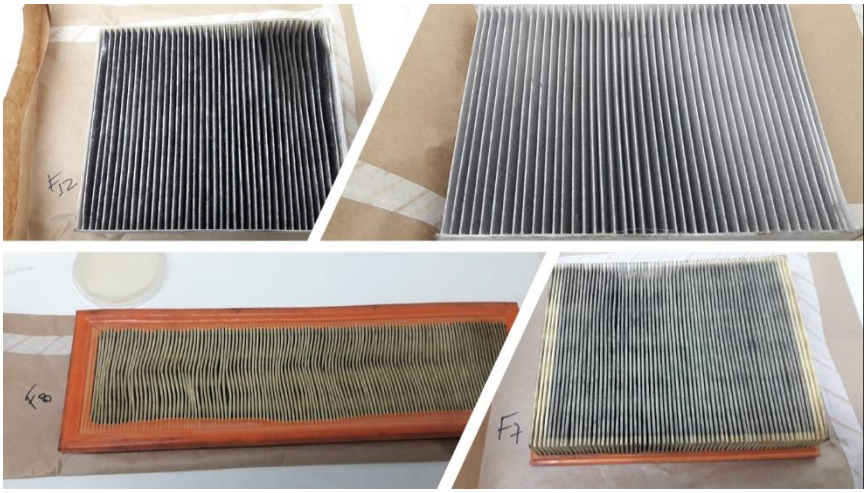

Figure 1: Filters of car air-conditioning collected in São Paulo (bioburden and irradiation).

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 (Pitt and Hocking 2009). 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 (2009). 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 Fig. 2.

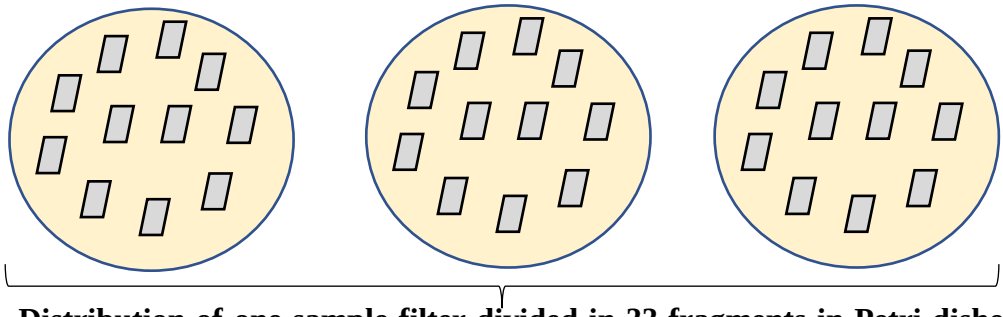

Figure 2. Distribution of one sample filter divided in 33 fragments in Petri dishes with PDA.

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 (Fig. 3).

Figure 3: Fungi incubation of filters samples in PDA media, at 25ºC for 7 days.

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. Differential counting of various genera is often possible, and as mentioned for each fungal genus, the results were also expressed in percentage (%), according to the technique described by Berjak (1984).

2.2. Irradiaton at cobalt source

The samples were individually protected by a Kraft paper envelope, and materials were maintained in a plastic box during irradiation at 5 kGy/h at a Multipurpose irradiator of IPEN/CNEN (Fig. 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 a Co^{60} source by gamma rays.

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

3. RESULTS AND DISCUSSION

3.1. Control samples

The present study showed that control samples were contaminated with fungi, diversity of genera. The results of the control group (0 kGy) were summarized at Table 1.

Table 1: Fungal genera and frequency (%) of control samples (0 kGy).

Fungi genera	Sample number (%)
Alternaria	2(5%); 5(33%); 6(24%); 8(45%); 9(3%)
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%).
Bipolaris	12(3%)
Chaetomium	12(3%); 13(8%); 16(7%); 17(1,5%); 18(4,5%)
Cladosporium	5(33%); 6(13%); 7(72%); 10(54%); 11(10%); 12(36%); 14(63%); 15(18%); 17(18%);
Curvularia	17(3%); 18(19%)
Fusarium	6(6%)
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%)
Nigrospora	9(27%); 12(7%); 18(30%)
Paecilomyces	13(8.5%);
Penicillium	2(3%); 4(40%); 5(7%); 12(2%); 13(12%); 15(3%); 16(33%); 18(1,5%); 20(10%)
Phoma	7(3%); 13(1.5%)
Rhizopus	1(61%); 2(58%); 3(21%); 5(3%); 6(33%); 9(20%); 16(9%); 19(4%)
Rhodotorula	1(12%); 3(48%)
Scytalidium	13(7%)
Syncephalastrum	1(3%);
Trichoderma	2(6%); 3(21%); 5(3%); 6(45%); 8(5%); 9(3%); 10(10%); 19(5%)
Ulocladium	8(6%)
Yeasts	1(15%); 4(34%); 5(12%); 6(3%); 7(12%); 8(3%); 10(21%); 12(46%); 13(53%); 17(60%); 18(20%)

The Figure 5 shows the genera of fungi isolated from fragments in agar Potato Dextrose of control samples

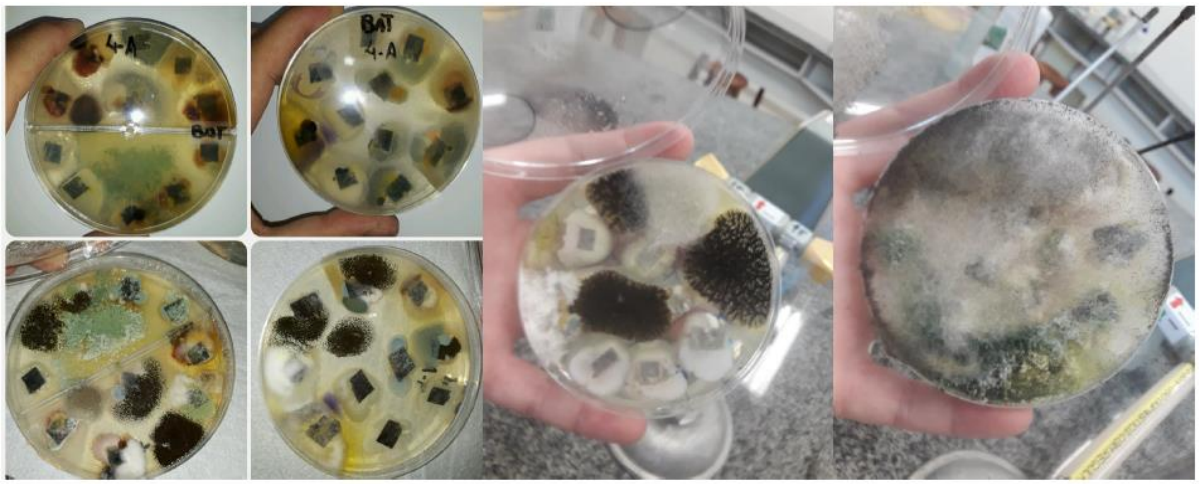

Figure 5: Samples of fragments in agar Potato Dextrose of control samples.

Previous studies found *Alternaria alternata*, *Aspergillus* spp., *Cladosporium cladosporioides*, *Penicillium* spp., *Trichoderma viride*, *Curvularia lunata*, and *Phoma* spp. and declared the highly possible that yeasts were also present in dust samples found in the vehicle filters in China. In another study performed by Simmons et al. (1997), the authors 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*.

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).

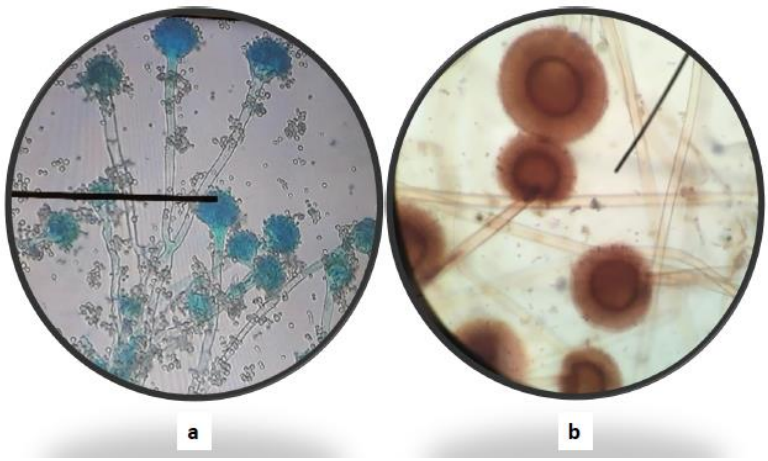

Figure 6: Microscopy of *Aspergillus fumigatus*(a) and *Aspergillus niger* (b) isolated from air-conditioning filters.

Schoenlein-Crusius et al. (2001) 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 (Gniadek 2012).

In a study performed by Li et al. (2013) in three Chinese cities (Beijing, Guangzhou, and Haikou), the dust from air-conditioning filters in vehicles demonstrated the presence of *Aspergillus niger, Aspergillus fumigatus, Aspergillus ustus, Aspergillus oryzae, Aspergillus ochraceus, Aspergillus terreus, Aspergillus restrictus, Aspergillus versicolor, Aspergillus sydowii* and *Aspergillus 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* (Gniadek et al. 2010).

3.2. Irradiated samples

Many studies have already reported the resistance of microorganisms to radiation (Daly, 2012; Slade and Radman, 2011; Williams et al., 2007; Liu et al., 2003). 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 (Daly, 2012). The results of the irradiated samples showed fungal growth in 10 samples (50%) as demonstrated in Table 2. Dose of 10 kGy was not enough to achieve the fungal decontamination in totality.

Table 2: Fungal genera isolated in irradiated samples (10 kGy).

Fungi genera	Sample number (%)
Aspergillus	3(6%); 5(12%); 7(3%); 13(2%)
Cladosporium	5(3%); 7(2%); 10(4%); 17(1%)
NSF	13(7%); 15(3%); 16(8%)
Penicillium	6(3%); 18(1%)
Rhizopus	1(1%); 5(3%); 6(6%); 17(1%)
Rhodotorula	3(2%); 5(8%); 6(5%); 7(5%); 10(7%); 18(2%)
Trichoderma	5(1%); 13(4%); 20(1%)
Yeasts	5(15%); 13(40%); 15(80%); 18(30%); 20(45%)

Concerning the fungal radiation resistance, Jung et al. (2016) 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 (Holloman et al., 2007).

Yeasts (unicellular and anaerobic fungi) are more resistant than filamentous fungi (aerobic and multicellular fungi). It is essential to observe that yeasts had an increase in growth in some samples after radiation exposure (arrows in Fig. 7).

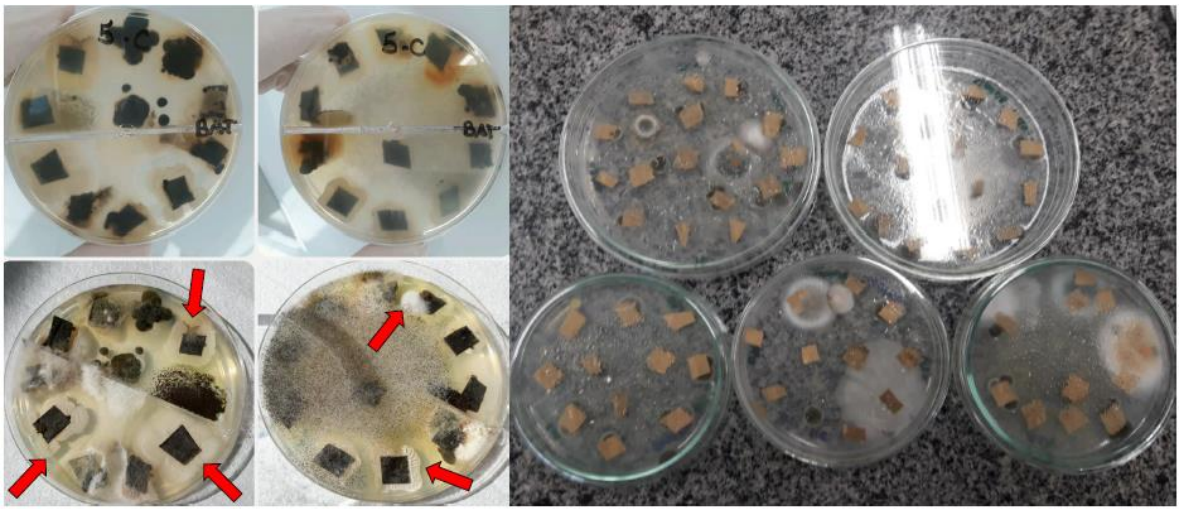

Figure 7: Macroscopic aspect of fungi isolated from irradiated air-conditioning filters.

The variation in gamma radiation resistance in filamentous fungus strains can be explained by multiple factors (Aquino et al., 2010; Aziz et al. 1997). Aziz et al. (1997) 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 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 (Lewis and Madhavesh, 1974; Linand and Dianese. 1976).

There are some indications that the radioresistance of microorganisms may result from melanization of their cells (Work et al., 1984). 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 (Dadachova et al., 2008).

4. CONCLUSIONS

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). Ionizing radiation may be a possible technology for reducing the amount of such a type of solid residue if part of car's filters may be reused after irradiation.

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