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# Analysis of fungal contamination in vehicle air filters and their impact as a bioaccumulator on indoor air quality

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## Abstract

Studies on air quality within automotive vehicles are an emerging research area in Brazil, especially in the city of São Paulo, one of the most polluted cities in the world and with the largest fleet of vehicles in the country. Indoor air quality is an indicator of environmental health that takes into account, in addition to thermal comfort, factors that interfere in precarious air conditions, such as the presence of fungi, bacteria and carbon dioxide in indoor air-conditioned environments. The objective of the present study was to analyse the fungal contamination in air-conditioning filters collected from 21 automotive vehicles and the study found 17 fungal genera in all samples collected (100%), including toxigenic fungi such as *Penicillium*, *Fusarim* and *Aspergillus*, indicating that indoor air quality can compromise the health of a portion of the population, such as professional drivers. Among the *Aspergillus* genus, the results showed the presence of the *A. flavus*, *A. niger*, *A. fumigatus*, *A. ochraceus* and *A. clavatus* species, which cause severe allergic and pulmonary respiratory diseases. Air in artificially heated environments should provide comfort to its occupants but it may pose a risk to human health if the car filtration system is contaminated by pathogenic fungi.

**Keywords** Air-conditioning · Filters · Vehicles · Contamination · Fungi

## Introduction

The economic activities and the disorderly formation of urban concentrations, resulting in unequal population distribution, are mainly responsible for pollution and loss of atmospheric air quality in cities. The disorderly growth of cities makes urban space more and more crowded, increasing the degradation of soil, water and air and deteriorating the quality of life of the population. In addition, problems related to the contamination of internal environments are strongly aggravated by urban, social and industrial issues, making environmental and human health conditions a concern in developing societies (Cartaxo et al. 2007). Quandt et al. (2014) pointed out the recent incorporation of the environmental problem in basic health care, by the World Health Organization (WHO), revealing the urgency of the intersectorial confrontation of health and environment. The concept of environmental health advocates the quality of life of people, influenced even by the quality of the air that is breathed within confined environments, i.e. the internal air quality has its importance based on the amount of time people spend indoors, especially in urban environments. Most of the day, many people spend up to 80% of their time indoors, which can affect the health of the occupants of these environments (Narciso et al. 2014).

Jodeh et al. (2018) reported that air pollution, both indoors and outdoors, is a major environmental health problem which affects both developed and developing countries and that understanding and controlling common indoor pollutants can help reduce the risk of indoor health concerns. The air citizens breathe every day is mostly indoor air with very little ventilation (at work, at home, at schools, at shopping, during transportation,

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etc.). Although it seems perfectly safe, this air contains pollutants as well as the smoke emitted by industries and cars. People spend most of their time in indoor environments (88.9%), with limited time spent outdoors (5.8%) or in vehicles (5.36%) (Matz et al. 2014), and as a consequence, they may be exposed to many pollutants of indoor origin (Błaszczyk et al. 2017).

Artificially air-conditioned environments are designed to offer maximum comfort to its occupants. However, they can be harmful to human health, since air-conditioning equipment can be contaminated by particles, dust or contain filters colonised by different microorganisms, such as bacteria, fungi and viruses, which are able to survive in dried environments for a long time (Narciso et al. 2014). Air conditioners can be microbial propagators (bioburden), since they usually only recirculate the air already present in a closed environment and, therefore, if the air is already contaminated in the filter, the air conditioner will have a biopropagating effect (Andrade et al. 2015). The results of a review carried out by Mota et al. (2014) on air quality and the factors that interfere with poor indoor air conditions in hospital units yielded some information about the subject, where some risk categories were delimited: the influence of fungi on indoor air quality; the influence of bacteria on indoor air quality; thermal comfort standard; and carbon dioxide ($CO_2$). According to the authors, the scientific productions that most approached the aspects of air quality, as to the themes cited, were fungal microbiota (37.5%); thermal comfort and $CO_2$ levels (37.5%); and detections of bacteria (25%).

Not only the risk of internal $CO_2$ concentration in air-conditioned environments, but also the accumulation of moisture and organic material in air-conditioning trays can turn them into powerful dispersing sources of fungal bioaerosols (Mota et al. 2014). Fungi disseminate their spores in the environment through the atmospheric air, water, insects, man and animals, but atmospheric air is the most widely used dispersion medium for fungi. Thus, spores and fragments of mould vegetative mycelium become viable portions of these organisms during the aerial dissemination process. Fungal spores constitute a large part of the biological material suspended in the air and their monitoring can provide important epidemiological information regarding the genera present and their quantification (Lobato et al. 2009). According to the Environmental Protection Agency (EPA), in several environments, indoor air can be up to 10 times more polluted than outside air, and that about 75% of the closed environments surveyed have problems with viruses, bacteria, mites, fungi and chemicals harmful to human health (Cerqueira and Guimarães 2017).

Compared to air supply systems in buildings, air-conditioning (AC) systems in vehicles have some disadvantages due to the small space available in cars. Tiny air conduits and frequent changes in the direction of air flow in cars support deposition of airborne particles and microorganisms within the air conduits (Simmons et al. 1999). To protect occupants, air-conditioning filters of vehicles are intended to retain aerial bioburden (microorganisms in sprinkler or bioaerosols). However, under favourable conditions, biofilm proliferation in air filters and consequent airflow release into the vehicle enclosure represents a potential source of exposure to bioaerosols, especially if it contains respirable fragments (< 1.1 μm). In addition, when the air-conditioning system is switched on, the airflow through the filter system could recirculate the biofuel containing aerosols and consequently lead into the vehicle (Li et al. 2013). 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). There is also some concern about persons with some kind of a severe immunodeficiency being at risk of airborne infection caused by an AC system (Kumar et al. 1984; Vonberg et al. 2010).

Apart from particulate matter (PM), volatile organic compounds (VOCs), hydrocarbons and tobacco smoke, bioaerosol exposure inside a vehicle has attracted great attention in recent years (Jo and Lee 2008; Vonberg et al. 2010; Wang et al. 2013). The small internal space (of most vehicles) causes concentration of various chemicals and organic compounds to be as much as or three times higher than in other closed environments (UL Library 2017). Studies on indoor air quality (IAQ) in vehicles address levels of $CO_2$ concentration as done by Quadros et al. (2008), where levels were found to be below the limit of 1000 parts per million (ppm) in vehicles without artificial air conditioning. However, it has been observed that the concentration levels of this compound are raised substantially when using the air-conditioning system within the means of transport, whether small vehicles or public transport buses. In a study by Quadros et al. (2008), the data obtained show that there was an increase of 431 ppm of $CO_2$ in the internal environment for each 1 °C of cooling produced.

There is a positive association between $CO_2$ and fungi, as increased $CO_2$ concentrations may stimulate fungal contamination. Studies have shown that some species of fungi are able to grow more in $CO_2$-enriched atmospheres. In some cases, the growing is twice as much as 10% $CO_2$, and it has been observed that with the $CO_2$ concentration of air at 670 ppm, the percentage of colonisation by fungi increased 4-fold (Brackmann et al. 1996; Lipson et al. 2014; Idso and Idso 2012). These indoor conditions in vehicles are favourable to anemophilous fungi that are those whose spores are spread by the atmospheric air. Qualitative and quantitative knowledge of these fungi in a given region is of great importance and concern because they can cause several respiratory diseases in persons, such as asthma and rhinitis when inhaled (Mezzari et al. 2002). During many years, little attention has been paid to indoor air quality, despite the fact that many people spend more than an hour every day in vehicles or even the exposure to professional drivers (truck drivers, bus drivers, taxi drivers,

etc.) with regular work shifts of 8 h a day, 5 days a week, representing considerably more time spent inside the vehicles than the passengers (Nowakowicz-Dębek et al. 2017).

São Paulo is a megacity in South America and the richest city in Brazil with a GDP of USD 158 billion. It is an immense urban complex and the centre of a metropolitan region of 21 million people, which are distributed in 39 municipalities. The city of São Paulo is located in the São Paulo State, the most populous and industrialised state in Brazil with about 45 million inhabitants. Moreover, the metropolitan area has the largest vehicular fleet (Caumo et al. 2018; Alarcón et al. 2017), corresponding to more than 11% of the total population of Brazil, covering an area of 7947 $km^2$ with a population density of 2653.9 inhabitants per $km^2$ (Andrade et al. 2017). According to Numbeo (2018), São Paulo is considered one of the most polluted cities in the world, being the second most polluted city in Latin America, after Lima (Peru), third in America, ranking 42nd worldwide with 82.81 pollution index.

There is a lack of studies about air contamination especially in relation to automotive vehicles, and there are no Brazilian standards or guidelines established for the inspection of vehicle air filters or for the quality of the air conditioning circulating inside cars. The objective of the present study is the analysis of vehicle air filters to detect fungal bioburden, especially pathogenic fungi (which cause respiratory diseases) and to evaluate their risks and impacts on the health of drivers in the city of São Paulo, Brazil.

## Materials and methods

### Sampling

For isolation of fungi, surface and air sampling techniques are used. Bulk sampling of materials such as settled dust, pieces of wall board, duct linings and carpets are tested to determine the contamination with biological agents (Khan and Karuppayil 2012; Asadi et al. 2011). AC filters need to be replaced or cleaned periodically since the filter can be clogged due to dust load and fungal infestation. These samples provide the hyphal fragments and the reproductive structures which may help for identification (Aydogdu et al. 2010; Ruping et al. 2011).

A total of 21 air-conditioning filters were collected from different models of motorised passenger vehicles, with 10 exchange stations located in the South (5 samples), North (4 samples), West (4 samples), Downtown (4 samples) and East (4 samples), of the city of São Paulo (Latitude 23° 32′ 51″ S and Longitude 46° 38′ 10″ W) in São Paulo State, Brazil (Fig. 1)) during the period from October 2017 to January 2018. Time of use of the filter removed and the model of the cruising vehicle have not been registered, since the partnership with the exchange stations was limited by the recollection of the samples, once a week, according to the customers' demand, without the presence of the owner of the vehicle. Samples of the filters were collected in polyethylene bags (individually packed after collection) and sent to the microbiology laboratory at room temperature (25 °C ± 2).

### Isolation and fungal counting

The procedures of isolation fungi from samples were 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). Identification procedures used have been designed to be simple and comprehensible, avoiding the use of specialised equipment or procedures unavailable in the routine laboratory. To this end, identification of fungal genus and some species is based entirely on inoculation of a single series of Petri dishes, incubation under carefully standardised conditions and examination by traditional light microscopy. A standard plating regimen has been used for the initial examination of all isolates, so that identification procedures can be 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).

An overview of culture media considered most suitable for particular purposes is given by Pitt and Hocking (1997) based in recommendations from ICFM (Hocking et al. 2006) and to the enumeration of yeasts and moulds, the non-selective medium used was Potato Dextrose agar (PDA), as a commercially prepared media. The PDA culture medium complies with the recommendations of the American Public Health Association for food, the European Pharmacopeia and the United States Pharmacopeia. Carbohydrate and potato infusion promote the growth of yeasts and moulds while the low pH value partially inhibits the growth of the accompanying bacterial flora. The composition (g/l) is potato infusion from 200 g potatoes; 20 g of D(+) glucose; 15 g of agar-agar and pH = 5.6. The suspension of the preparation is 39 g/l and thereafter the suspension is placed in the autoclave for 15 min at 121 °C and distributed in Petri dishes with 90 mm of diameter. Plating should be carried out immediately and the fungi growing in this solid medium develop a typical morphology (Beever and Bollard 1970).

The approach used here has been to examine every isolate by a single system: inoculation onto a standard set of Petri dishes and examination of them culturally and microscopically after 7 days incubation. Most of the genera and species can be identified immediately, at that point. Only in exceptional cases has it been found necessary to reinoculate isolates onto a further set of media in order to complete identification (Pitt and

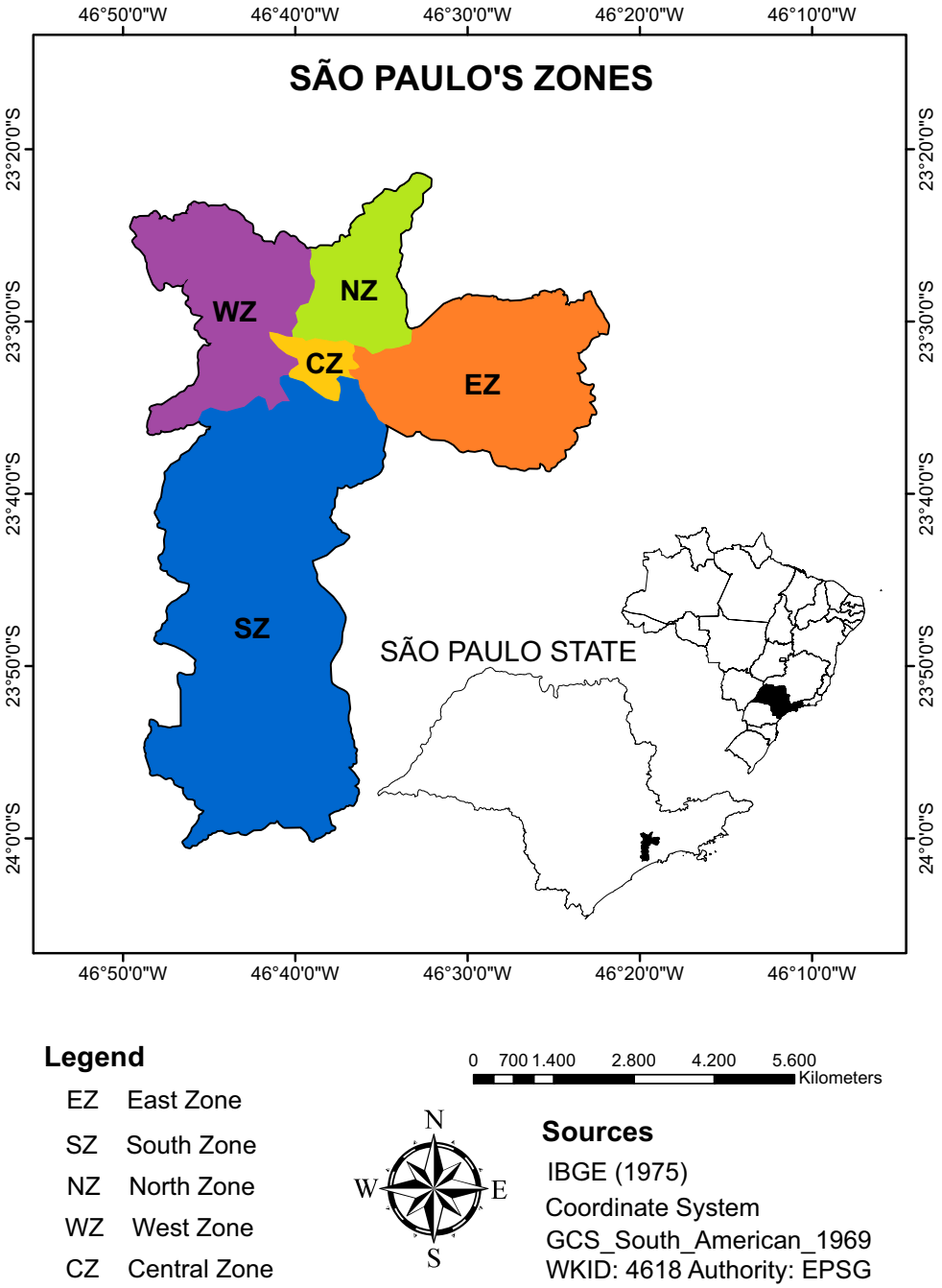


**Fig. 1** The map of São Paulo city zones in São Paulo State, Brazil

Hocking 2009). Contributions at the international workshops emphasised the use of direct plating as the preferred method for detecting, enumerating and isolating fungi from particulate substrates. In direct plating, particles are placed directly on solidified agar media and the results from direct plating analyses are expressed as percentage (%) infection of particles (Pitt and Hocking 2009; Berjak 1984). The samples particles should be plated onto solidified PDA, at the rate of 6–20 particles per Petri dish, depending on particle size. 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 PDA. For the analysis of variance between groups, the ANOVA and Tukey tests were applied through SPSS software (2007).

## Incubation and microscopes and microscopy examination

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, in accordance with the standard incubation conditions specified by International

Commission on Food Mycology (Hocking et al. 2006). After incubation, the examination of fungal culture in the plates is made by visually, and the total counting of infected fragments is express in results as a percentage. Differential counting of various genera is often possible, and as mentioned for each fungal genera, the results were also expressed in percentage (%), according to the technique described by Berjak (1984). According to Pitt and Hocking (2009), the correct choice of media, a stereomicroscope and experience will all assist in this process. Each sample was observed macroscopically (after the incubation period) for the morphological and macroscopic characteristics of the mycelia (colour, texture, back and front of the colony) as demonstrated in Fig. 2a–c.

Fungi should always be examined microscopically as wet mounts rather than fixed and stained like bacteria, using an inoculating needle to cut out a small portion of the colony which includes sporing structures. The portion of the colony or mycelium withdrawn with the needle is disposed between slide and cover slip, stained with a drop of lactophenol cotton blue. For the microscopic observation of the fungal morphology, the direct technique of mycological examination of the aerial mycelium (with the reproductive structures) was applied to the structures of hyphae, conidia and conidiogenic cells under a light microscope with an increase of 100- and 400-fold (100–400 ×) (Fig. 2d). The taxonomic identification of fungi was performed considering the morphological characteristics of the vegetative mycelium and the reproductive structures (Hoog and Guarro 1995; Alexopoulos et al. 1996; Pitt and Hocking 2009).

## Results and discussion

### Fungal genera isolates

The present study demonstrates a great variety of fungal biocharges in the analysed vehicle filters, where all samples showed fungal contamination (100%) and 17 fungal genera were observed. It was isolated fungi such as *Aspergillus* spp., *Cladosporium* spp., *Rhizopus* spp., *Trichoderma* spp., *Alternaria* spp., *Nigrospora* spp. (Fig. 3a), *Chaetomium* spp., *Curvularia* spp. (Fig. 3b), *Bipolaris* spp., *Phoma* spp., *Rhodotorula* spp., *Fusarium* spp., *Paecillomyces* spp., *Penicillium* spp. (Fig. 3c), *Scytalidium* spp., *Syncephalastrum* spp., *Ulocladium* spp., yeasts (unicellular fungi) and non-sporulating fungi (NSF).

The data are considerably similar to the study of airborne fungi in the city of Cubatão, in the State of São Paulo, performed by Schoenlein-Crusius et al. (2001) where the number of genera found corresponding to 19, such as *Aspergillus*, *Cladosporium*, *Penicillium* and *Trichoderma*, including the NSF. About 300 species of fungi have already been described as allergenic and among the fungi groups that spread air spores as important aeroallergens are *Zygomycota*, *Ascomycota*, *Basidiomycota* and *Chytridiomycota*. *Zygomycota* are represented by *Rhyzopus* and *Mucor*. *Ascomycota* deliver asci carrying ascospores and are represented by yeasts and *Chaetomium*. *Basidiomycota* deliver basidiospores and are represented by fungi that are pathogenic for plants, i.e., rusts and smuts. *Chytridiomycota* (before known as *Deuteromycota*) have the greatest number of fungi in their assexual reproduction phase, such as the *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria*. *Ascomycota*, *Basidiomycota*, *Zigomycota* and *Chytridiomycota* have the greatest number of well-known causative agents of allergic symptoms. In the world, species belonging to *Alternaria*, *Cladosporium*, *Aspergillus* and *Penicillium* are the most frequent ones (Burge et al. 1997; Mezzari et al. 2002; Pitt and Hocking 2009).

According to Mezzari et al. (2002), there is a difference between fungi present in indoor environments and in the atmospheric air and these conditions determine a greater or lesser probability of fungal growth. Several studies on fungal contamination have already been described in air-conditioning systems, especially for sick building syndromes. Contaminated air conditioning has already been blamed for the emergence of hospital infections, which are responsible for causing pneumonia, rhinitis, allergic sinusitis, lack of concentration and fatigue, both to patients and to other users (Mobin and Salmito 2006).

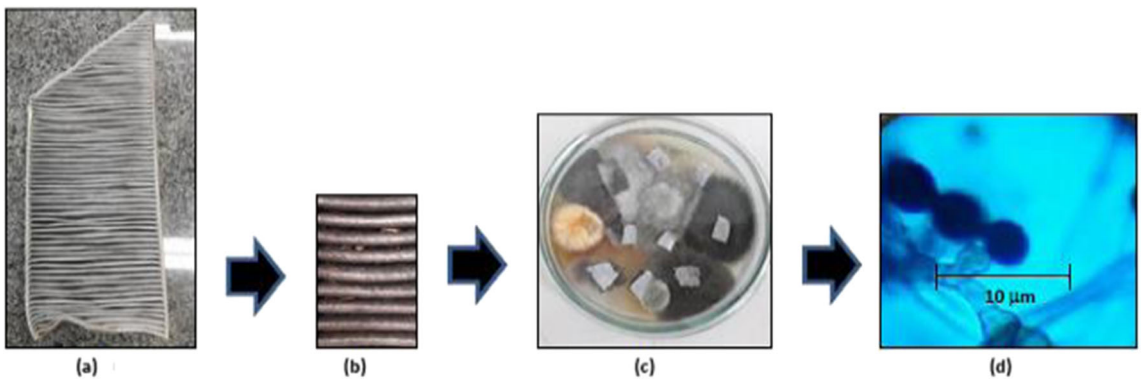


**Fig. 2** Laboratory procedures for filter fragment analyses (**a**, **b**, **c**) and fungi microscopic picture (**d**)

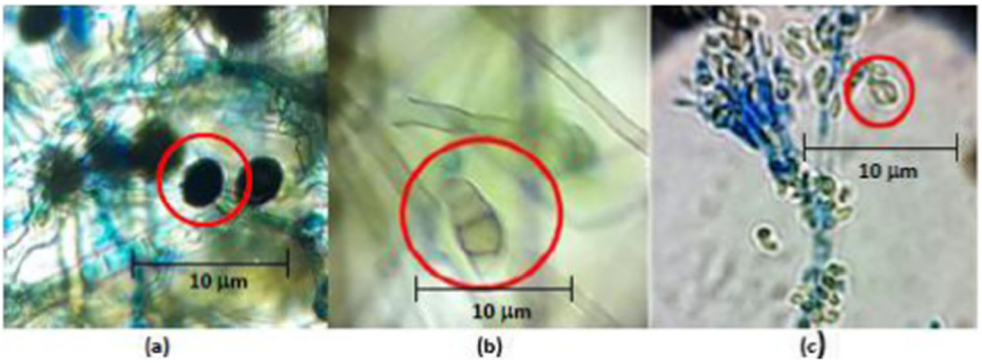


**Fig. 3** Conidia or spores of *Nigrospora* spp. (**a**), *Curvularia* spp. (**b**) and *Penicillium* spp. (**c**). Source: Prepared by the authors

In the study of Santana and Fortuna (2012), the authors reported *Fonsecaea* spp., *Penicillium* spp., *Candida* spp. (yeast) and *Aspergillus* spp. in air-conditioners of hospitals. Cartaxo et al. (2007), who verified the high growth of fungi such as *Penicillium* spp., *Paecillomyces* spp., *Cladosporium* spp., *Rhizopus* spp., *Mucor* spp., *Aspergillus* spp. and *Rhodotorula* spp. in the filters of air conditioners collected from households in Manaus (Brazil). Cerqueira and Guimarães (2017) evaluated the IAQ of an air-conditioned environment in a petrochemical industry, researching fungi as biological indicators and found the genera *Aspergillus*, *Trichoderma*, *Penicillium* and yeasts at all points studied. The same authors found a fungal load above the limit in some places, causing discomfort to workers who were daily in the place.

Li et al. (2013) collected in four different geographical locations in China both AC and engine 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 an important source of respiratory allergies or infections. The authors showed data that clearly revealed that automobile AC filters harboured significant amounts of biological agents including diverse bacteria and fungi, and high levels of endotoxin; and some of these agents could reproduce under high humidity conditions. The authors 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 colonised by odour-producing fungi such as *Penicillium viridicatum*.

These results demonstrate not only the presence of the same fungal genera in buildings, but also as contaminants of air filters in vehicles, since the same fungi were found in the present study as *Aspergillus*, *Penicillium*, *Trichoderma* and yeasts. The group of moulds described in this study cause negative impacts and consequences for human health. Fungi are often associated with clinical conditions that can reach high severity, causing several aggravated opportunistic fungal diseases such as respiratory diseases, such as asthma, rhinitis, sinusitis and chronic obstructive pulmonary disease (Cartaxo et al. 2007).

## Fungal genera total counting

In relation to the frequency of genera by total samples, the NSF, yeasts, *Cladosporium* spp., *Aspergillus* spp. and *Penicillium* spp. as the most frequent fungi are contaminants of air filters of automotive vehicles. In the present study, NSF were predominant in 81% of all analysed filter samples, being the second highest contamination observed by *Aspergillus* spp. (76%), followed by yeasts (62%) and *Cladosporium* spp. (52%), considered outliers, as shown by Fig. 4.

In relation to the relative frequency per sample individually analysed in the present study, the fungi *Aspergillus* spp., *Cladosporium* spp., NSF, yeasts, *Penicillium* spp., *Rhizopus* spp. and *Trichoderma* spp. were the most frequent per samples filters fragments. Samples car filters were contaminated at least two or more different genera of fungi, such as the sample n.5 and n. 13 with seven genera fungal contaminants, besides NSF, and in both samples there were two toxigenic genera (*Penicillium* and *Aspergillus*) as demonstrated in Table 1.

Knowledge of anemophilous fungi or airborne fungi is important for the ecological diagnosis and specific treatment of allergic manifestations induced by inhaled allergens. In order to evaluate the air quality and to diagnose the presence of anemophilous fungi in the city of Porto Alegre (Brazil), Mezzari et al. (2002) found in all seasons of the year that the prevalence of spores was as follows (in descending order): ascospores (50.48%), *Cladosporium* (17.86%), *Aspergillus/Penicillium* (15.03%), basidiospores (3.84%), rusts (3.82%) and *Helminthosporium* (2.49%). Less prevalent were *Botrytis* (1.22%), *Alternaria* (1.19%), smuts (0.90%), *Curvularia* (0.87%), *Nigrospora* (0.61%) and *Fusarium* (0.08%). Melo et al. (2009) verified that more than 40% of the isolated colonies of air conditioners of hospitals belonged to the genus *Penicillium* spp., followed by *Cladosporium* spp. and *Chrysosporium* spp.

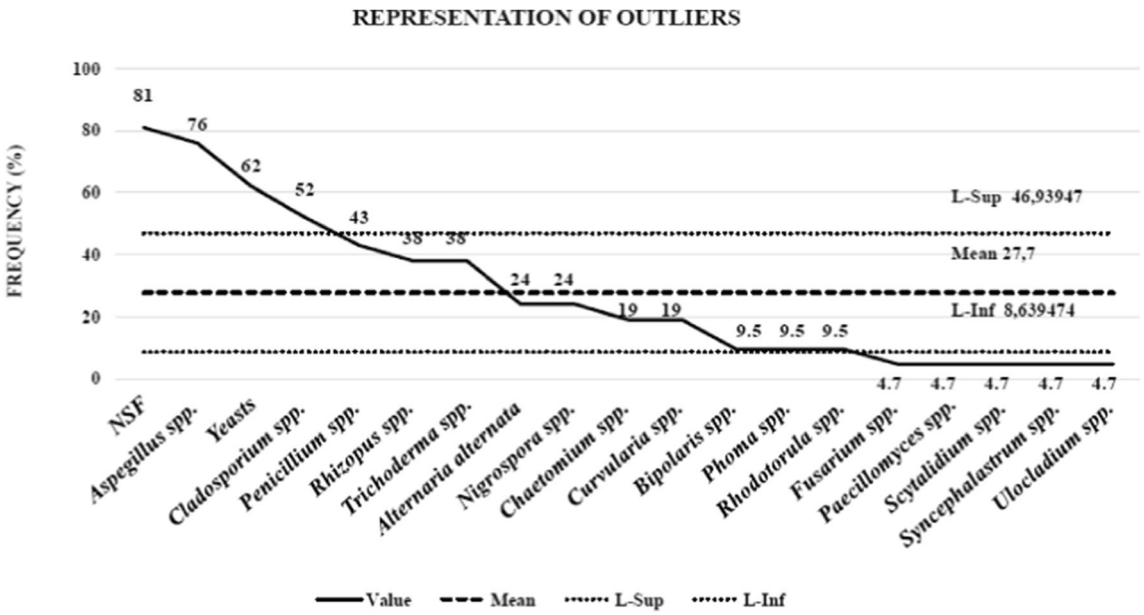


**Fig. 4** Frequency (%) of fungal genera in the total of 21 vehicle filter samples. Note: Quartile 1 = 4.7; Quartile 3 = 43; Interquartile range (IQR) = 38.3

Ross et al. (2004) analysed the indoor air in sites as a public auditorium, a hospital, a company and a shopping centre during the year 2001. The relative frequency of fungi varied according to the genera and sites. The genera that occurred most in the indoor public auditorium were *Penicillium* (57.3%), *Aspergillus* (11.24%) and *Epicoccum* (5.63%). In the hospital

**Table 1** Fungal genera and frequency (%) per sample

| Samples | Fungal genera and frequency per sample (%) |
|---|---|
| n. 1 | *Syncephalastrum* spp. (3%); NSF (3%); yeasts (15%); *Rhizopus* spp. (61%); *Rhodotorula* spp. (12%); *Aspergillus* spp. (36%); |
| n. 2 | *Alternaria* spp. (15%); NSF (24%); *Penicillium* spp. (3%); *Rhizopus* spp. (58%); *Trichoderma* spp. (6%) |
| n. 3 | *Trichoderma* spp. (21%); *Rhizopus* spp. (21%); *Rhodotorula* spp. (48%); *Aspergillus* spp. (6%); |
| n. 4 | Yeasts (34%); *Penicillium* spp. (40%); *Aspergillus* spp. (60%); |
| n. 5 | *Alternaria* spp. (33%); *Cladosporium* spp. (33%); NSF (9%); yeasts (12%); *Penicillium* spp. (7%); *Rhizopus* spp. (3%); *Trichoderma* spp. (3%); *Aspergillus* spp. (12%); |
| n. 6 | *Alternaria* spp. (24%); *Cladosporium* spp. (13%); NSF (36%); yeasts (3%); *Rhizopus* spp. (33%); *Trichoderma* spp. (45%); *Fusarium* spp. (6%) |
| n. 7 | *Cladosporium* spp. (72%); NSF (54%); yeasts (12%); *Phoma* spp. (3%); *Aspergillus* spp. (3%); |
| n. 8 | *Alternaria* spp. (45%); NSF (33%); yeasts (3%); *Trichoderma* spp. (5%); *Ulocladium* spp. (6%); *Aspergillus* spp. (20%); |
| n. 9 | *Alternaria* spp. (3%); NSF (45%); *Nigrospora* spp. (27%); *Rhizopus* spp. (20%); *Trichoderma* spp. (3%); *Aspergillus* spp. (21%); |
| n. 10 | *Cladosporium* spp. (54%); yeasts (21%); *Trichoderma* spp. (10%); *Aspergillus* spp. (15%); |
| n. 11 | NSF (4.5%); *Cladosporium* spp. (10%) |
| n. 12 | *Bipolaris* spp. (2.5%); *Chaetomium* spp. (3%); *Cladosporium* spp. (36%); *Nigrospora* spp. (7%); *Penicillium* spp. (2%); yeasts (46%) |
| n. 13 | *Scytalidium* spp. (7%); *Paecilomyces* spp. (8.5%); *Phoma* spp. (1.5%); *Chaetomium* spp. (8%); yeasts (53%); *Penicillium* spp. 13 (12%); NSF (7%); *Aspergillus* spp. (1.5%); |
| n. 14 | *Cladosporium* spp. (63%); yeasts (1.5%); NSF (3%); *Aspergillus* spp. (3%); |
| n. 15 | *Cladosporium* spp. (18%); yeasts (16%); *Penicillium* spp. (3%); NSF (9%) |
| n. 16 | *Chaetomium* spp. (7%); NSF (18%); *Penicillium* spp. (33%); *Rhizopus* spp. (9%); *Aspergillus* spp. (15%); |
| n. 17 | *Chaetomium* spp. (1.5%); *Cladosporium* spp. (18%); *Curvularia* spp. (3%); NSF (15%); yeasts (60%) |
| n. 18 | Yeasts (20%); *Chaetomium* spp. (4.5%); *Curvularia* spp. (19%); NSF (30%); *Nigrospora* spp. (30%); *Penicillium* spp. (1.5%) |
| n. 19 | *Aspergillus* spp. (24%); *Cladosporium* spp. (50%); *Curvularia* spp. (24%); NSF (32%); *Trichoderma* spp. 19 (10%) |
| n. 20 | *Cladosporium* spp. (37%); *Curvularia* spp. (12%); NSF (35%); *Nigrospora* spp. (23%); *Penicillium* spp. (5%); *Aspergillus* spp. (1.5%); |
| n. 21 | *Alternaria* spp. (4.5%); *Rhizopus* spp. (17%); NSF (21%); *Nigrospora* spp. (4.5%); *Penicillium* spp. (4.5%); *Trichoderma* spp. (1.5%); *Aspergillus* spp. (7.5%); |

the genera found in air were *Aspergillus* (51.56%), *Penicillium* (31.96%), *Microsporum* (3.09%), *Emmonsia* (2.06%) and *Drechslera* (2.06%). In the company, the most frequent fungi genera were *Aspergillus* (59.62%), *Penicillium* (20.51%) and *Myriodontium* (5.13%). In the shopping centre there, was predominance of the genera *Aspergillus* (55.02%), *Penicillium* (20.12%), *Drechslera* (5.33%) and *Epicoccum* (3.55%) in the indoor air samples. A descriptive study was developed by Viegas et al. (2011) to monitor air fungal contamination in one Portuguese maternity and the authors identified 23 species of fungi in indoor air, being the two most commonly isolated the genera *Penicillium* (41.5%) and *Cladosporium* (28.4%). Regarding yeasts, only *Rhodotorula* spp. (45.2%), *Trichosporon mucoides* (51.6%) and *Cryptococcus neoformans* (3.2%) were found.

The maintenance of equipment and the cleaning of air circulation systems in the interiors are important practices to reduce the potential of both chemical and biological contamination (Cartaxo et al. 2007). Ross et al. (2004) pointed out that the lack of cleaning and checking out of the heating, ventilation and air-conditioning systems (HVAC) may allow for microbial growth, which causes rhinitis, bronchitis, pharyngitis, pneumonia, conjunctivitis and keratitis in users.

As seen in modern societies, people spend 90% of their time indoors or inside the home, and it is not surprising that factors contributing to poor indoor air quality are receiving significant attention from researchers, government and public in general (Cerqueira and Guimarães 2017). The influence of AC and heating systems on the levels of airborne fungi inside automobiles has been assessed. Soon after the start of the AC systems, there was an increase in the levels of airborne microbes due to the purging of their pipes and also as a result of the resuspension of accumulated dust inside the cars (Sattar et al. 2017). The Brazilian Resolution n. 09 (2003) published by the National Agency of Sanitary Vigilance defined the technical guidance on IAQ in artificially air-conditioned environments for public and collective use and determines that the presence of pathogenic and toxigenic fungi is unacceptable, according to the Reference Standards. According to Motta et al. (2015), the genera *Aspergillus*, *Fusarium* and *Penicillium* are considered as the most important toxigenic fungi.

It is noteworthy that in the present study the three toxigenic genera mentioned above (*Penicillium*, *Fusarim* and *Aspergillus*) were isolated in the cars filters, since such fungal genera produce secondary metabolites known as toxins or mycotoxins. These results corroborate with data found by Varga et al. (2015) in other occupational environments of coexposure by means of filters of air-conditioning systems in addition to the bioburden, that is, an additional risk factor such as the presence or inhalation of toxic fungal metabolites, the mycotoxins. The authors also observed fungi of the genera *Penicillium* and *Aspergillus* with toxigenic potential in taxicab filters and ordinary vehicles.

### *Aspergillus* isolation and health risks

In the present study, pathogenic *Aspergillus* species such as *Aspergillus* section *Flavi* and *Aspergillus* section *Nigri* isolates were found, as well as*Aspergillus fumigatus*, *Aspergillus clavatus* and *Aspergillus ochraceus*. Figure 5 shows the microscopic aspect of *Aspergillus clavatus* (a), *A. flavus* (b), *A. fumigatus* (c), *A. ochraceus* (d) and *A. niger* (e).

Human infections caused by members of the *Aspergillus* genus are considered as a major health problem worldwide. From among moulds, fungi of the *Aspergillus* species demonstrate highest pathogenicity and the most often isolated species from the indoor air belong to *A. fumigatus* and *A. niger* (Nielsen 2003). As pointed out by Viegas et al. (2018), the identification of *Aspergillus* spp. taxis filters and personal vehicles is a concern, with greater relevance in the case of taxis intended for the transport of patients. The present study demonstrated that *Aspergillus niger* were the most frequent species (43%) isolated in samples filters, as shown by Fig. 6.

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 moulds isolated, and the most frequently identified species was *A. fumigatus* (Gniadek et al. 2010).

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). Many individuals are at higher risk of succumbing to aspergillosis, a condition that encompasses a variety of diseases caused by members of the *Aspergillus* genus, such as pulmonary aspergillosis, that are increasing and pose a significant health risk to the population, especially to immunocompromised individuals (Vermeulen et al. 2015; ARPEM Association 2017). Very often, prior to development of aspergillosis, a patient's oronasal cavity is subject to fungal spore colonisation. A developing fungus located in the lungs (incubation period spans between 2 days to 3 months) results in haemorrhagic infarctions, which cause a further transmission of the infection through the bloodstream to the brain, liver, spleen, kidneys, pericardium or skin (Mortensen et al. 2011).

Among the relevant species and pathogenic potential of fungi found in ICU air conditioners, the most frequent are as the following: *Aspergillus flavus*, that can cause allergic bronchial aspergillosis, pneumonia in immunocompromised patients, external otitis and fungal sinusitis; *Aspergillus fumigatus*, with

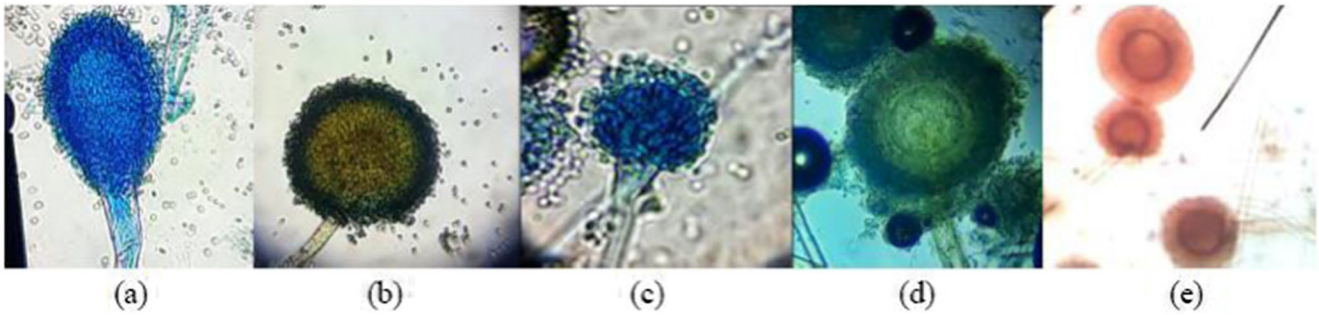


**Fig. 5** *Aspergillus clavatus* (**a**), *A. flavus* (**b**), *A. fumigatus* (**c**), *A. ochraceus* (**d**) and *A. niger* (**e**) isolated in vehicle air-conditioning filters. Source: Prepared by the authors

inhaled mycosis, chronic sinusitis, allergic reactions and chronic inhalatory respiratory tract infections (aspergillosis, aspergilloma) as well as infections of the haematopoietic system, digestive system, genitourinary tract, skeletal muscles and nervous system; *Aspergillus niger* may cause onychomycosis, peritonitis and endocarditis and may also cause infections of the inner and outer ear (otomycosis) as well as pulmonary aspergillosis; *Aspergillus clavatus* (commonly found in soil and animal manure) is allergenic and causes hypersensitivity pneumonitis, an occupational hazard also known as malt-worker's lung; *Aspergillus ochraceusis important* in the context of immunocompromised and asthmatic patients and may be the cause behind antromycosis and onychomycosis (Nadumane et al. 2016; Mobin and Salmito 2006; Moazamand Denning 2017).

However, according to ARPEM Association **(**2017**)** of all*Aspergillus* species, *Aspergillus fumigatus* is the most frequently isolated from animal and human infections, with most significant clinical relevance for humans. *Aspergillus fumigatus* belongs to the *Aspergillus subgenus Fumigati*, section *Fumigati*. Conidial heads are columnar with short, smooth-walled, 300-μm-long conidiophores. Vesicles are broadly clavate, green in upper part, usually fertile on the upper half only, 20–20 μm in diameter. They bear only phialides that are often pigmented green, 6–8 × 2–3 μm. Conidia are green, rough-walled to echinulate, subglobose to globose, 2.5–3.0 μm in diameter, which are easily inhalable and the primary focus of infection in such cases is usually located in the lungs.

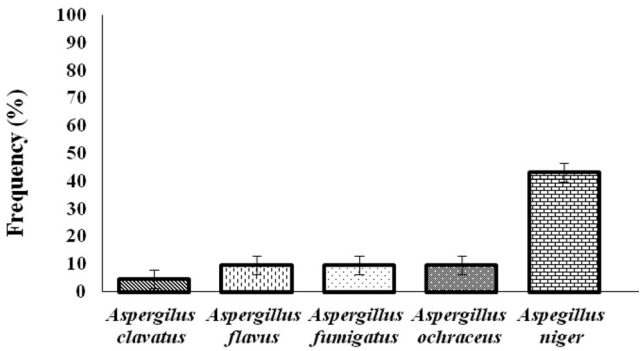


**Fig. 6** *Aspergillus* specimens isolated from air filter samples. Note: Average = 16.66667; Standard deviation = 14.48678; Error = 3.636965

An automobile becomes a necessity in modern life, especially in developed nations, and a significant fraction of the population spends a considerable amount of time in passenger cars on a daily basis. Exposure to biological aerosols can cause numerous adverse health effects including lung impairment and respiratory allergies or infections. Thus, ensuring biologically good air quality inside the vehicle is of vital importance to the health of the in-vehicle occupants. For taxi drivers, such a time could be up to 10 h or more per day (Li et al. 2013).

A study by Vonberg et al. (2010) demonstrated that 67% increase in bacterial counts and even doubling for fungal concentration when the AC turned on with an old filter. However, when the old filter was replaced with a new one, the in-vehicle microbiological air quality was significantly improved. This clearly implied that the old filter was an important source of microbial agents. Since the AC filter is directly exposed to the outside air, those filter-borne biological agents could rapidly reproduce when the humidity level is high, e.g., during or after rain. Another study about the contamination air of taxi vehicles, performed by Viegas et al. (2017) demonstrated the presence of *Penicillium* and *Aspergillus* indicating that preventive and protective measures should be implemented to protect the health of taxi drivers due to occupational risk due to exposure time.

Workers' health should also be taken into consideration for drivers of professional vehicles (transport companies, bus fleets, taxis, etc.), and who spend hours inside automotive cabins under air-conditioning. According to Silva and Gomes (2015), the Brazilian Safety Regulatory Norms and Occupational Health Regulations are mandatory to private and public companies in São Paulo city and determine the ideal conditions and requirements of work environments, as the Normative Instruction n. 01 (2005) that considers as place of work the area where the work activities are performed, including vehicles.

## Conclusions

Control of fungi in the indoor environments has traditionally focused on identifying the source of contamination control, use of filters, cleaning etc. It is necessary to take some

measures to eliminate and prevent fungi in the AC car system. Even when changing the filters, it is important to observe the minimum cleaning frequency for the vehicle air system, such as cleaning the building air conditioners, which involves removing the grilles and diffusers for washing, removing dirt main ducts and their extensions and the application of disinfectant agents inside the ducts, to eliminate the foci of microorganisms.

The limitation of this study is due to the lack of information about the time of use of the filters, which could contribute to future recommendations for the renewal or cleaning of the air circulation system included in the vehicle manufacturer's manual. Future studies are needed, mainly on the dynamics of pollutants and the variation of physical and microbiological parameters together (such as $CO_2$ concentration and fungal growth), as well as the kinetic study of the reactivity of airborne contaminants from internal vehicle environments. The present study demonstrated that the presence of fungi causing respiratory diseases (and potentially toxigenic) deserves the attention of researchers in environmental health and even the auto industry.

The IAQ for vehicles is an emerging research area in Brazil and there is still a number of gaps to be filled regarding vehicles that use air conditioning, types of users (professional or not), and how often filters are changed, for air renewal performed by the ventilation system can eliminate part of the bioaerosols. The filters can be indoor sources of fungal allergens for drivers and passengers in the city of São Paulo. The results of this study demonstrated that vehicle air-conditioning filters are environments favourable to the bioaccumulation of several fungi genera, including toxigenic moulds and the genus *Aspergillus* that cause pulmonary diseases.

The contribution of the present study refers to the originality to be performed in the city of São Paulo and that can be replicated in other capitals with a high rate of atmospheric pollution, since the contamination observed by fungi in the internal air in vehicles may be associated with air quality captured. New standards, studies or Brazilian recommendations on the exchange of air filters, and the frequency of cleaning and disinfection of air conditioners system should be implemented in order to avoid risks to the health of people who spend much of their time in inside passenger vehicles as well as in public transport.

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