The Open Public Health Journal

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RESEARCH ARTICLE

The Survey of Fungal Contamination in the Air Flowing Out of Air Conditioners (Coolers) in a Car

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Abstract:

Background:

Fungi are among the most important microorganisms in the air. The air conditioner (cooler) in a car can be an important reservoir for infectious agents, especially fungal species. The present study aimed to measure the fungal contamination in the air flowing out of air conditioners.

Objective:

In this study 138 cars including 69 Iranian-made cars and 69 cars made by foreign companies were examined.

Methods:

The plating technique was used. In this study, in each car, 2 plates were placed for 5 minutes when the air conditioner was on and also 2 plates were placed for 5 minutes when the air conditioner was off.

Results:

A total of 2442 colonies were isolated. The most common isolated fungi were *Cladosporium spp.* (25.55%), *Penicillium spp.* (6.14%) and *Aspergillus niger* (3.03%). The highest number of isolated fungal elements was observed in the Iranian-made cars during summer season (*P-value*<0.05). Furthermore, the growth rate of colonies on the plates placed on the front and rear seats of cars did not vary considerably (*P*-value > 0.05).

Conclusion:

Air conditioners of Iranian-made cars can be an important source for fungal transmission to the human respiratory system that is important, especially in susceptible people to allergic diseases and patients with immunodeficiency.

Keywords: Air fungal contamination, Air conditioning systems, Cooler, Cars, Epidemiology, Fungi.

Article History	Received: March 16, 2021	Revised: August 20, 2021	Accepted: September 21, 2021

1. INTRODUCTION

The air we breathe is composed of millions, even billions of microscopic bioaerosols. Bioaerosols are small airborne particles (ranging from 0.001 to 100 µm) including microorganisms, as well as fragments and released molecules like endotoxin, 1-3 β-glucans, and spores [1]. After inhalation of these tiny elements, hosts may have no symptoms or may cough up blood or have a fever or chest pain, or symptoms ranging from allergies to life-threatening invasive infections [2 - 4]. The outcome depends on the immune status of the host. During recent decades, pulmonary fungal diseases (consist of fungal colonization, allergy, and infection of the pulmonary tract and lungs) are being diagnosed with increasing frequency, largely because of the increasing size of the population at risks,

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such as patients receiving immunosuppressive therapy, those undergoing bone marrow transplantation or solid-organ transplant (SOT) and those with HIV infection, hematologic malignancies, tuberculosis or cystic fibrosis [2 - 5]. Several human diseases are known to be caused or triggered by exposure to fungi or their metabolites. These include Allergic Bronchopulmonary Mycosis (ABPM), Allergic Fungal Sinusitis (AFS), and Hypersensitivity Pneumonitis (HSP) [5]. The results of a study conducted by Fisk *et al*. showed that the presence of fungal growth is associated with increases of 30–50% in respiratory symptoms such as cough, wheeze, and asthma [6]. Furthermore, results of a study conducted by Baxi *et al*. demonstrated that indoor exposure to fungal elements appears to be associated with increased risk of developing asthma in young children and asthma morbidity in individuals who have asthma [5]. Studies showed that with an increase in temperature and humidity during hot seasons, and an increase in the number of air pollutants, the prevalence and growth rate of fungal agents rise in the environment [7]. Consequently, exposure to high concentrations of spores can cause health effects such as asthma attacks [5]. Among fungal elements, *Aspergillus spp.* are very common in both indoor and outdoor environments. The most important clinical *Aspergillus spp.* are *A. fumigatus, A. flavus, A. terreus,* and *A. niger*. Often the primary infection route for aspergilloses is through inhalation. The diseases caused by *Aspergillus spp.* represent a continuum of clinical forms, including allergies, fungal sinusitis, and invasive aspergillosis [15]. On the other hand, *Cladosporium spp.* contribute to and often dominate in atmospheric bioaerosols. The spores of *Cladosporium spp.* are biological air pollutants that pose a threat to human health. They irritate the respiratory tracts and contain numerous proteins that cause inhalant allergies [16]. Also, *Penicillium spp.* are a part of the normal fungal flora of indoor air. The spores of *Penicillium* genus play a role in the pathogenesis of asthma [17]. Besides, *Nattrassia spp., Cunninghamella spp*., *Aureobasidium spp*., and *Hetrosporium spp.* are frequently occurring in the air environment as bioaerosols and often cause allergic and immunotoxic diseases [18]. The use of air conditioning and ventilation systems in confined spaces such as cars can be a useful method for reducing the exposure of humans to fungal agents and pollutants. In air conditioning systems, filters are installed in the air outlet to prevent the flow of a large volume of pollutants into the car [8, 9]. However, these filters have to be examined and replaced regularly at the recommended intervals because fungal spores settle in them and enter the cabin environment once the air conditioner is turned on [10]. Furthermore, a dirty filter reduces the ventilation system efficiency, increases power dissipation, and reduces the flow of air in the cabins of cars. Since car air conditioners (coolers) are an important source of contamination from fungal spores, we decided to measure the fungal contamination and identify the fungal agents in the air flowing out of these. These are important issues because they provide a rationale for interventions that might be considered for cars in which people are at increased risk for fungal exposure.

2. MATERIALS AND METHODS

2.1. Sampling and Mycological Examinations

This descriptive cross-sectional study was carried out for 6 months (during the summer and autumn of 2019) in northern Iran. We aimed to measure the fungal contamination and identify the fungal agents in the cabins of cars with air conditioners. In this study, samples were collected from a total of 138 cars including 69 Iranian-made cars and 69 cars made by foreign companies using the open plate method. Plates contained Sabouraud Dextrose Agar (SDA) with chloramphenicol (SC, Merck, Germany). It is worth mentioning that half the cars were sampled during summer and the other half were sampled during autumn. Besides, half the cars were sampled in the daytime and the other half were sampled at night. Also, the car owners reported that the filters in half the cars had been replaced less than 6 months ago, while the filters in the other half had been replaced more than 6 months ago.

For sample collection, two plates were placed at the average sitting height (height of 85 cm above the floor seat) on the front seats of the car cabins, while the other plate was placed on the rear seats. Five minutes after turning the air conditioner on, the plate lids were closed. Thereafter, two other plates were placed in the cabins when the air conditioner was on and the windows were opened for 5 minutes [11, 12]. After closing the plate lids and recording the results, the plates were transferred to the laboratory and were stored at the ambient temperature. Afterward, the growth of fungi on the plates was monitored periodically and daily. Any growth obtained was further identified by its rate of growth, colony morphology and lactophenol cotton blue mounts. Slide culture w performed as required [13 - 18].

2.2. Statistical Tests

The data analysis was performed by SPSS software (IBM SPSS Statistics for Windows, Version 21.0, IBM Corp, Armonk, NY, USA). The study was assessed by using standard Chi squared and 95% Confidence intervals (CI). Statistically, *P*-value<0.05 was considered as a significant difference or correlation.

3. RESULTS

Of the 552 plates placed inside the cars when the air conditioners were on and off, a total of 2442 colonies related to 27 fungal genera or species were isolated. The most common isolated fungal elements were *Cladosporium spp.* (25.55%), *Penicillium spp.* (6.14%), *Aspergillus niger* (3.03%), and *Nattrassia spp.* (0.16%). Besides, *Aspergillus nidulans, Cunninghamella*, *Aureobasidium*, and *Hetrosporium* were the least isolated fungal genera with a frequency of 0.08%.

The results of the current study showed that the total numbers of the isolated fungal colonies in the cars made by foreign companies were 946 (36.47%), and the total numbers of the isolated fungal colonies in Iranian-made cars were 1478 colonies (60.52%). This finding showed that type of the studied cars was significantly effective in their fungal contamination (*P-value*<0.05) (Fig. **1A**).

The total number of isolated fungal colonies in the summer season was 1760 colonies (72.07%) and the total number of isolated fungal colonies during autumn was 682 colonies (27.92%) (Fig. **1B**).

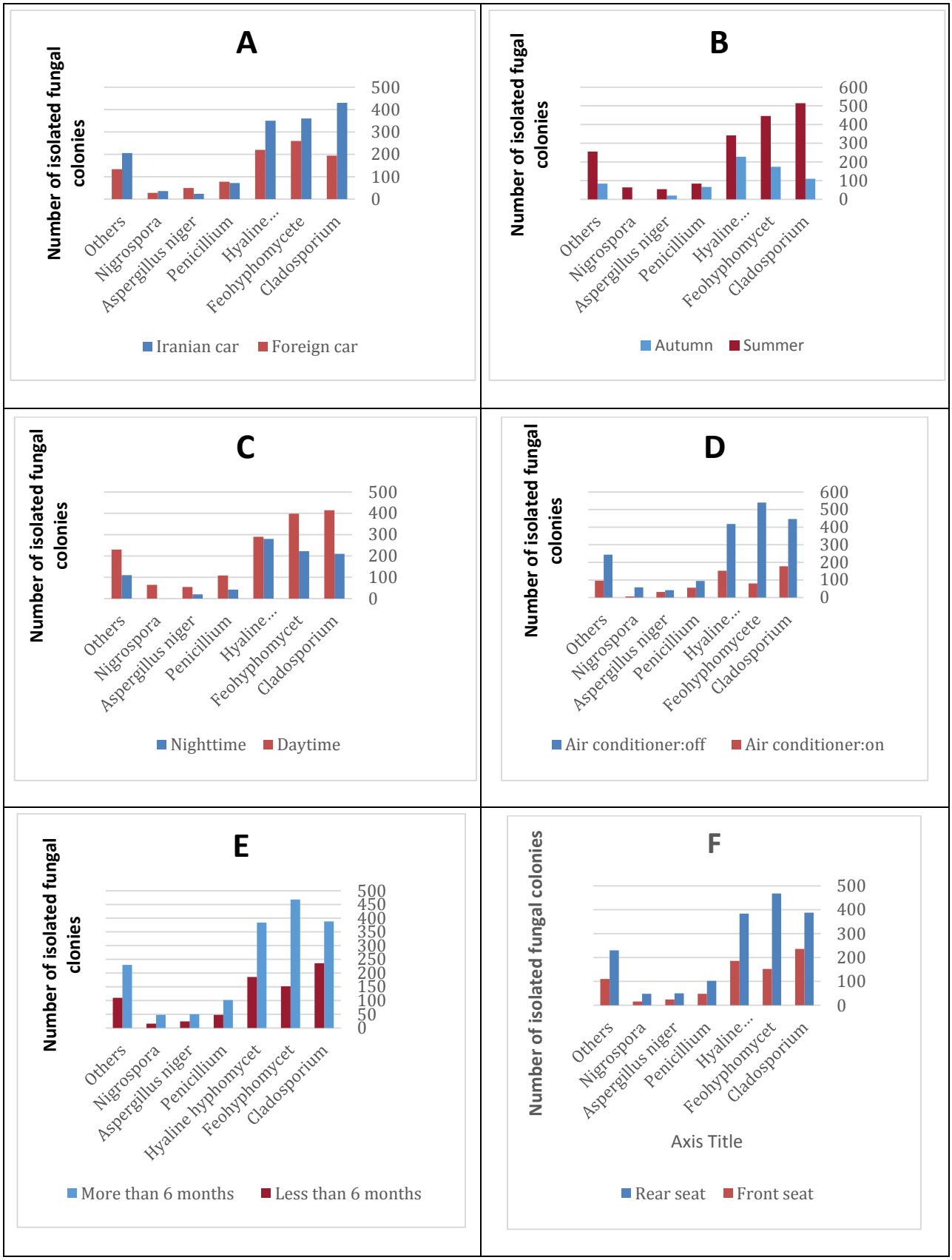

Fig. (1). The frequency of the isolated fungal colonies in the air inside the automobiles according to: the automobile's type (Iranian-made cars; Cars made by foreign companies) (**A**); the season of sampling (autumn, summer) (**B**); the time of day that sampling was done (nighttime, daytime) (**C**); the air conditioner's mood (on-off) (**D**); the time elapsed since the filter's exchange (more than 6 months, less than 6 months) (**E**); the place of the plates on the seat (front seat, rear seat) (**F**).

The total number of colonies obtained in the daytime was 1558 colonies (63.8%) and the total number of colonies obtained in the nighttime was 884 colonies (36.19%), respectively (Fig. **1C**). These results were statistically significant (*P-value*<0.05).

Also, regarding the samples which were collected from the air inside the cabins, with the air conditioners in on and off modes, the highest number of fungal isolated colonies was related to cars in which the air conditioner was in off-mode and the window was open, with 1842 fungal colonies (75.42%), while the number of fungal isolated colonies from cars in which the air conditioner was in off-mode and the windows were closed as well was 600 (24.57%). This finding was statically significant (*P-value*<0.05) (Fig. **1D**).

Furthermore, 1670 colonies were isolated from cars whose filters had not been replaced in more than 6 months, whereas 772 colonies were isolated from cars whose filters had been replaced in less than 6 months (31.61%). This finding was statistically significant(*P-value*<0.05) (Fig. **1E**).

After plating on the front and rear seats of the cars when the windows were closed, there was no significant statistical difference between the numbers of grown colonies resulting from the two states. In other words, the number of colonies identified on the front seats of the cars was 1240 (50.77%), while the number of colonies grown on the rear seats of the cars was 1202 colonies (49.22%). These results suggest that the difference between the numbers of colonies grown in the two states was not statistically significant(*P*-value>0.05) (Fig. **1F**).

4. DISCUSSION

In this study, samples were collected from the air inside the cabins of Iranian-made cars and cars made by foreign companies in northern Iran with a temperate climate, making it a highly suitable area for rice cultivation and farming. In this study, 2442 fungal colonies were isolated. An increase in the growth of fungal agents in this condition is not unexpected due to the mild weather in the north of Iran and high humidity in these areas. Similarly, the results of a study conducted by Zalewska *et al*. (2015) showed that in regions with increased humidity, an increase in the growth rate of fungal agents occurs [19]. In the present study, the most frequently isolated fungal genera were *Cladosporium, Penicillium,* and *Aspergillus niger*. In accordance, Sowiak *et al*. (2018) reported high levels of molds belonging to *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria genera* after a 6-minutes operation of the air-conditioning system relative to the state before turning on [12]. Furthermore, Weryszko-Chmielewska *et al*. (2017) demonstrated that the spores of *Cladosporium spp.* are often present in the air in high quantities. Also, they reported that the highest concentrations of *Cladosporium* spores were associated with intensive farming activities in the surrounding regions, which provide plant residues suitable for the development of these fungi [16]. In this study, the results of sampling during summer and autumn indicated that the number of colonies during summer was more than in autumn (when the air temperature was lower). Similarly, at night the number of fungal agents was less than the daytime due to the decrease in air temperature. Reddy *et al*. (2015) carried out a study during the 12 months of the year 2015 and concluded that the number of saprophytic fungal colonies during the hot months of the year was higher than the number of these colonies during November and February [20]. The studies by Mannaa *et al*. (2012) revealed that the number of bacterial and fungal agents in grains was significantly enhanced with increasing temperature [7]. The study by Belli *et al*. (2006) also showed that the number of fungal agents during the day is higher than the number of fungal agents at night due to the relatively high temperature [21]. Our results showed that the frequency of the fungal agents isolated from the air inside the Iranian-made cars and cars made by foreign companies that were equipped with air conditioning systems was 8147 and 964, respectively. Hence, the Iranian-made cars played a significant role in the dispersion of fungal agents in the cabins of the car (*P-value*<0.05). The lack of competition and supervision in Iranian manufactures and the reduction in the safety and quality of components of Iranian-made cars due to sanctions can be reasons for this finding [23].

The analysis results suggested that when the air conditioner was off and the car windows were closed, the number of colonies was 1842. However, the number of colonies reduced to 600 when the air conditioner was on under the effect of the filters. Moreover, in cars whose filters had been replaced less than 6 months ago, the number of colonies was 772 and in cars whose filters had been replaced in more than 6 months, the number of colonies was 1670. Although filters play a substantial role in reducing the fungal contaminants, failure to their timely replacement increases the propagation of fungal agents. Results of a study conducted by Sowiak *et al*. (2018) showed that when the air-conditioning or ventilation system was switched on, the air contamination with fungal microflora was decreased by 68–78% [12]. A lower level of fungi after the air-conditioning or ventilation was turned on was also observed in the study carried out by Wang *et al*. (2013) [22]. Besides, the resulting *P*- value <0.05 reflects the statistically significant difference between the number of fungal agents in the aforesaid 2 states.

CONCLUSION

In this study, plates containing culture mediums were placed along the air conditioner direction and in the area between the front and rear seats. The analysis results indicated that the number of grown colonies in the plates on the front seats and rear seats was 1240 and 1202 colonies, respectively. Hence, the statistical tests indicated that the difference between the numbers of colonies in the aforesaid two states was statistically insignificant and there was not a significant difference between the numbers of colonies with regard to the position of the plates inside the car cabin. In conclusion, air conditioners can be an important source for fungal colonization and fungal transmission to the human respiratory system. This important finding should be noted, especially in susceptible people to allergic diseases and patients with immunodeficiency and protective strategies should be adopted. Reduced exposure by a variety of interventions primarily aimed at reducing moisture, killing fungi, and removing contaminated materials (especially filters installed in air conditioning systems) must be used to decrease this risk of morbidity.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

STANDARDS OF REPORTING

STROBE guidelines and methodologies were followed in this study.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available within the article.

FUNDING

This work was supported by funding from Tehran University of Medical Sciences, Tehran, Iran (Grant No: 43968).

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Kim KH, Kabir E, Jahan SA. Airborne bioaerosols and their impact on human health. J Environ Sci (China) 2018; 67: 23-35. [http://dx.doi.org/10.1016/j.jes.2017.08.027] [PMID: 29778157]

[2] Rafat Z, Hashemi SJ, Ashrafi K, *et al.* Fungal isolates of the respiratory tract in symptomatic patients hospitalized in pulmonary units: A mycological and molecular epidemiologic study. J Multidiscip Healthc 2020; 13: 661-9. [http://dx.doi.org/10.2147/JMDH.S252371] [PMID: 32801730]

[3] Sasani E, Rafat Z, Ashrafi K, *et al.* Vulvovaginal candidiasis in Iran: A systematic review and meta-analysis on the epidemiology, clinical manifestations, demographic characteristics, risk factors, etiologic agents and laboratory diagnosis. Microb Pathog 2021; 154: 104802. [http://dx.doi.org/10.1016/j.micpath.2021.104802] [PMID: 33741400]

[4] Ardi P, Daie-Ghazvini R, Hashemi SJ, *et al.* Study on invasive aspergillosis using galactomannan enzyme immunoassay and determining antifungal drug susceptibility among hospitalized patients with hematologic malignancies or candidates for organ transplantation. Microb Pathog 2020; 147: 104382. [http://dx.doi.org/10.1016/j.micpath.2020.104382] [PMID: 32663605]

[5] Baxi SN, Portnoy JM, Larenas-Linnemann D, Phipatanakul W, Barnes C, Baxi S, *et al.* Exposure and health effects of fungi on humans. J Allergy Clin Immunol Pract 2016; 4(3): 396-404. [http://dx.doi.org/10.1016/j.jaip.2016.01.008] [PMID: 26947460]

[6] Fisk WJ, Lei-Gomez Q, Mendell MJ. Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. Indoor Air 2007; 17(4): 284-96. [http://dx.doi.org/10.1111/j.1600-0668.2007.00475.x] [PMID: 17661925]

[7] Mannaa M, Kim KD. Effect of temperature and relative humidity on growth of Aspergillus and Penicillium spp. and biocontrol activity of Pseudomonas protegens AS15 against aflatoxigenic Aspergillus flavus in stored rice grains. Mycobiology 2018; 46(3): 287-95. [http://dx.doi.org/10.1080/12298093.2018.1505247] [PMID: 30294490]

[8] Alipour V, Mahvi AH, Rezaei L. Quantitative and qualitative characteristics of condensate water of home air-conditioning system in Iran. Desalination Water Treat 2015; 53: 1834-9. [http://dx.doi.org/10.1080/19443994.2013.870724]

[9] Wu M, Dick WA, Li W, Wang X, Yang Q, Wang T, *et al.* Bioaugmentation and biostimulation of hydrocarbon degradation and the microbial community in a petroleum-contaminated soil. Int Biodeterior Biodegradation 2016; 107: 158-64. [http://dx.doi.org/10.1016/j.ibiod.2015.11.019]

[10] Kwan SE, Shaughnessy R, Haverinen-Shaughnessy U, Kwan TA, Peccia J. The impact of ventilation rate on the fungal and bacterial ecology of home indoor air. Build Environ 2020; 177: 106800. [http://dx.doi.org/10.1016/j.buildenv.2020.106800]

[11] Bardeen CR. General relations of sitting height to stature and of sitting height and stature to weight. Am J Phys Anthropol 1923; 6: 355-88. [http://dx.doi.org/10.1002/ajpa.1330060403]

[12] Sowiak M, Kozajda A, Jeżak K, Szadkowska-Stańczyk I. Does the air condition system in busses spread allergic fungi into driver space? Environ Sci Pollut Res Int 2018; 25(5): 5013-23. [http://dx.doi.org/10.1007/s11356-017-0830-4] [PMID: 29209965]

[13] Abe M, Ogawa Z, Tanuma H, Kume H. [Study of mycological examination methods in clinical laboratories--specimen pretreatment and isolation]. Med Mycol J 2009; 50(4): 235-42. [http://dx.doi.org/10.3314/jjmm.50.235] [PMID: 19942795]

[14] Winn WC. Koneman's color atlas and textbook of diagnostic microbiology. Lippincott williams & wilkins 2006.

[15] Viegas C, Almeida B, Gomes AQ, Carolino E, Caetano LA. *Aspergillus spp*. prevalence in primary health care centres: Assessment by a novel multi-approach sampling protocol. Environ Res 2019; 175: 133-41. [http://dx.doi.org/10.1016/j.envres.2019.05.015] [PMID: 31121528]

[16] Weryszko-Chmielewska E, Kasprzyk I, Nowak M, *et al.* Health hazards related to conidia of Cladosporium-biological air pollutants in Poland, central Europe. J Environ Sci (China) 2018; 65: 271-81. [http://dx.doi.org/10.1016/j.jes.2017.02.018] [PMID: 29548398]

[17] Jussila J, Komulainen H, Kosma VM, Pelkonen J, Hirvonen MR. Inflammatory potential of the spores of *Penicillium spinulosum* isolated from indoor air of a moisture-damaged building in mouse lungs. Environ Toxicol Pharmacol 2002; 12(3): 137-45. [http://dx.doi.org/10.1016/S1382-6689(02)00018-2] [PMID: 21782633]

[18] Kakde UB. Study of biodiversity of fungal bioaerosols in mumbai metropolis. Int J Res BioScience Agri Technol 2014; 2: 196-208.

[19] Zalewska ED, Machowicz-Stefaniak Z, Król ED. Fungi colonizing caraway (*Carum carvi L.*) in different regions of cultivation. Acta Sci Pol Hortorum Cultus 2015; 14: 175-88.

[20] Reddy K, Sarita P, Srinivas T. A study of fungi in air in selected areas of Visakhapatnam city, India. Eur J Exp Biol 2015; 5: 10-4.

[21] Bellí N, Ramos AJ, Sanchis V, Marín S. Effect of photoperiod and day-night temperatures simulating field conditions on growth and ochratoxin A production of *Aspergillus carbonarius* strains isolated from grapes. Food Microbiol 2006; 23(7): 622-7. [http://dx.doi.org/10.1016/j.fm.2005.12.007] [PMID: 16943060]

[22] Wang YF, Tsai CH, Huang YT, *et al.* Size distribution of airborne fungi in vehicles under various driving conditions. Arch Environ Occup Health 2013; 68(2): 95-100. [http://dx.doi.org/10.1080/19338244.2011.650798] [PMID: 23428059]

[23] Mohajer B, Azmin M, Mohebi F, Ahmadi N, Farzadfar F. Low-quality domestic automobiles continue to threaten lives in Iran: Economic instability as the potential contributor. Arch Iran Med 2020; 23(11): 764-5. [http://dx.doi.org/10.34172/aim.2020.101] [PMID: 33220694]