



Characterization and concentration of airborne fungi in public transport vehicles in Istanbul

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Received: 6 April 2021 / Accepted: 17 October 2021 / Published online: 30 October 2021
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Abstract

The study investigated the concentration and distribution features of culturable airborne fungi (CAF) in indoor air for four public transport vehicles (bus, metro, metrobus, and ferry) in Istanbul. The collection of indoor air samples was performed from each transport vehicle in both directions once a month from March 2017 to February 2018. The highest and lowest CAF concentrations measured during the study were determined to be 40–660 CFU/m³ in bus, 20–400 CFU/m³ in metro, 40–360 CFU/m³ in metrobus, and 20–260 CFU/m³ in ferry. It was determined that no fungal colonies were observed on the ferry on the inbound route in March. The findings demonstrated that CAF concentrations in bus were higher compared to the indoor air in other transport vehicles, and there was a significant correlation between fungal levels and the number of passengers ($p < 0.05$; $r = 0.68$) and RH% ($p < 0.05$; $r = 0.43$). In line with the density order of fourteen fungal species determined, *Penicillium* spp., *Paecilomyces* spp., and *Aspergillus* spp. were identified. The results of this study also showed that there were high levels of fungi in public transport vehicles that could affect human health in many sampling periods. Many countries are trying to or have established their national ambient air quality standards, following the WHO Air Quality Guidelines. Unfortunately, Turkey does not have official standards for indoor air quality (IAQ). Therefore, monitor, control, and maintenance of IAQ-related research in such public transport vehicles in Turkey are necessary as much as development of standards related to it.

Keywords Bioaerosol risk · Culturable airborne fungi (CAF) · Indoor air quality · Public health · Public transportation · Istanbul

Introduction

Humans spend the majority of their time indoors (88.9%) while spending limited time outdoors (5.8%) or in vehicles (5.36%) (Nieuwenhuijsen et al. 2007; Matz et al. 2014). In this case, conditions of the indoor environment make a significant contribution to the well-being of humans (Leech et al. 2002). As stated by Jodeh et al. (2018), both indoor and outdoor air pollution represents the main environmental health problem influencing both developing and developed countries, and it may be beneficial to understand and control common indoor pollutants for decreasing the risk of indoor health concerns. Despite the fact that it seems completely safe, this air has high pollutant content and also contains the smoke emitted by cars and industries. As a result, they can be exposed to different pollutants of indoor origin (Kim et al. 2011; Błaszczyk et al. 2017). One of the most important groups of these pollutants is bioaerosols, which have been

Responsible Editor: Amjad Kallel

Presented: the part of the study explained in the manuscript was presented at the 20th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region on the 25–28th of April 2020 in Athens Greece

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defined as ubiquitous suspended airborne particles of biological origin (bacteria, viruses, fungi, toxins, pollen, etc.) (Lee 2011).

It is known that studies on indoor air quality are mostly carried out indoors of common areas such as homes, hospitals, schools, or public institutions (Hargreaves et al. 2003; Kalogerakis et al. 2005; Sarbu and Sebarchievici 2013; Cincinelli and Martellini 2017; Vilcekova et al. 2017; Kim et al. 2018; Xin et al. 2021). As in other countries, the areas selected in the indoor air quality (IAQ) studies carried out in Turkey are generally these common areas (Mentese et al. 2009; Sivri et al. 2016, 2020; Onat et al. 2017; Tan and Erdoğan 2017; Bağcıgil et al. 2019). It is seen that there are very limited studies on the evaluation and characterization of these areas in terms of bioaerosols. For finding the motivation of this study, the research was conducted using the keywords related to the subject [TITLE-ABS-KEY] without any limitations (year, the field of study, article type, etc.) through the Scopus database. In the literature review, 49 studies were identified with the keywords [bioaerosol AND transport AND fungal]. When the search was limited to the keywords [bioaerosol AND public AND transport], it was identified that 36 studies were conducted. However, only 12 of these studies are directly related to public vehicles. When the search is done with the keywords [bioaerosol AND fungus AND vehicle], the total results are 27. It was determined that only 13 of these studies included fungus results. The number of studies carried out in the field of bioaerosols in Turkey under this title, in which such limited studies are conducted, is only 11. In the survey, there are only 5 studies directly related to vehicles and bioaerosol. However, this study was both a special study for Turkey, and it was the first of its kind in terms of indoor air quality in different transportation vehicles widely used in Istanbul.

Fungi are present everywhere in indoor air and leading to poor indoor air quality and implicate health risks to humans (Priyamvada et al. 2017; Xin et al. 2021). Their presence reduces the IAQ of buildings and affect building occupant's well-being, causing health problems (Bongomin et al. 2017; Casadevall 2018). With the widespread use of metro, train, bus, and similar transport vehicles, the ratio of people being exposed to air pollutants has increased, and the evaluation of the health risk related to the indoor environment has become an important public issue. In a study conducted in St. Petersburg, fungal and bacterial aerosols were examined at four metro stations, and it was reported that the airborne fungal concentration increased significantly at all stations, especially in spring (Bogomolova and Kirtsideli 2009). In a study performed in China, it was reported that there was a considerable increase in culturable airborne fungi (CAF) concentration depending on the human density during the morning and evening rush hours, and these values decreased during the day (Cho et al. 2006). In a study carried out in

the Tehran metro, the CAF concentration was found to be 21 CFU/m³ in the aboveground station air and 1402 CFU/m³ in the underground station air (Hoseini et al. 2013). *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., and *Chrysosporium* spp. were reported as genera frequently isolated in the studies conducted in the indoor air of this type of transport vehicles (Bicakci et al. 2001; Tatlıdıl et al. 2001; Kim et al. 2011; Khan and Karuppayil 2012; Hoseini et al. 2013; Arikoglu et al. 2016).

This study aimed to determine indoor CAF concentrations and fungal genus distribution in different types of public transport vehicles (metrobus, bus, metro, and ferry) in Istanbul province. Furthermore, it was aimed to evaluate the relationship between the determined CAF concentrations and the important variables (temperature, humidity, and the number of passengers) measured during sampling, statistically.

Materials and methods

Study area and route description

The study was carried out in the center of Istanbul, which is a city with a population of 14.4 million people and 38 districts situated in the northwest of Turkey (lat. 41° 00' N, long. 28° 97' E, alt. 40 m) (TurkStat 2018). The selection of sampling routes and types of public transport vehicles was planned in such a way to include districts and neighborhoods where people spend a lot of time commuting to work, the population density is high, and there are commercial and residential areas. Along the routes in Avcılar, Bakırköy, Bostancı, Söğütlüçeşme, and Yenikapı neighborhoods, four main types of public transport vehicles representing the out-bound–inbound models in Istanbul and one line for each vehicle type were selected for sampling. The type of public transport vehicle selected in the study and the selected line properties are summarized in Table 1. The routes of the lines are presented in Fig. 1.

Other lines (R2 and R3), except for route 1 and route 4 (R1 and R4), are on the European side of the city. R1 starts from Avcılar station on the European side and ends at Söğütlüçeşme station on the Anatolian side, and R4 starts from Bakırköy pier and ends at Bostancı pier.

The Metrobus line (R1) The Metrobus line (R1) was created by the Istanbul Electricity, Tram, and Tunnel General Directorate (IETT) by combining the metro and bus working principles to prevent heavy traffic congestion on the main roads and provide fast and comfortable transportation and provided service to approximately 3,500,000 people daily with 6169 buses in 2018. The most significant difference between metrobus and bus is that metrobus goes on a separate road

Table 1 Public transport vehicles and routes selected for sampling

Transport vehicle	Line	Route length (km)	Route duration (one way) (min)	Number of stops	Daily number of trips	Line route
<i>Metrobus</i>	R1	42	63	33	72	The express road parallel to the D100 highway
<i>Bus</i>	R2	20	50	39	42	The bypass of the D100 highway
<i>Metro</i>	R3	26.8	35	18	46	Commercial and residential areas
<i>Ferry</i>	R4	20	50	1	69	Along the coastline of the city

R1 Metrobus (34 AS; The Avcılar–Söğütliçeşme line), R2 Municipality Bus (76B; The Avcılar–Bakırköy line), R3 Metro (M1; The Airport–Yenikapı line), R4 Istanbul Sea Bus–IDO, (The Bakırköy–Bostancı line) were selected for indoor sampling

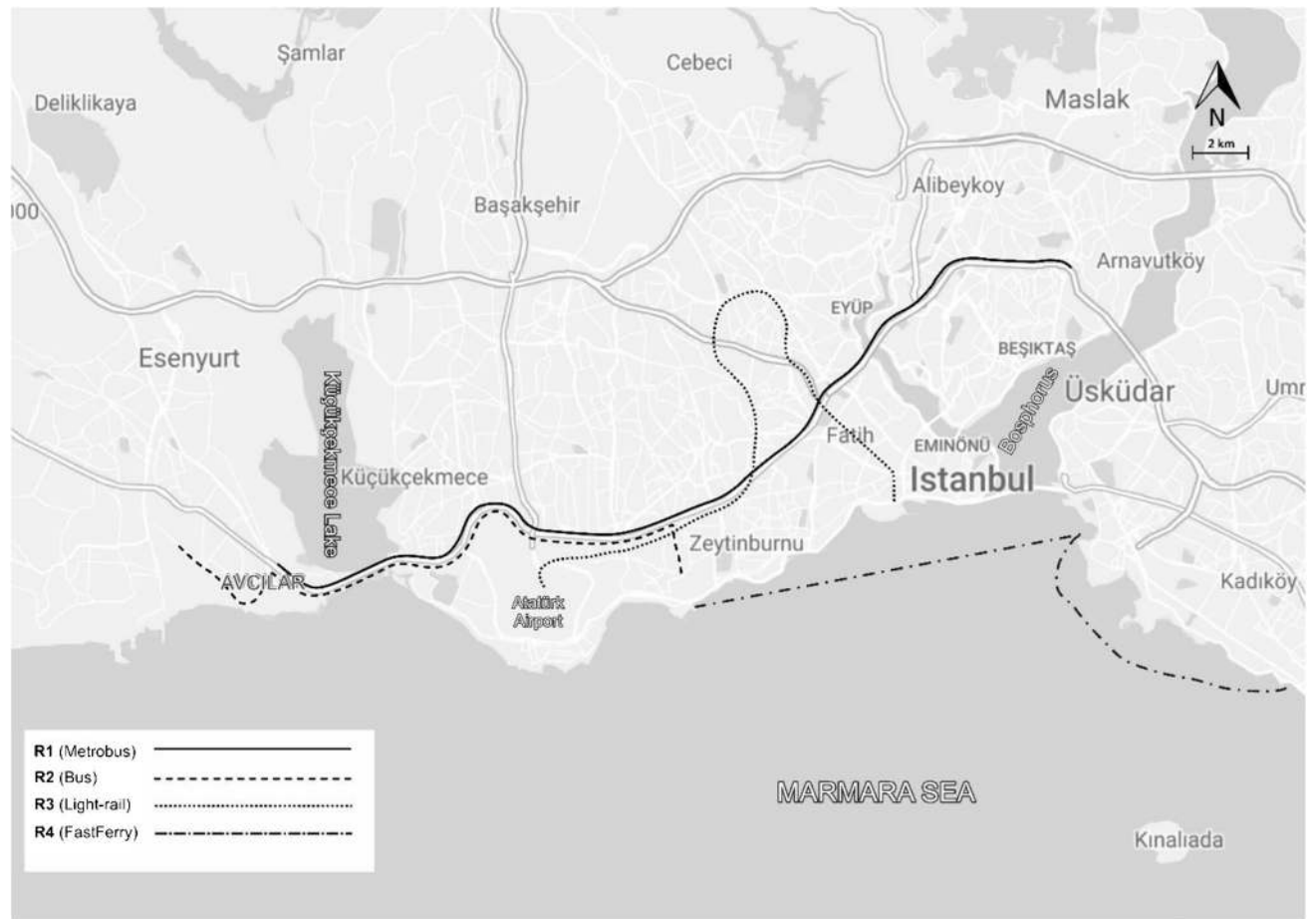


Fig. 1 Study area and routes for each transport vehicle

independent of traffic, just like rail systems. Since metrobus uses the express road parallel to the D100 highway, it is not affected by the traffic density on the D100 highway, and its route takes a shorter time. The metrobus is one of the most preferred transport vehicles with a daily capacity of 700,000 passengers (IETT 2018).

The bus line (R2) The bus line (R2) starts from Avcılar district on the European side and ends in Bakırköy district. In

this type of public transport vehicle, which is a classical bus line, passengers get off and on at each designated stop.

The metro line (R3): the metro line (R3) extends along commercial and residential areas, consists of 7 tunnels and 11 aboveground stations, and 400,000 passengers are transported daily.

Ferries (R4) Ferries (R4) carry an average of 160,000 passengers per day in Istanbul with 52 vehicles. Bakırköy–Bostancı

route, which is preferred in the current study, is the longest route running on the Anatolian-European side, and it picks up passengers from Bakırköy pier and unload them at Bostancı pier without stopping at another pier during the trip.

Metrobus and buses have diesel engines, and mechanical ventilation is performed in these vehicles. In buses, windows can also be opened, and natural ventilation can also be provided. The metro is powered by electricity and mechanically ventilated. The ventilation of ferries is provided by cooling and heating air conditioning systems.

Selection of sampling location

Indoor air sampling was performed once a month for 12 months between March 2017 and February 2018 from four transport vehicles. To ensure the homogenization of sampling days, the third week of each month was preferred. Parallel sampling was performed on the outbound and inbound routes of each transport vehicle.

Sampling was carried out at the predetermined fixed points in each transport vehicle. These places were determined as the first row of seats opposite the middle door in metrobus (R1) and bus (R2), between the second and third rows of seats from the front door in the second wagon of train in metro (R3), and the first row of seats opposite the main door in ferry (R4). To minimize the air inlets and outlets in the sampled environment during sampling, stops with the longest distance between two stations were preferred. Before sampling, care was taken to avoid natural or mechanical ventilation in the environment. After air samples were collected, the sides of petri dishes were taped to keep the lids closed and placed in sample carrying bags. To ensure suitable long-term conditions (+4 °C), petri dishes in the carrying bag were supported by cool packs and were delivered to the laboratory as soon as possible (within a maximum of 6 h).

Sampling and measurement equipment

To identify the concentration of CAF, the “Active Sampling (Impaction) Method” was employed (Mandal and Brandl 2011). To this end, an AES Sampl’air (Aes Laboratoire Sampl’air Lite-France) Air Sampler Device, consisting of 258 holes having a diameter of 0.7 mm and adjusted in such a way to draw 50 L of air in a minute, was utilized. The British Health Protection Agency tested and validated the air sampler following ISO 14698–1 (URL 1: 2003; URL 2: 2018). Before air samples were collected, the disinfection of the device’s top cover with alcohol (70%) was repeated for every sampling area to prevent the risk of contamination between sample collections. To perform sampling, the

device’s position was fixed at a minimum human breathing level of 1.5 m and at a 45° angle to the indoor environment’s center. During sampling, the measurement and recording of the number of passengers in a 2-m radius, relative humidity, and temperature were performed.

Sampling was carried out between 10:00 and 12:00 a.m. when passengers commuted actively in public transport vehicles in Istanbul. Simultaneously with air quality sampling, the measurement of the temperature (T °C) and relative humidity (RH, %) of the sampling section was performed in situ at the identical sampling point by utilizing temperature and humidity meters (Fluke 971).

Isolation and identification of fungi

Potato dextrose agar (PDA, HiMedia, M096) was utilized for sampling. The sampled PDA plates were incubated at a temperature of 25 ± 1 °C for a period of 7 days, and the count of colonies was performed. Various colonies formed in the PDA, examined under a microscope and identified on the basis of genus level (De Hoog GS et al. 2001). The recording of counts for all incubation results was performed by averaging. In all samples, CAF values were expressed as “CAF concentration CFU/m³” (colony forming units (CFU) per cubic meter of air) by utilizing the Feller correction table (URL 2).

Statistical analysis

The sectional properties of fungal loads (CFU/m³) were found by computing the similarity index of the sections. The similarity between the sections with regard to fungal loads was computed by employing the Bray–Curtis similarity index [$\log(x + 1)$] using the MultiVariate Statistical Package (MVSP 3.0) software. Due to the difference in the amount of the data acquired from measurements in the sampling sections for every parameter, an average of the values measured in every sampling section on every sampling day was taken for factor analysis (Greenacre and Primicerio 2013). In addition, as in the researches on indoor air quality, statistical results related to the parameters were presented in the relevant headings.

Results and discussion

A lot of countries have set their national standards for the quality of ambient air in line with the WHO Air Quality Guidelines. However, there is an important gap in broad legally binding force on indoor air quality standards, not to mention that on cabins of vehicles (Nehr et al. 2017). In Turkey, to the present day, the relevant standards or norms related to indoor environments have

been promulgated municipality-wide, e.g., the hygienic standard for metro and metrobus and the indoor environmental pollution control of civil corporation buildings. The sampling was determined according to the literature and current national standards and then interpreted with the measured data. The parameters measured in four public transport vehicles between March 2017 and February 2018 in this study are presented in Table 2. It was observed that fungal concentrations in the samples taken from different vehicles varied according to the transport vehicle, outbound–inbound routes, and the season when sampling was performed. Among the four transport vehicles in the study, the CAF concentration measured as 660 CFU/m³ in bus on the inbound route in June was determined as the highest value of the study. No fungal colony was observed in the air sampling performed on ferry on the inbound route in March. Apart from this, the CAF concentrations determined in the sampling performed on the ferry outbound route in May and again on the outbound route in metrobus in August were recorded as the lowest values of the sampling with a value of 20 CFU/m³. When the mean CAF concentrations were evaluated on the outbound routes by seasons, it was revealed that the highest CAF concentration among all vehicles was in metro with 340 CFU/m³ in spring, and the lowest CAF concentration was 55 CFU/m³ in ferry in autumn. On the inbound route, the highest mean CAF concentration by seasons was 430 CFU/m³ in bus in summer, and the lowest mean CAF concentration was detected to be 95 CFU/m³ in ferry in winter.

Variation of fungal concentrations according to environmental parameters

In this study, upon examining the minimum and maximum temperature ranges in vehicles, they were determined to vary between 14.9 and 33.4 °C in metrobus, 11.7 and 33.3 °C in bus, 16.6 and 29.4 °C in metro, and between 16.5 and 29.1 °C in ferry. Although the determined temperature values seem to be within the normal range, it was observed that the interior temperature of the vehicle was below room temperature (22–25 °C) on the outbound and inbound routes of all transport vehicles in winter. The CAF concentration determined in metro, metrobus, bus, and ferry in winter was 100 CFU/m³, 90 CFU/m³, 85 CFU/m³, and 75 CFU/m³, respectively, and was determined to be lower compared to other seasons.

It was observed that the average temperature values of Istanbul for the summer and autumn seasons were above the seasonal norms in the entire region compared to the previous years (URL 3), and this temperature increase caused a significant increase in the CAF concentration in the summer and autumn seasons compared to other seasons (Fig. 2). In the stacked column graph in Fig. 2, the contributions of each transport vehicle to the whole on the outbound–inbound route are given. When the contribution of each vehicle to the total is examined, the dominance of metro in the spring period and bus in the summer period on the inbound route is remarkable.

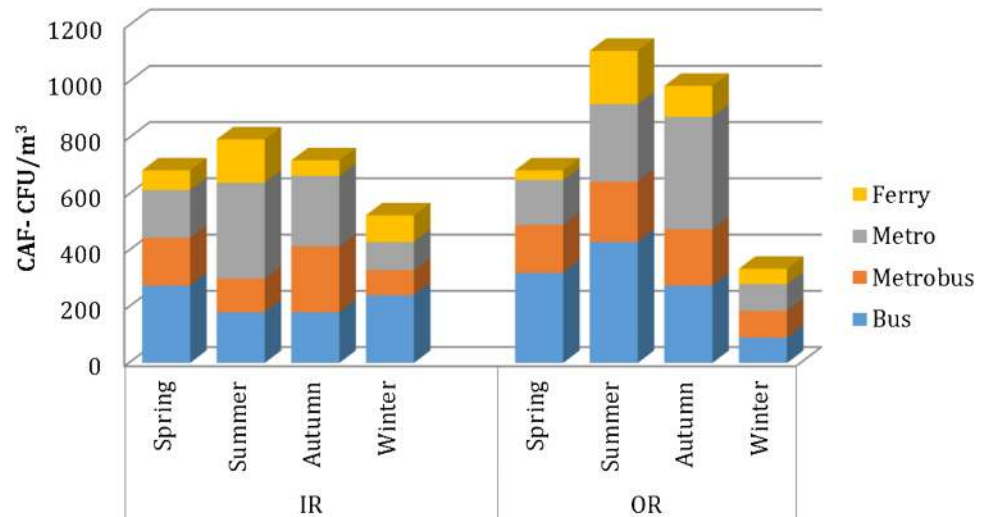
Many studies have stated that bioaerosol concentrations are strongly correlated with seasonal temperature changes,

Table 2 Distribution of environmental parameters in four transport vehicles

Vehicle	Sampling	Outbound passenger	Inbound passenger	Min–max	RH (%) OR	RH (%) IR	Min–max	Temp (°C) OR	Temp (°C) IR	Min–max
Bus	Spring	25	45	11–50	47.0	47.0	33.6–54.3	24.7	25.6	16.3–28.2
	Summer	15	25	15–50	54.4	46.8	37.8–56.6	28.8	26.7	23.1–33.3
	Autumn	20	30	15–30	56.0	55.7	41.7–68.3	23.8	24.2	14.7–31.4
	Winter	25	40	15–70	47.2	47.8	32.4–63.5	19.2	17.5	11.7–23.2
Metrobus	Spring	55	75	50–90	52.0	49.0	31.7–72.8	20.0	24.8	14.9–33.4
	Summer	90	85	50–150	62.4	60.6	51.1–77.2	27.5	26.4	20.8–30.2
	Autumn	45	130	50–150	65.6	57.4	49.6–74.1	22.3	24.7	19.9–26.8
	Winter	90	110	80–120	57.6	53.1	41.2–71.0	19.8	20.0	16.9–21.8
Metro	Spring	60	60	35–90	58.4	59.1	36.4–64.5	21.8	23.0	18.7–24.7
	Summer	55	75	50–80	56.7	58.6	48.4–74.6	26.6	26.1	22.7–29.4
	Autumn	85	75	60–100	57.1	57.5	47.9–68.7	23.9	22.3	21.7–27.5
	Winter	65	70	55–90	51.1	51.3	41.3–60.1	19.0	17.8	16.6–24.3
Ferry	Spring	220	150	100–350	57.7	56.1	39–70.9	21.4	21.2	17.8–24.3
	Summer	95	140	60–250	58.4	61.5	46.6–72.4	25.1	26.2	23.5–29.1
	Autumn	125	170	100–225	66.0	62.2	59.1–71.8	23.1	24.4	18.7–27
	Winter	70	90	25–120	51.7	46.4	40–58.6	19.7	19.9	16.5–22.2

IR inbound routes, OR outbound routes, T °C temperature, RH% relative humidity

Fig. 2 Seasonal distribution of CAF concentrations. IR, inbound routes; OR, outbound routes



and generally low CAF concentrations are detected in the winter months, and high CAF concentrations are detected in the summer months (Ren et al. 2001; Lee and Wan 2005; Liao and Luo 2005; Aydogdu and Asan 2008). The study conducted at four metro stations in St. Petersburg reported that airborne fungal concentration increased significantly in parallel with the temperature increase in spring (Bogomolova and Kirtsideli 2009). Kim et al. (2020) stated that the fungal concentrations in summer were higher than those determined in autumn, and they were 252.0 CFU/m³ in metro, 45.1 CFU/m³ in train, and 111.9 CFU/m³ in bus. On the contrary, Hernández et al. (2014) stated that although the bacterial and fungal concentrations determined in spring were higher than in winter, this difference was statistically insignificant. Heo and Lee (2016) indicated that fungal concentration in the air was not affected by seasonal changes. Hernández et al. (2014) stated in their study that although the bacterial and fungal concentrations determined in spring were higher than in winter, this difference was statistically insignificant.

In this study, the determination of the low CAF concentration in winter and the high CAF concentration in summer was parallel with temperature change. This result is consistent with many studies on the subject (Ren et al. 2001; Lee and Wan 2005; Liao and Luo 2005; Aydogdu and Asan 2008; Bogomolova and Kirtsideli 2009). The distribution of the CAF concentrations determined in transport vehicles by seasons is presented in Fig. 2.

It has been stated that fungi are well adapted to the environment both indoors and outdoors, especially in humid areas (Lin and Li 2000). It has been reported that fungal formation increases in indoor environments where the humidity rate is above 70% (Çobanoğlu et al. 2005; Çobanoğlu and Kiper 2006), and a positive correlation has been found between relative humidity and temperature and

CAF concentration at metro stations (Cho and Paik 2009; Ghosh et al. 2011; Hoseini et al. 2013; Wang et al. 2013). CAF concentrations were measured in air samples of different environments, and the effect of humidity was evaluated (Mentese et al. 2009; Sivri et al. 2020). A study conducted in the home environment stated that fungal concentrations were also high in kitchens and bathrooms with high humidity (Mentese et al. 2009), while a study carried out in a primary school revealed that the presence of high relative humidity in indoor environments increased the formation of fungi (Sivri et al. 2020).

In the present study, low humidity rate and CAF concentrations were determined in all transport vehicles in winter. The determination of low humidity rate and low CAF concentration in winter is similar to the previous studies (Sowiak et al. 2018; Pastuszka et al. 2000; Lin and Li 2000; Jones and Harrison 2004; Chang et al. 2019). In this study, although the humidity rates in different transport vehicles differed according to seasons, they did not show a correlation with the CAF concentrations determined in other transport vehicles other than bus. However, as a result of the statistical analysis of the data, CAF concentrations in bus were found to be higher compared to the indoor air in other vehicles, and there was a significant correlation between fungal levels and RH% ($p < 0.05$; $r = 0.43$).

The correlation between CAF concentration and passenger density in the vehicle

In this study, the lowest CAF concentration (85 CFU/m³) was detected in a bus with 70 people with high passenger density in winter. Although the maximum number of passengers in metrobus was 150 in both summer and autumn, the average CAF concentrations of seasonal values were determined as 170 CFU/m³ and 220 CFU/m³, respectively. The

highest CAF concentration for the same period was measured from a metrobus as 360 CFU/m³ in July. Although the number of persons in this vehicle was equal in summer and autumn, it was determined that CAF concentrations were variable.

While the maximum number of passengers in metro was 100 in autumn, the highest CAF concentration was found to be 400 CFU/m³. Although the maximum number of passengers on ferry was 350 in spring, the highest CAF concentration reached 100 CFU/m³ in April.

Numerous studies have indicated a correlation between airborne bioaerosol and human activity. These studies have stated that the indoor CAF concentration is significantly affected by human population and human activity, and high human density can resuspend dust and cause higher fungal levels in indoor environments (Cho et al. 2006; Ayanbimpe et al. 2010; Kim et al. 2011; Hoseini et al. 2013; Hwang et al. 2016; Onat et al. 2017). Heo and Lee (2016) stated that the effects of seasons and human density on the fungal concentration in underground train stations were low. The data acquired in the current research demonstrated that bus and metrobus have the highest concentration levels during certain periods. The high CAF concentration determined in the bus indoor environment can be attributed to the number of passengers in the bus and the low vehicle interior airflow. Although the number of passengers on ferry was more than 100 in each sampling period, a lower CAF concentration was revealed compared to other transport vehicles. This result can be associated with the new interior equipment of ferries and the modern ventilation system.

The findings demonstrated that CAF concentrations in bus were higher compared to the indoor air in other vehicles, and there was a significant correlation between fungal levels and the number of passengers ($p < 0.05$; $r = 0.68$).

Correlation between CAF concentration and vehicle interior cleaning and disinfection

One of the most important factors affecting the number of airborne microorganisms in the indoor environment is disinfection protocols performed in that environment (Sivri et al. 2020). According to the information provided by the IETT General Directorate regarding the vehicles sampled in this study, it was stated that routine internal and external cleaning was performed at the end of each working day in metrobus, bus, and metro; detailed cleaning was done to vehicles taken to the parking area once a week, and they were disinfected by special teams once a month (IETT 2020). It was indicated that simple surface cleaning materials (e.g., sodium hypochlorite and liquid detergents) were preferred for cleaning, and the microorganism load of the vehicle interior air of 5–10 CFU/m³ was far below the limit determined

by the WHO (500 CFU/m³) owing to the applied cleaning and disinfection process (IETT 2020).

Despite regular cleaning and disinfection in metrobus, bus, and metro indoor environments, the CAF concentrations determined in these transport vehicles were found to be in the range of 150–1000 CFU/m³, which is considered risky for human health in some periods (Lieberman et al. 2006). The CAF concentration increase rates were different in the sampling performed during the day, and a considerable increase was detected in some samples. The notable CAF concentration increases in this study were determined as the CAF concentration of 80 CFU/m³ on the outbound route in metrobus in July and 360 CFU/m³ on the inbound route, the CAF concentration of 120 CFU/m³ on the outbound route in bus in April and 520 CFU/m³ on the inbound route, the CAF concentration of 120 CFU/m³ on the outbound route in metro in November and 400 CFU/m³ on the inbound route. In the IETT operating system, cleaning and disinfection are performed at the end of the day. In this study, it was thought that the low level of CAF concentrations in the first air samples might be due to sampling after cleaning and disinfection, and the noticeable increase in CAF concentrations, especially on the inbound routes, might originate from the lack of any other disinfection process during the day. The increase in CAF concentration indicates the need to review the frequency of cleaning in vehicles.

The Directorate of the Turkish Maritime Lines stated that the daily routine cleaning of ferries was done at the end of the day, detailed cleaning and disinfection were applied once a week, and simple surface cleaning agents not affecting human health adversely (non-allergic) were used in cleaning. This study determined low CAF concentrations in ferries compared to other transport vehicles. The CAF concentrations detected in ferries on the outbound and inbound routes in spring, summer, and winter months were close to each other, and increases on the inbound routes in October and November drew attention. The close CAF concentrations determined on the outbound and inbound routes in ferries may be due to the long time interval between trips and the low number of trips.

The correlation between CAF concentration and natural and mechanical ventilation

Studies have not detected strong connections between indoor air pollution caused by fungi and air conditioners. However, it has been determined that the longer operation of the ventilation system causes an increase in the fungal concentration in buses (Pastuszka et al. 2000; Sowiak et al. 2018). It has been stated that the indoor air quality and the amount of particles in vehicles are quickly balanced with the outdoor environment when windows or doors are opened. However, it has been indicated that the

situation varies depending on many different factors for vehicles with completely closed windows and doors, and the best air quality is provided when the air conditioner and fan are turned on at the same time with regular disinfection (Xu et al. 2018). In their study conducted in 2010, Vonberg et al. determined that when the air conditioning (AC) was turned on with an old filter, the fungal concentration doubled.

The buses and metrobus sampled in this study are vehicles with a similar structure. The most significant difference in terms of ventilation is that windows are designed to be opened in buses, while the ventilation in metrobuses is completely performed by air conditioners, and windows cannot be opened. The average CAF concentration (300 CFU/m³, 305 CFU/m³) determined in buses in the spring and summer months was higher than the average CAF concentration (170 CFU/m³) detected in metrobuses in spring and summer. In this study, it was thought that the reason for determining the lower CAF concentration in metrobuses compared to buses was that the windows in metrobuses were designed not to be opened and that the vehicle interior air conditioning was provided with air conditioners in both summer and winter months.

Among the transport vehicles, high CAF concentration values in summer and autumn (310 CFU/m³, 325 CFU/m³) were found in metro. Since some of the stops in metro are located underground and some are above the ground, natural ventilation occurs, especially during the waiting periods at stops, and mechanical ventilation is provided underground and during travel. It was thought that the high CAF concentration detected in metro could be caused by the old ventilation used in transport vehicles and the natural ventilation by opening doors at the stops (Awad 2002; Hoseini et al. 2013; Yukselen et al. 2013).

Since there was no relationship with the outdoor environment during the trip on ferries, it was determined that indoor heating, ventilation, and air conditioning systems were the main source of contamination considering air quality, and disinfection performed on the ventilation system significantly affected air quality (Webster and Reynolds 2005).

In this study, it draws attention that the average CAF concentration (170 CFU/m³) found in ferry in summer is higher than the average CAF concentrations determined in ferry in other seasons. This result is correlated with the study results obtained by Sowiak et al. (2018), Pastuszka et al. (2000), Vonberg et al. (2010), and Webster and Reynolds (2005). This suggests that air conditioners operated at high capacity for a long time due to the increasing temperature in the summer months may cause a bioaerosol increase in the indoor environment.

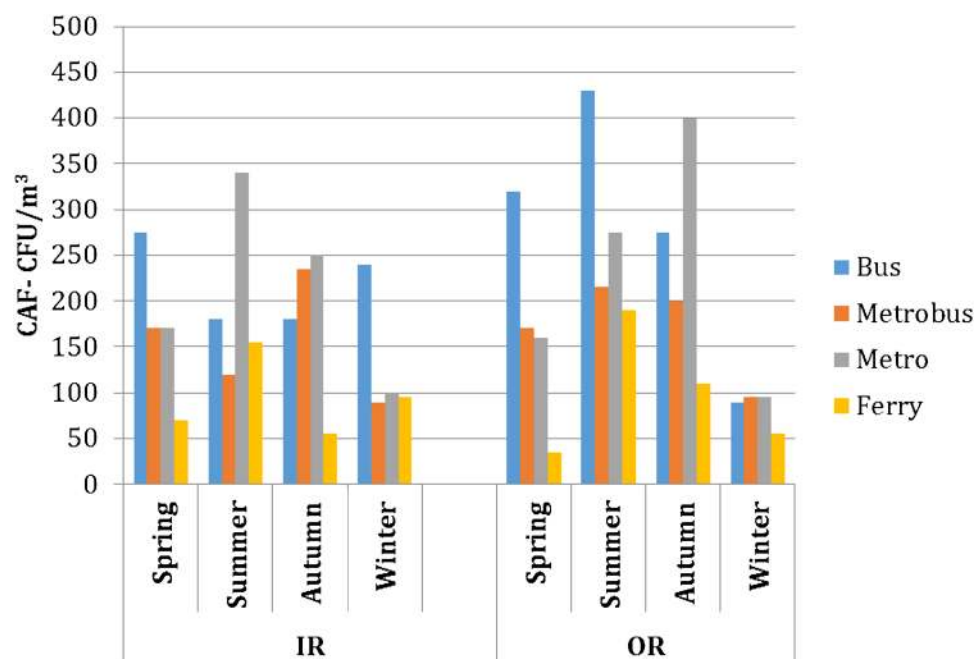
Change in CAF concentrations according to vehicles' outbound and inbound routes

Upon examining the CAF concentrations on the outbound and inbound routes of vehicles in this study, there was usually an increase in the CAF concentration on the inbound route. According to transport vehicles, CAF concentration values increased in all seasons, except winter, in bus, only in summer in metrobus, in autumn in metro, in summer and autumn in ferry. The change on the inbound route during the summer and autumn months and the difference between the two routes were also found to be statistically significant ($p < 0.05$; $r = 0.429$). The CAF change according to vehicles' outbound and inbound routes is presented in Fig. 3. In the literature review, no study in which separate samples were taken for outbound and inbound routes could be found. Since there was no similar study, it was not possible to compare these data. Although it is not an indoor air quality study, Tan and Erdoğan (2017) examined surface swab samples taken from tramway, metrobus, and bus in Istanbul in the morning and evening periods. Similarly to our study results, it was determined that the number of fungi isolated in the samples collected in the evening was higher than the samples taken from the tramway and metrobus in the morning.

Furthermore, the seasonal similarity analysis of the outbound and inbound routes of all vehicles was applied, regardless of the vehicle difference in Fig. 4. It is observed that the CAF concentrations obtained in the winter months are clustered, especially on the outbound route. On the outbound route, the CAF data, except for ferry, in autumn and summer are at a statistically significant distance from the other seasons. The fact that the values of ferry always took low values caused them to cluster within themselves outside the winter season (shown with the blue arrow), and they were at a statistically significant distance from the high values of bus (shown with the red arrow). On the inbound route, just like the outbound route, it is observed that the CAF concentrations obtained in the winter months in all transport vehicles are clustered. It is remarkable that the spring and summer seasons take close values, except for ferry, and there is a significant distance of metro and bus from ferry.

Figure 5 presents the similarity analysis of monthly changes of transport vehicles. The remarkable situation in all transport vehicles is that there is clustering in CAF concentrations in the winter months. Significant groups were formed in terms of similarity in bus and metrobus due to the high values in CAF concentrations for the spring and autumn months. In ferries with the lowest values, August and September are at a significant distance compared to other months. Uniform data resulting from the increase in the use of metro and bus in autumn can be observed as statistically significant similarity in Fig. 5.

Fig. 3 Distribution of CAF concentrations according to vehicles' outbound and inbound routes. IR, inbound routes; OR, outbound routes



Airborne fungal identification

The fungi isolated in the study were also identified at the genus level. The identified genera were *Alternaria* spp., *Artrographis* spp., *Aspergillus* spp., *Candida* spp., *Cephalosporium* spp., *Chaetomium* spp., *Cladosporium* spp., *Helminthosporium* spp., *Malassezia* spp., *Mucor* spp., *Paecilomyces* spp., *Penicillium* spp., *Scopularis* spp., and *Trichoderma* spp.

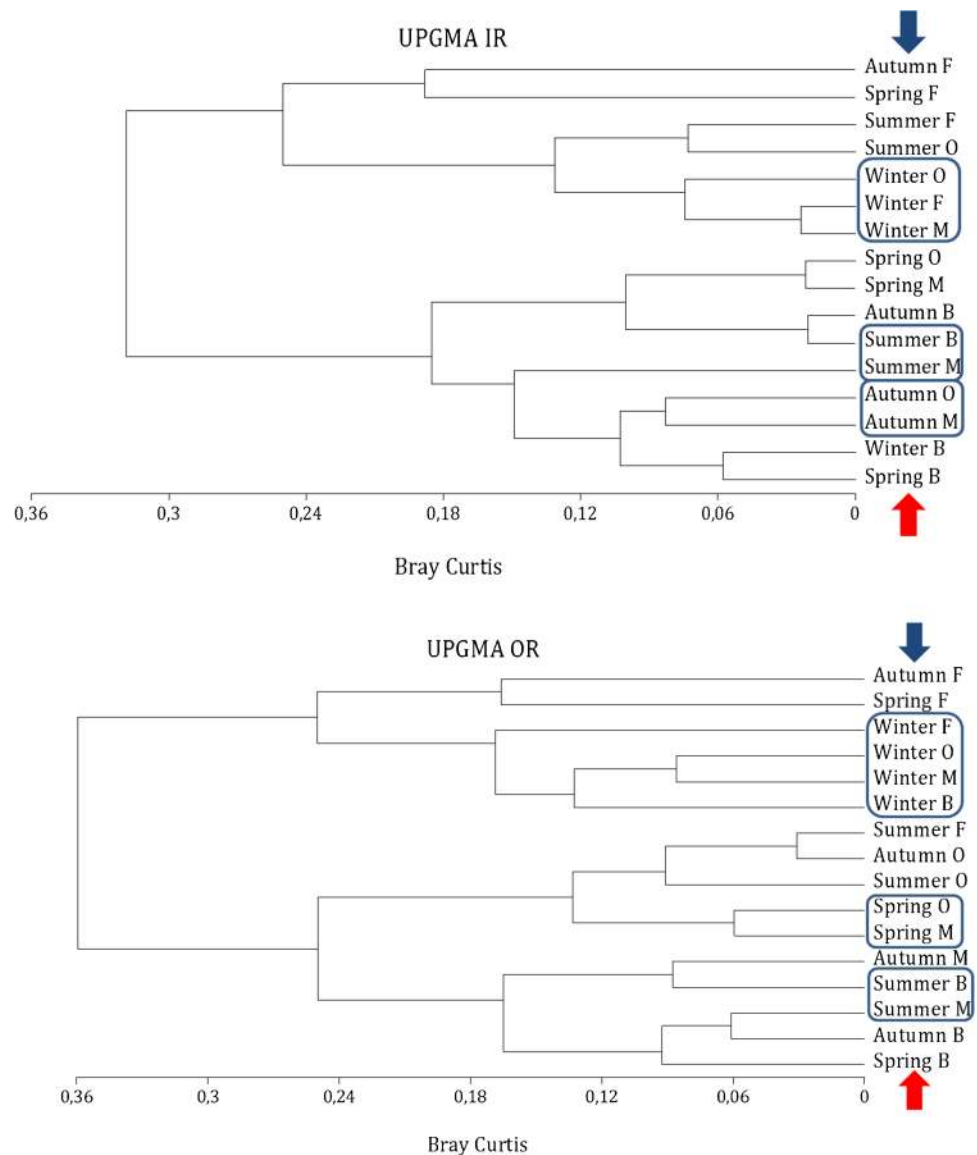
Researchers studying airborne fungal identification have worked with different indoor air samples. Studies carried out in different environments such as school indoor environments (Jo and Seo 2005; Aydoğdu and Asan 2008), museum indoor air (Chen et al. 2010), offices (Burge and Rogers 2000), university campus air (Stryjawska et al. 2007; Lou et al. 2012) determined that the dominant fungal genera identified were usually *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp.

Similar dominant genera draw attention in studies performed in transport vehicles. The dominant fungal genera were determined at metro stations to be *Penicillium* spp., *Cladosporium* spp., and *Aspergillus* spp. (Kim et al. 2011; Kim et al. 2020; Madhawal et al. 2020) and by Hoseini et al. (2013) on the train indoor and outdoor air in the Tehran metro. Among the fungi identified in this study, *Penicillium* spp., *Paecilomyces* spp., and *Aspergillus* spp. were revealed as the most dominant fungi. It drew attention that *Penicillium* spp. (75%) and *Aspergillus* spp. (4.3%), which can cause a series of allergic, inflammatory, and toxic reactions in humans, were identified at a higher

rate than other fungi. Fungi isolated in transport vehicles and their isolation rates are shown in Fig. 6.

Cladosporium spp. (Ren et al. 2001; Mentese et al. 2009), frequently isolated as airborne, was isolated at low rates in this study, such as 0.35% in bus, 0.35% in metro, and 0.9% in ferry. Although there are studies on bacteria and fungi in indoor and outdoor air in Turkey, there are a limited number of studies on biological pollutants in the air of public transport vehicles, especially fungi in indoor environments (Onat et al. 2017; Sivri et al. 2020). It has been determined that eighty different fungi cause the allergy of the respiratory system, and the spores of *Alternaria* spp., *Aspergillus* spp., and *Penicillium* spp. have been stated as the most common allergen fungi in Turkey (Bicakci et al. 2001; Tatlidil et al. 2001; Arikoglu et al. 2016). It has been reported that long-term exposure to fungi can cause skin and respiratory irritation, infection, and toxicity (Fung and Hughson 2003). *Penicillium* spp. and *Paecilomyces* spp. have been isolated most frequently in four transport vehicles, respectively. The third intensely isolated fungus in bus, metro, and ferry was *Aspergillus* spp. and *Candida* spp. in metrobus. Other fungal genera, many of which can be qualified as saprophytes, have been isolated at varying rates according to vehicles (Fig. 7). This has been thought to be due to the passenger population density, mobility, the loading and unloading of passengers by vehicles at a high number of stops, or saprophytic fungi, which are commonly found in the outdoor environment (Nazaroff 2016), being transported into the vehicle with passenger clothing and belonging.

Fig. 4 The seasonal similarity analysis of all vehicles' outbound and inbound routes. IR, inbound routes; OR, outbound routes



Conclusion and recommendations

This study aimed to determine changes in 12-month CAF concentrations in the indoor air of different transport vehicles depending on both season and transport vehicle because in many countries, including Turkey, there is a significant gap in the legal binding force related to the standards for the indoor air quality of vehicles. Many countries have established or are trying to establish their national ambient air quality standards, following the WHO Air Quality Guidelines. Turkey does not have official standards for indoor airborne microorganism concentrations. However, it is stated that an indoor fungal level of 150–1000 CFU/m³ is considered sufficient to cause health problems in humans (Lieberman et al. 2006). According to the European Union criteria, the limit bioaerosol values are determined as 5×10^2 CFU/m³ for fungi, and values above these limits are stated to

cause severe health problems (Gorny and Dutkiewicz 2002). The results of this study also demonstrated that there was a high rate of fungi in public transport vehicles that could affect human health in many sampling periods. The highest CAF concentration was determined in bus, while the lowest CAF concentration was detected in the indoor environment of ferry. It was observed that changes due to differences in the design, operation, and maintenance of transport vehicles, ventilation type, air heating systems, air conditioners, population density, environmental conditions, and climates could be associated with CAF concentrations. When CAF concentrations on the outbound and inbound routes of vehicles were examined in this study, an increase in the CAF concentration was usually determined on the inbound route.

Different fungal genera were determined at different rates in all transport vehicles. Among the fungal genera detected in all four transport vehicles, *Penicillium* spp., *Paecilomyces*

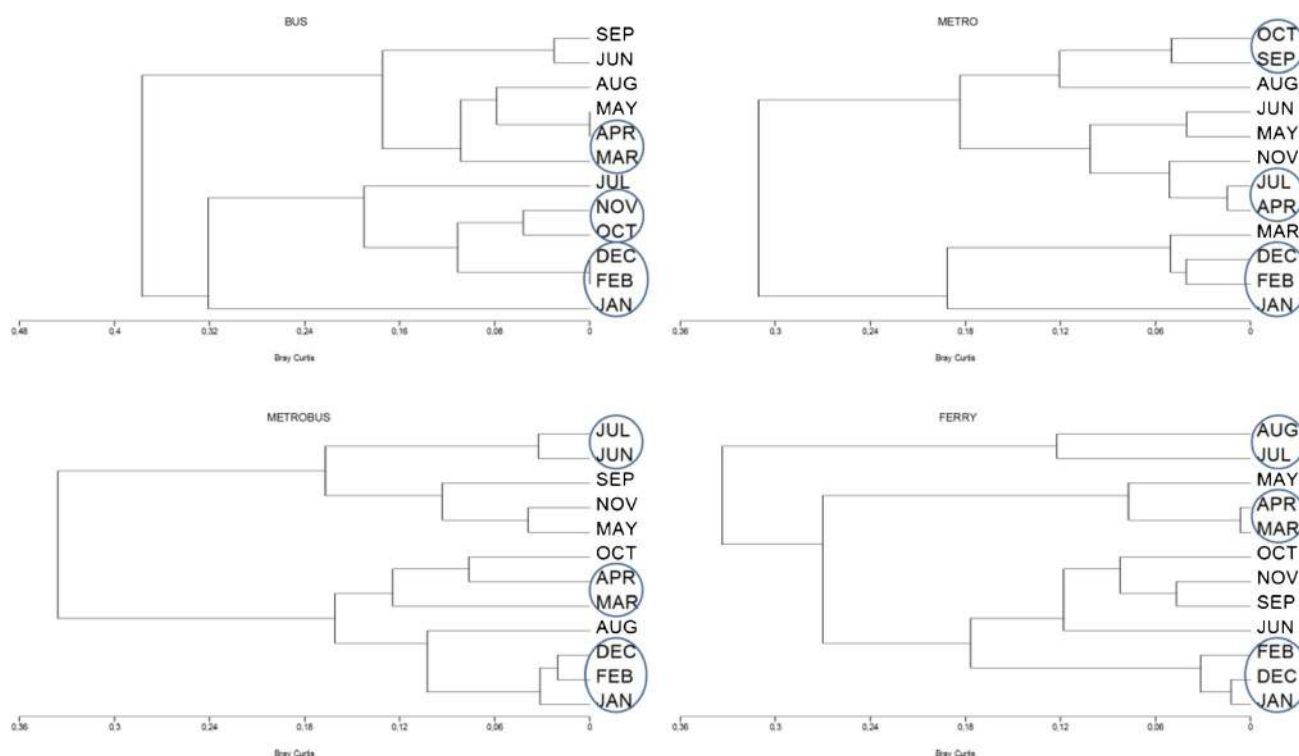
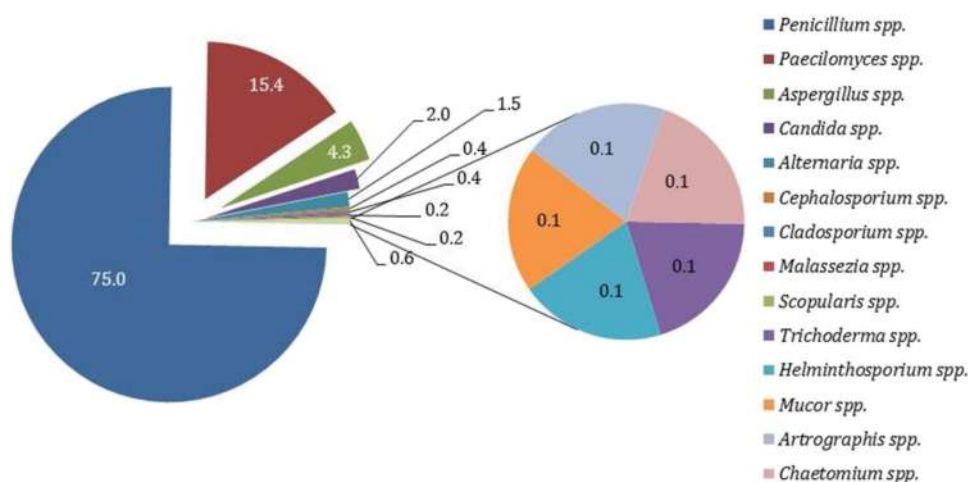


Fig. 5 The similarity analysis of different transport vehicles by months

Fig. 6 Fungal genera and percentages isolated from transport vehicles



spp., and *Aspergillus* spp. stood out with their high concentration. In addition to this study, it is necessary to conduct comprehensive research and epidemiological studies on the health effects of fungi in the air of different transport vehicles in the future.

To prevent health problems that may occur in transport vehicles, heating, ventilation, air conditioning systems (HVAC) should be cleaned and controlled regularly, as stated by the researchers (Ross et al. 2004; Prussin and Marr 2015). In the study carried out by Zhang et al. (2011), it was thought that it would be beneficial to commonly use

ultraviolet-operated (UVGI: ultraviolet germicidal irradiation) systems, stated to be safe, in transport vehicles. In this study, it was determined that there was no UVGI system in any vehicle from which the air sample was taken.

It is known that agents are always taken into the body through the mucous membranes (eyes, nose, and mouth) in droplet infection. As the COVID-19 pandemic continues, it is possible that more health problems will occur, along with fungal and bacterial agents.

As a result, studies to reduce the concentrations of bacteria or fungi should be increased to reduce the risks that

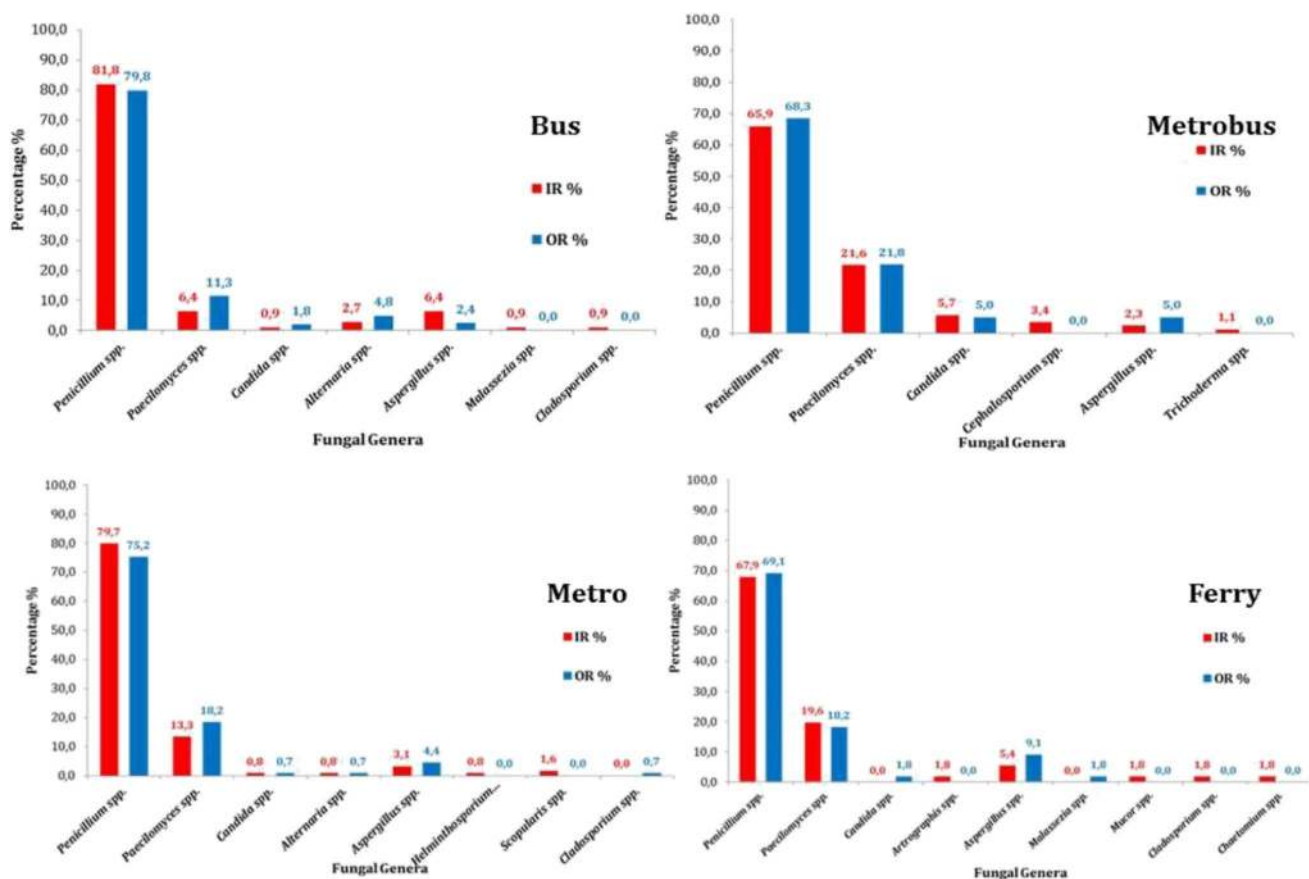


Fig. 7 Fungal genera and isolation rates detected in four transport vehicles. IR, inbound routes; OR, outbound routes

threaten the health of people who spend most of their time in public transport vehicles, and measures should be taken against possible health threats. Moreover, as stated under the bioaerosol-cleaning relationship title, the frequent change of both vehicle interior cleaning conditions and air filters is closely related to the health of vehicle users. It has been observed that it is necessary to establish new standards regarding the cleaning and disinfection frequency of especially the air conditioning and ventilation system.

Acknowledgements We gratefully acknowledge Canan KENAR, Can PAMUKÇU, Enes Efe GÜL, and Sibel ÇAKIROĞLU for their assistance with the field work.

Author contribution KM: Literature search, data acquisition, experimental studies (fungi), manuscript preparation, corresponding author.

AIK: Literature search, experimental studies (bacteria and fungi), manuscript editing.

BH: Experimental studies (bacteria), data analysis, manuscript editing, and manuscript review.

AFB: Concept, design, literature search, data acquisition, experimental studies (*Staphylococcus*), manuscript preparation, manuscript editing.

BM: Literature search, experimental studies (bacteria and *Staphylococcus* and fungi), manuscript editing.

NS: Concept, design, definition of intellectual content, experimental studies (bacteria), data acquisition, data analysis, statistical analysis, manuscript preparation, “guarantor”.

BO: Project leader, design, manuscript editing, and manuscript review.

Funding This study was supported by the Scientific Research Project Coordination Unit of Istanbul University, Project Number: İÜ BAP 25952.

Declarations

Conflict of interest The authors declare no competing interests.

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