

Concentration and distribution characteristics of airborne fungi in indoor and outdoor air of Tehran subway stations

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Abstract The concentration and distribution characteristics of airborne fungi were investigated in indoor and outdoor air of two metro stations (Imam Khomeini and Sadeghiyeh stations) in Tehran subway. Samples were taken from indoor air at each station from platform and ticket office area also from adjacent outdoor air of each station. Indoor sampling was conducted for two types of trains, old and new. The concentration of airborne fungi ranged from 21 CFU/m³ at the outdoor air of Imam Khomeini station to 1,402 CFU/m³ in the air samples collected from the platform of this station. Results showed that airborne fungi concentrations at indoor air were higher than the outdoor air ($p < 0.05$), and fungal levels significantly correlated with the number of passengers ($p < 0.05$;

$r = 0.68$) and RH % ($p < 0.05$; $r = 0.43$). Sixteen genera of fungi were isolated in all sampled environments. The predominant genera identified in indoor and outdoor air were *Penicillium* spp. (34.88 % of total airborne fungi) and *Alternaria* spp. (29.33 % of total airborne fungi), respectively. The results of this study showed that the indoor air quality in subway is worse than the outdoor air.

Keywords Subway system · Airborne fungi · Indoor air · Air pollution

1 Introduction

Subway systems are gradually being used as a principal method of public transportation in large cities around the world, a place where daily commuters spend significant part of their time (Nieuwenhuisen et al. 2007). In addition, the potential exposure of subway employees or passengers to various airborne contaminants could be extremely high considering the amount of time spent underground during travel or work (Kim et al. 2011).

Countries with subway systems have performed many qualitative and quantitative studies with various air pollutants in indoor air of these systems. These studies have been mainly focused on assessing the concentrations and characteristics of pollutants such as carbon monoxide and carbon dioxide (Chan et al.

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2002; Gomez-Perales et al. 2004; Kim et al. 2006), nitrogen oxide (Chan et al. 1999), volatile organic compounds (VOCs) (Shiohara et al. 2005), metals (Chillrud et al. 2005; Kang et al. 2008), polycyclic aromatic hydrocarbons (PAHs) (Velasco et al. 2004), particulate matter (PM) (Furuya et al. 2001; Awad 2002; Johansson and Johansson 2003; Aarnio et al. 2005) and bacterial and fungal bioaerosol (Cho et al. 2006; Kim et al. 2006; Bogomolova and Kirtsideli 2009). There are, however, very limited information and studies related to airborne biological contaminants, especially airborne fungi, in Tehran's subway system.

Airborne biological contaminants known as bioaerosols include bacteria, fungi, viruses and Pollens (Naddafi et al. 2011). Some bioaerosols are hazardous since they are infectious and/or produce allergens and toxins (Curtis et al. 2006). Epidemiological studies show that high concentration of bioaerosols can result in several adverse health effects such as respiratory disorders, allergic reactions, infections and toxic responses (Gorny et al. 2002; Fracchia et al. 2006; Mandal and Brandl 2011). Among the different types of bioaerosols, fungi represent a heterogeneous group, presence of which plays an important role in human health, especially in indoor environments. Since concentration of airborne fungi is an important factor influencing Indoor Air Quality (IAQ), this study aimed to determine the fungal concentration level and identify the fungi genera as well as of the association between important variables (air humidity and temperature, number of passengers) and airborne fungi concentration levels in indoor air of Tehran's subway stations.

2 Materials and methods

2.1 Selection of sampling location

This research was performed at 2 stations—with different location, structure and crowding—of lines 1 & 2 of Tehran subway system. The first station, “Imam Khomeini,” is an underground station located at cross point of two most busy lines (lines 1 & 2). It is equipped by mechanical ventilation systems and maintained under positive pressure. The second station, “Sadeghiye,” is a surface station except for its ticket office area which is underground. It is

characterized by good natural ventilation and less passengers. For each station, measurements were taken at the platform and ticket office area. For the comparative assessment of indoor subway pollution levels, one additional outdoor spot was investigated adjacent to each station. Besides, indoor air of trains (both old and new ones) was studied.

2.2 Sampling and measurement equipment

Air samples were collected for 2 min using Quick Take 30 sample pump equipped with the BioStage® single-stage cascade impactor (SKC, USA). The pump was set for a flux of 28.3 L/min, and sampling took place once every 6 days for 4 months from December to March 2011. Before collecting samples, the sampler's internal part was sterilized with 70 % alcohol. The measured point was in respiratory height (about 1.5 m above floor level), and each point was measured twice. Measurements were taken between 9 a.m. and 3 p.m. During sampling period, number of passengers in 2 m radius, relative humidity and temperature were measured and recorded (Kim et al. 2011; Naddafi et al. 2011).

2.3 Isolation and identification of fungal flora

To examine airborne fungi, Sabouraud Dextrose Agar (SDA) was used as the growth medium with 50 mg chloramphenicol added to suppress any bacterial growth. After a fungal sample was collected, the media was moved to the laboratory and airborne fungi were cultured for 3–7 day at room temperature (20–25 °C). The simple method of slide culturing was established to identify the fungal species by performing some levels of microscopic study by using optical microscopes. Airborne fungal genera were then identified according to Pitt and Hocking (1997), Carmichael et al. (1980) and Samson et al. (2000). The concentration of airborne fungi (CFU/m³) was calculated by dividing the value that was counted for the colony formed in the media after culturing by the air volume (m³).

2.4 Statistical analysis

The concentration difference of airborne fungi between the outdoor, office area and platform of the stations was calculated with Kruskal–Wallis *H* test by

SPSS version 16 (SPSS Inc., Chicago). The concentration difference between two stations as well as new and old trains was analyzed for statistical significance with Mann–Whitney U test. To evaluate the relationship between the recorded parameters (number of passengers, relative humidity and temperature) and the concentration of airborne fungi (as CFU/m³), Bivariate Correlations study (Spearman's correlation coefficient test) was performed. A significance level of p value <0.05 was used.

3 Results and discussions

3.1 Concentration of airborne fungi in different sampling locations

The concentrations of fungal aerosols at the indoor and outdoor air of different sampling locations are shown in Table 1. As represented in the table, the concentrations of airborne fungi varied widely at different sampled environments, and ranged from 21 CFU/m³ at the outdoor air of Imam Khomeini station to 1,402 CFU/m³ at the platform of this station. As shown in this table, the maximum fungal concentration was at the platform of Imam Khomeini station with an average of 1,017.50 CFU/m³ and the minimum was at outdoor air of this station with an average of 154.57 CFU/m³. Based on the results of statistical analyses, there were significant differences between the concentration of airborne fungi (CFU/m³) at the three sampling locations of Imam Khomeini station (Kruskal–Wallis H test, $p < 0.05$), but no significant difference was observed between the concentration of airborne fungi at the office area and the platform (Mann–Whitney U test, $p > 0.05$). The concentration

of airborne fungi at the indoor air of Imam Khomeini station (both platform and office areas) was higher than those of the outdoor (Mann–Whitney U test with, $p < 0.05$). At the Sadeghiye station, the difference between the concentration of airborne fungi at the office area and the platform as well as indoor (only office area) and outdoor was statistically significant (Mann–Whitney U test, $p < 0.05$). The average concentration of airborne fungi of all sampling locations at the Imam Khomeini station was significantly higher than at the Sadeghiye station (Mann–Whitney U test, $p < 0.05$). According to $p > 0.05$ obtained from the Mann–Whitney U test, there was no significant difference between the concentration of airborne fungi at two types of trains.

According to the results, all underground areas and indoor air of trains had higher airborne fungi concentrations than the outdoors. Ayanbimpe et al. (2010) have reported that the high population density affects the occurrence of high indoor fungal contamination. Also, it is reported that more population density might cause re-suspension of dust and result in higher bioaerosol levels (include airborne fungi) in indoor environments, so the reasons of high concentration of fungi at the Imam Khomeini station platform are most likely due to the high population density at this place, which affects the indoor fungal contamination and re-suspension of dust, and also the fact that the station platform is located at a lower level the other area, which results in poor ventilation and little air current at this station.

There is documented information related to concentration of airborne fungi in subway stations; also, several studies have been performed in measuring concentration of fungi at different other indoor and outdoor environments. In a similar study performed at the Seoul metropolitan subway stations, the concentration

Table 1 Concentrations of airborne fungi at different sampling locations

Station	Point of sampling	Number of samples	Total airborne fungi (CFU/m ³)			
			Min	Max	Mean	S.D
Imam Khomeini	Platform	19	487	1,402	1,017.50	244.26
	Office area	19	127	1,060	673.47	271.70
	Outdoor	19	21	275	154.57	78.05
Sadeghiye	Platform	19	53	512	241.68	125.18
	Office area	19	441	1,095	766.00	197.55
	Outdoor	19	14	589	197.89	134.32
Trains	New	19	71	756	387.42	214.72
	Old	19	123	919	497.47	243.34

of airborne fungi ranges from 9 to 459 CFU/m³ (Kim et al. 2011). In another study performed at indoor air of subway station in Cairo, the mean concentration of airborne fungi ranged from 0 to 1,250 CFU/m³ with an average of 800 (Awad 2002). According to Kawasaki et al. (2010), the concentration of airborne fungi in subway stations in Tokyo was 25–2,445 CFU/m³, an average (\pm SD) of 486 (\pm 430) in underground platform. They have reported that the concentration of airborne fungi in above-ground platforms ranged from 0 to 580, an average (\pm SD) of 148 (\pm 155) CFU/m³. Figure 1 presents a descriptive comparison of concentrations of airborne fungi in this study with other subway stations studied by other researchers in different countries. As shown in this figure, the concentration of airborne fungi at underground platform of Tehran subway (measured in this study) is higher than other platforms. Concentration of airborne fungi at above-ground platform in Tehran subway (platform of Sadeghiye station) is higher than above-ground platform in Tokyo's subway, but lower than Cairo's. Differences in design, operation and maintenance as well as type of ventilation, forced air-heating systems, air conditioners, variety of climates and environmental conditions, and also population density and its distribution pattern can attribute to this dissimilarities. In other public areas such as hospital environment, high fungal concentration was observed ranging from 3,419 to 7,701 CFU/m³ (Sharma et al. 2010). In offices, the range was much lower, for example, according to Burge et al. 2000, indoor fungal counts in the air of a large office building vary from 106 to 1,113 CFU/m³ (Burge et al. 2000).

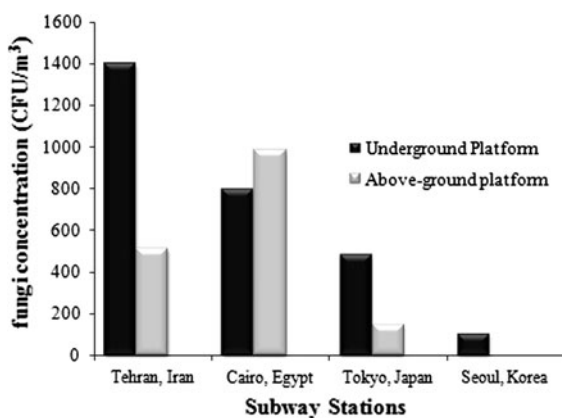


Fig. 1 Descriptive comparison of airborne fungi concentration between different subway stations

There is no uniform international standard available on levels and acceptable maximum fungal bioaerosols in indoor environments, and each country has unique guidelines; for instance, the guideline values of fungal bioaerosol in Canada, the United States and the European Union for indoor environments are 150, 1,000 and 2,000 CFU/m³, respectively. The standards reach 20,000 CFU/m³ in Germany (Mandal and Brandl 2011); therefore, it is impossible to compare the results with a standard value.

Regarding the trains, the higher concentration of fungi in old ones (mean 497.46 CFU/m³ vs 387.42 CFU/m³) can be attributed to the fact that the separated and confined interior space of these trains cause high population density and lower the air movement, subsequently increasing the fungal concentration, while in new trains this interior space is interconnected along all parts of the trains, as a result the passengers move relatively more evenly in different parts of the train, and the air movement is better therefore the fungal concentration will be lower. In addition, old trains might have more fungal contamination compared to the new ones, which can also contribute to higher fungal levels.

3.2 Association between recorded parameters and fungal concentrations

In this study, the number of passengers in different sampling points varied from 5 passengers at the outside of Imam Khomeini station to 120 passengers in old trains. Also, relative humidity and temperature ranged from 17 to 39 % and 6 to 27 °C, respectively. The distributions of number of passengers, relative humidity and temperature at each sampling location are shown in Table 2. According to the results of Spearman's correlation coefficient test, in general, there were positive correlation between the concentration of airborne fungi (as CFU/m³) and the number of passengers in the 2 m radius ($p < 0.05$; $r = 0.68$) and relative humidity ($p < 0.05$; $r = 0.43$); but there was no significant correlation between the concentration of airborne fungi and temperature ($p > 0.05$; $r = 0.06$).

The two most important factors affecting growth of fungi in indoor air are moisture and available carbon sources (Oppliger et al. 2008). In confined environments, human activity and population density affect concentration of bioaerosols (Naddafi et al. 2011). So

Table 2 Distributions of number of passengers, relative humidity and temperature at each sampling location

Station	Point of Sampling	Mean/range		
		No. of passengers	Relative humidity (%)	Temperature (°C)
Imam Khomeini	Platform	79/42–120	28.4/25–39	18.9/17–23
	Office area	52/35–70	26.1/21.5–29	16.4/15–22
	Outdoor	16/5–26	24.2/21–26	10.7/6–16
Sadeghiye	Platform	30.6/14–60	24.3/22–27	12.5/10–17
	Office area	57.8/32–85	26.4/23–29	15.9/13–20
	Outdoor	16.2/10–25	24.2/17–28	11.4/9–16
Trains	New	81.6/55–100	32.4/31–35	22.9/20–26
	Old	95.6/75–115	35.7/33–39	23.9/21–27

in this study, in addition to the correlation between population density and the concentration of airborne fungi, significant correlation was also observed between relative humidity and concentration of fungi. Comparison of this analysis with results of other studies on indoor air quality shows acceptable consistency. Different authors have found a pattern variation in fungal concentration which relates to population density and relative humidity. According to Kim et al. (2011), there was a significant correlation between the concentration of airborne fungi and number of residence in Seoul metropolitan subway stations. Buttner and Stetzenbach (1993) found that in an experimental room, human activity resulted in retrieval of significantly higher concentrations of airborne fungi spores. In another study, Wemedo et al. (2012) have found that in one of their study areas, a room characterized by very poor ventilation and large number of people; the concentration of airborne microorganisms was significantly higher than other less populated sampled points. Kawasaki et al. (2010) found at a railway station, which had both underground and above-ground concourses and platforms, there were positive correlations between the concentration of airborne fungi and relative humidity. Also, the result of this study is consistent to research reports that studied the concentration of airborne fungi in other indoor environments (Aydogdu et al. 2005; Cho et al. 2006; Nasir and Colbeck 2010).

3.3 Airborne fungal identification

Based on diagnostic tests, a total 16 genera of fungi were isolated in all sampled environments. Table 3 shows percentage contribution in percent (%) and

mean concentration of different fungi genera as CFU/m³ in each sampled location. The predominant genus identified in indoor air was *Penicillium* (34.88 % of total airborne fungi) which was mostly represented by *P. chrysogenum*, *P. spinulosum*, *P. pinophilum*, and *P. glabrum*. Other genera identified, with lower concentrations in this environment, were *Cladosporium* spp. (*C. cladosporioides*, *C. sphaerospermum* and *C. herbarum*) and *Alternaria* spp. In outdoor air, the predominant genus was *Alternaria* spp. which comprises 29.33 % of the total identified fungi in this environment and *A. alternata* was the only species identified. In the air samples collected from the trains, *Aspergillus* was the principal genus (32.15 % of total identified fungi) which was represented by *A. flavus*, *A. niger*, *A. fumigatus* and *A. ochraceus*. Figures 2, 3, 4 show the percentage contribution of particular genera of fungi with a percentage contribution >2 % in different sampled environments.

Typical and most important fungal genera found in indoor air are *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. which are proportionally of importance in indoor air samples collected from different environments such as schools (Aydogdu et al. 2005; Jo and Seo 2005; Stryjakowska-Sekulska et al. 2007), museums (Chen et al. 2010) and offices (Burge et al. 2000, Chao et al. 2002). Also in Seoul metropolitan subway stations, the predominant fungal genera were *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and *Chrysosporium* spp. (Kim et al. 2011). In our study, however, *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. were the dominant captured fungi in indoor air including indoor air of trains. In outdoor air, *Alternaria* spp. and *Cladosporium* spp. were dominant genera which

Table 3 Mean concentration of different fungi genera as CFU/m³ and percentage contribution in % at different sampling locations

Genus	Fungal concentration and percentage contribution; CFU/m ³ (%)							
	Imam Khomeini station			Sadeghiye station				
	Outdoor	Office area	Platform	Outdoor	Office area	Platform		
<i>Penicillium</i> spp.	22.58 (14.61)	232.41 (34.51)	410.15 (40.31)	32.02 (16.21)	214.86 (28.05)	30.38 (12.57)	93.42 (24.11)	98.70 (19.84)
<i>Cladosporium</i> spp.	17.48 (11.31)	122.98 (18.26)	194.14 (19.08)	34.85 (17.64)	177.41 (23.16)	68.54 (28.36)	73.85 (19.06)	81.04 (16.29)
<i>Aspergillus</i> spp.	15.09 (9.84)	70.58 (10.48)	123.73 (12.16)	30.01 (15.19)	97.74 (12.76)	22.40 (9.27)	109.11 (28.16)	167.70 (33.71)
<i>Alternaria</i> spp.	33.07 (21.40)	48.62 (7.22)	72.34 (7.11)	61.73 (31.25)	79.05 (10.32)	79.34 (32.83)	23.56 (6.08)	41.04 (8.25)
<i>Chrysosporium</i> spp.	8.28 (5.36)	34.21 (5.08)	41.51 (4.08)	5.97 (3.02)	33.40 (4.36)	3.67 (1.52)	8.45 (2.18)	11.59 (2.33)
<i>Fusarium</i>	12.69 (8.21)	13.20 (1.96)	11.40 (1.12)	2.15 (1.09)	9.73 (1.27)	2.08 (0.86)	4.69 (1.21)	5.37 (1.08)
<i>Drechslera</i>	15.75 (10.19)	14.28 (2.12)	19.94 (1.96)	9.80 (4.96)	28.27 (3.69)	9.72 (4.02)	22.71 (5.86)	23.78 (4.78)
<i>Mucor</i>	2.33 (1.51)	23.57 (3.50)	20.96 (2.06)	4.15 (2.1)	12.10 (1.58)	1.76 (0.73)	8.25 (2.13)	9.75 (1.96)
<i>Acremonium</i>	4.97 (3.22)	7.95 (1.18)	13.43 (1.32)	2.25 (1.14)	7.51 (0.98)	3.26 (1.35)	1.59 (0.41)	3.23 (0.65)
<i>Nigrospora</i>	0 (0)	5.72 (0.85)	6.92 (0.68)	1.34 (0.68)	8.27 (1.08)	0 (0)	0 (0)	0 (0)
<i>Epicoccum</i>	7.14 (4.62)	32.46 (4.82)	17.20 (1.69)	0.97 (0.49)	47.42 (6.19)	9.93 (4.11)	4.22 (1.09)	5.47 (1.10)
<i>Ulocladium</i>	3.92 (2.54)	14.68 (2.18)	22.18 (2.18)	2.31 (1.17)	8.12 (1.06)	2.56 (1.06)	3.18 (0.82)	0 (0)
<i>Sporothrix</i>	0 (0)	7.27 (1.08)	5.49 (0.54)	0 (0)	2.83 (0.37)	0 (0)	0 (0)	0 (0)
<i>Geotrichum</i>	0 (0)	5.12 (0.76)	6.41 (0.63)	0 (0)	2.37 (0.31)	0.68 (0.28)	3.25 (0.84)	6.22 (1.25)
Yeasts	6.69 (4.33)	21.62 (3.21)	32.25 (3.17)	7.55 (3.82)	20.31 (2.65)	4.79 (1.98)	11.08 (2.86)	15.47 (3.11)
Unidentified molds	4.43 (2.87)	19.26 (2.86)	19.74 (1.94)	2.55 (1.29)	16.78 (2.19)	2.63 (1.09)	20.26 (5.23)	28.16 (5.66)
Total	154.5 (100)	673.47 (100)	1,017.50 (100)	197.55 (100)	766.00 (100)	241.68 (100)	387.42 (100)	497.47 (100)

Fig. 2 Concentration of different fungal genera in the outdoor air

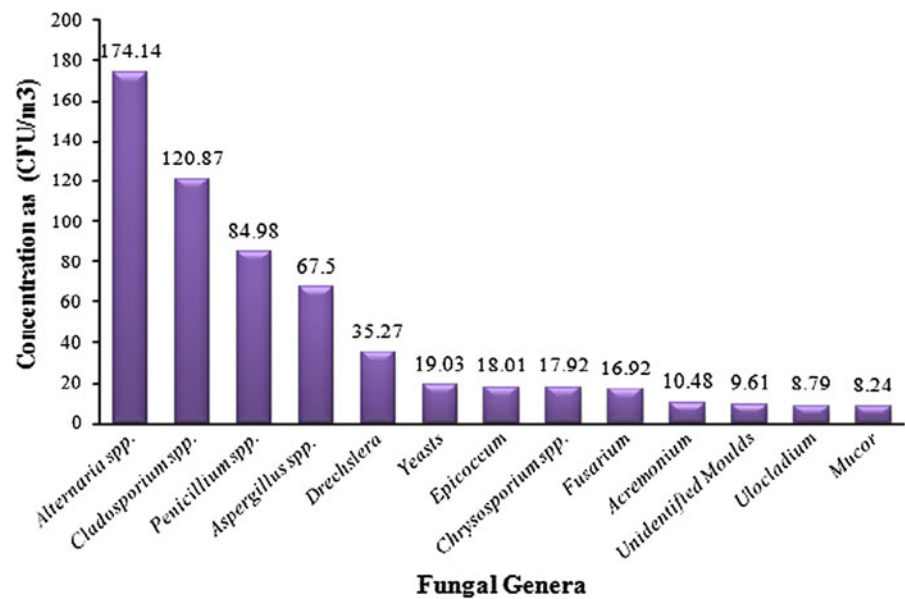
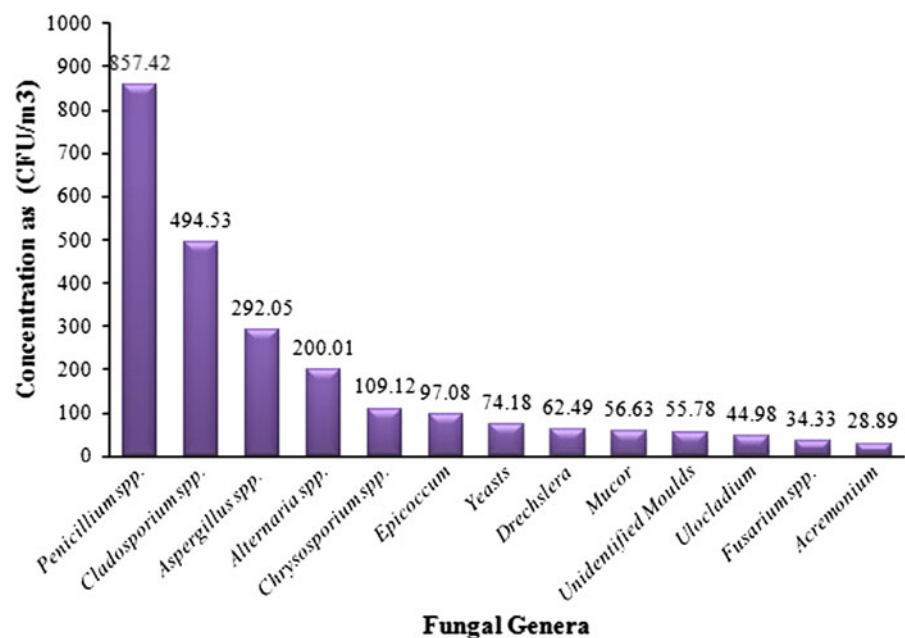


Fig. 3 Concentration of different fungal genera in the indoor air

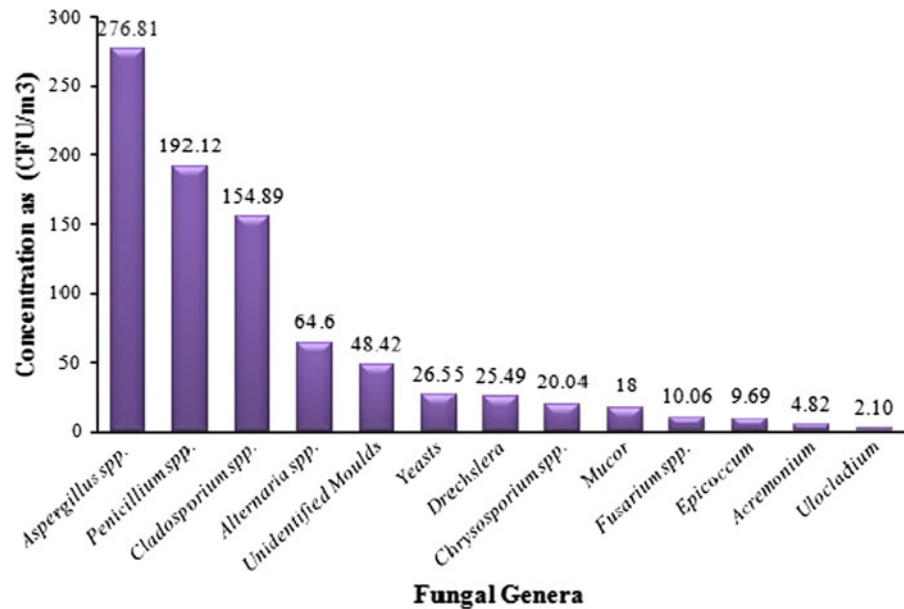


collectively comprised 49.69 % of the total identified fungi. All these identified fungal genera are able to form spores which are resistant to changes in environmental conditions (Mandal and Brandl 2011). So the dominance of these genera can be attributed to these the metabolic capabilities which facilitate their distribution and survival in harsh environmental conditions such as UV radiation, desiccation, lack of nutrients or extreme temperatures.

4 Conclusion

This study describes the level and distribution characteristics of airborne fungi in indoor and outdoor airs of two subway stations in Tehran. As the results show, the higher fungal concentration in indoor air compared to the outdoor could be associated with high passenger population in closed spaces; therefore, operational modifications such as increasing the number of trains

Fig. 4 Concentration of different fungal genera in the indoor air of trains



to prevent overcrowding and also regular cleaning of various indoor environments can be considered as preventive measures. In addition, programmed and periodical inspection and monitoring of ventilation systems and air conditioners seems to be necessary to adjust relative humidity and ventilation and consequently reduce fungal concentration. Although this study provides the first set of ranges of the concentration of airborne fungi reported at the indoor air of Tehran subway system which can be used for comparative purposes in future studies, however, one of the limitations of this study may be using only one sampling method which might not sufficiently document fungal amplification and contamination in these indoor environments. In addition, for a comprehensive assessment of the health effects of airborne fungi, gathering complementary information by performing epidemiological studies to evaluate personal exposure to the airborne fungi and establish a causal relationship between exposure to the airborne fungi and systemic effects is necessary in addition to air sampling.

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