

Aerobiological study in the Mexico City subway system

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Abstract Monitoring campaigns in two different seasons were carried out at two underground stations (Tacubaya and Azcapotzalco) of the subway of Mexico City, in order to assess airborne bacterial and fungi concentrations, as well as their relationship with several factors, such as depth of the station, sampling site, temperature, and relative humidity. Sixteen sampling sites were selected according to the depth of the lines and the transit of passengers in the corridors, concourses, and platforms. In addition, samples were also collected in the carriages when they were in movement. Outdoor samples were taken at the two stations for comparison. Two-stage multi-orifice cascade impactors were used to collect aerobiological particles, and with the aid of macroscopic and microscopic characterization were found 57 fungi and 61 bacteria colonies. Outdoor bacteria concentrations ranged from 1 to 68 CFU m⁻³, while fungi concentrations varied from 6 to 80 CFU m⁻³. The indoor concentration levels of bacteria and fungi ranged from 1 to 484 CFU m⁻³ and from 51 to 715 CFU m⁻³, respectively. Fungi and bacteria indoor concentrations in the subway were higher than outdoor, up to 8 times. Most of bacteria were identified

as Gram-positive nonsporulating short *bacillus*, while the most abundant fungi genera identified were *Aspergillus*, *Penicillium*, and *Alternaria* in that order of frequency. Statistical analysis showed significant differences between the stations and the lines of different depths, showing a greater microorganisms' concentration with a greater depth. Although bacteria and fungi concentrations were higher in the spring than in winter, this difference was not significant. Even if indoor microbiological pollution in underground stations was higher than outdoor, the concentrations found in this study were lower than indoor air international standards.

Keywords Subway · Airborne bacteria · Airborne fungi · Air quality · Mexico City

1 Introduction

Currently, the metropolitan area of Mexico City has around 21 million inhabitants and the subway system has 12 lines, thus serving large areas of the Distrito Federal and part of the State of Mexico, offering its services to about 5.5 million people daily (STCMAL 2011). As a result of this large number of passengers, the indoor air quality in the subway system is an important health issue.

Gomez-Perales et al. (2004) and Mugica-Alvarez et al. (2012) carried out indoor air quality studies in the

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Mexico City subway system and reported that PM_{2.5} and PM₁₀ concentrations within were 20 % higher than outdoor levels. Bioaerosol adverse health effects such as allergies, asthma, and toxic effects have been assessed by Kurup et al. (2000), Douwes et al. (2003) and Srikanth et al. (2008), among others. Because the subway system is an environmental enclosure, indoor air can be affected by the presence of microbiological particles, as reported for other indoor areas such as houses, offices, and universities (Hargreaves et al. 2003; Kalogerakis et al. 2005; Yassin and Almouqatea 2010). Regarding recent researches related to transport systems, Lee and Jo (2005) studied in Korea the presence of bacteria and fungi in passenger cars and public buses. Wang (2011) measured the size distributions of aerobiological concentrations in commuter buses in northern Taiwan, finding fungi and bacteria concentrations up to 2,193 and 601 CFU m⁻³, respectively. The presence of bacteria in airplanes was investigated by Mckernan et al. (2008), determining that bacteria levels were higher during the boarding and deplaning processes than during the flights.

Although several air quality studies related to the presence of airborne nonbiological particles in subways have been reported worldwide (Pfeifer et al. 1999; Adams et al. 2001; Johansson and Johansson 2003; Aarnio et al. 2005; Branis 2006; Nieuwenhuijsen et al. 2007; Gomez-Perales et al. 2004; Mugica-Alvarez et al. 2012, among others), the available information on the presence of airborne microorganisms in subway stations is limited. Awad (2002) studied the presence of microorganisms in El Cairo subway finding that viable bacteria, staphylococci, dust, and oxidants had higher levels in the air of a tunnel station, whereas *Candida* spp, fungi, and actinomycetes were slightly higher in a surface station than in the tunnel; Picco and Rodolfi (2000) reported the presence of *Cladosporium*, *Penicillium*, *Epicoccum*, and *Alternaria* in Milan underground stations showing variations throughout the year; Seino et al. (2005) reported that bacteria counts in an underground public concourse ranged from 50 to 1,380 CFU m⁻³ and correlated with the number of pedestrian, relative humidity, and particles (PM_{2.5}); Cho et al. (2006) found high concentrations of fungi in the settled dust samples in the subway of Seoul; meanwhile, Bogomolova and Kirtsideli (2009) obtained that the most abundant genera of 50 fungal species were *Acremonium*, *Aspergillus*, *Cladosporium*, and *Penicillium* in the St. Petesburg underground railway system;

Hwang (2010) and Kim et al. (2011) investigated the presence of microbes in Seoul reporting up to 4,997 CFU m⁻³ of total airborne bacteria in subway stations, and recently, Dybwad et al. (2012) identified 37 different genera of bacteria in an Oslo's subway station, most of them belonging to genera *Bacillus*, *Micrococcus*, and *Staphylococcus*. Finally, Hoseini et al. (2012) measured airborne fungi ranging from 21 CFU m⁻³ at the outdoor air up to 1,402 CFU m⁻³ in two Tehran subway stations, finding that *Penicillium* spp was the most abundant genera.

To the best of our knowledge, there is no information concerning the presence of microorganisms in the Mexico City subway system; therefore, the main objective of this investigation was to quantify and identify the indoor microbial population present in this environment, as well as investigate the conditions that could influence microbial counts, in two dry seasons (winter and spring) and in two different subway stations, one of which serves three underground lines located at different depths. Therefore, the influence of temperature, relative humidity, percent moisture, and dew point on microbial concentration levels was investigated.

2 Materials and methods

2.1 Sampling sites

Two different types of subway stations were selected. The first one is Tacubaya, a very crowded station where three different lines at different depths concur: Line 9 located approximately 10 m below ground level, inaugurated in 1988, that presently serves 85,242 passengers daily (labor day average); Line 1 located approximately 25 m below ground level, inaugurated in 1972, that serves 51,557 users daily (labor day average); and Line 7, 40 m below ground level, inaugurated in 1985, that serves 8,061 passengers daily (labor day average). The reference station, Azcapotzalco, was built in 1983. Nowadays, it is a part of Line 6 located approximately 10 m below ground, and it is one of the least used stations serving only 7,530 users daily (labor day average) (STCMAL 2011). There is neither heating nor air conditioning systems installed at the stations.

There were 16 sampling sites selected in different zones of Azcapotzalco and Tacubaya stations

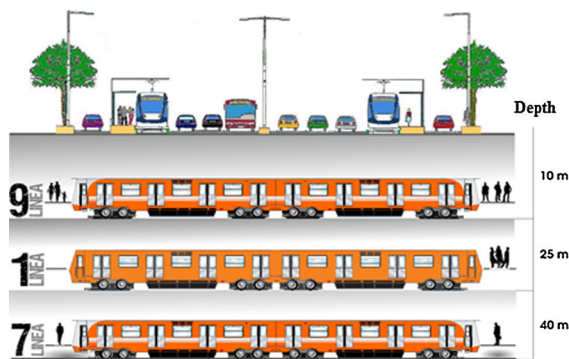


Fig. 1 Subway lines in Tacubaya station

according to the depth of the lines (as illustrated in Fig. 1) and passengers' transit at platforms, concourses areas, and corridors. Samples were also collected within the carriages in transit to identify differences between carriages and stations. Finally, samples were taken at outdoors of each of the two stations.

2.2 Microorganisms analysis

Two sampling campaigns were carried on during December 2010 and April 2011, corresponding both to the dry-cold season and dry-warm season, respectively. Sixty-eight aerobiological samples were collected, using two Andersen samplers with two-stage cascade of multiple orifices, which operate at a flow rate of 28.3 L min^{-1} under the impaction principle. Each stage includes a plate with 200 uniform diameter holes (sharp cut of diameter in the first stage is $8 \mu\text{m}$ and above, while in the second stage is $0.8\text{--}8 \mu\text{m}$); thus, airborne particles are separated into two fractions: a large-particle fraction that is retained in the upper airways on inhalation and is considered non-respirable, and a small-particle fraction that can penetrate to the gas exchange tissue of the lung that is considered respirable. Petri dishes containing the selective culture medium Sabouraud Dextrose Agar (Lot 1280573, Becton–Dickinson and Company) and m-Endo-Agar Les (Lot 7242834, Becton–Dickinson and Company) were placed for simultaneous capture of fungi and bacteria, respectively, with an air flow of 28 L min^{-1} for a sampling time of 10 min. Both equipments were placed at a height of 1.5 m above ground level, that being the average height vulnerable to aspiration of particles. Plates were incubated at

37°C during 24–48 h for coliform bacteria and 27°C during 48–72 h for fungi for further determination of the number of colony-forming units (CFU) with a Darkfield Quebec colony counter. Bacteria colonies that presented a rose color with green metallic shining were selected and identified with the Hucker Gram staining technique. Fungi colonies were selected by observing the form, shape, texture, and color through the optical microscope and were isolated in malt extract agar (Lot 0189493, Becton–Dickinson and Company) for further identification through comparison with identification manuals (Ulloa and Hanlin 1991; Barnett and Barry 2003). The airborne microorganisms were reported as CFU m^{-3} and were calculated by dividing the number of the counted colonies formed in the media after culturing (CFU) by the air volume (m^3) during the sampling time.

2.3 Meteorological parameters

The data from the meteorological parameters temperature and relative humidity percent were obtained and recorded with an EA20 Easy View Hygro-Thermometer with a dual display showing relative humidity, temperature, as well as dew point and wet bulb. The range and resolution for relative humidity is from 10 to 95 % of relative humidity (RH) with an accuracy of $\pm 3 \%$ RH (at 25°C , from 30 to 95 % of RH) and of $\pm 5 \%$ RH (at 10°C , from 30 to 95 % of RH). The range and resolution for temperature (T) is from -20 to 60°C with an accuracy of $\pm 1^\circ\text{C}$. Dew point temperature is calculated automatically by the Hygro-Thermometer based on humidity/temperature, as well as wet bulb that is calculated based on humidity/temperature measurements.

2.4 Statistical analysis

Statistical analysis was performed with SPSS software, version 12.0. Pearson's correlation coefficients were estimated in order to determine the relationship among bacteria and fungi airborne concentrations with depth, temperature, and relative humidity. The Mann–Whitney test was applied to determine differences between the two seasons. Differences between bacteria and fungi concentrations related to depth, sites, and other parameters were analyzed for statistical significance with two-way and one-way ANOVA and Fisher LSD (least significant difference) post hoc test. Levine

Table 1 Meteorological parameters

Metro station	Sampling site	Dry-cold season (winter)				Dry-warm season (spring)			
		T (°C)	RH %	WB °C	D.P. °C	T (°C)	RH %	WB °C	D.P. °C
Tacubaya (Line 7)	1. Platform	20.6	24.2	12.5	1.4	27.7	34.5	17.3	10.2
	2. Concourses	24.2	40.0	11.2	0.4	25.0	27.6	16.6	10.4
	3. Corridors	20.1	26.5	11.6	0.8	24.8	43.4	16.0	11.5
	4. Carriage	22.6	16.0	13.1	4.8	31.2	28.0	18.5	10.1
Tacubaya (Line 1)	1. Platform	27.7	34.5	17.3	10.2	30.7	23.6	16	5.8
	2. Concourses	25.0	27.6	16.6	10.4	28.7	24.0	17.2	6.4
	3. Corridors	24.8	43.4	16.0	11.5	28.5	15.8	14.3	1.0
	4. Carriage	31.2	28.0	18.5	10.1	31.7	14.3	15.9	1.3
Tacubaya (Line 9)	1. Platform	26.2	20.8	12.1	4.3	25.3	33.6	15.3	8.0
	2. Concourses	24.2	20.5	10.2	5.0	25.1	32.6	15.7	8.0
	3. Corridors	26.5	20.0	9.0	2.0	25.3	32.9	15.1	7.6
	4. Carriage	26.2	10.7	13.3	4.0	28.6	31.6	17.5	9.4
Azcapotzalco (Line 6)	1. Platform	16.8	36.0	9.5	1.5	29.2	24.5	15.7	4.8
	2. Concourses	17.7	25.5	9.3	2.4	28.2	22.1	15.5	5.3
	3. Corridors	19.2	26.4	10.0	0.5	28.2	24.0	15.5	5.6
	4. Carriage	20.5	32.0	12.1	4.1	29.3	19.6	15.5	3.9

T (°C) temperature, (*RH* %) relative humidity percentage, *WB* (°C) wet bulb temperature, *D.P.* (°C) dew point

test was used to determine the homogeneity of variance.

3 Results

3.1 Variation of climatological parameters and microorganisms' concentrations

Average values of temperature (T), relative humidity percent (%RH), wet bulb temperature, and dew point temperature are displayed for each line in Table 1. These parameters are very variable, since each line has different ventilation systems, passenger's transit, depth, and light levels. The highest temperatures were measured within the carriages, and in the different sites located at Line 1, which has the poorest and oldest ventilation system. The temperature variations in Tacubaya station were in general from 3 to 5 °C higher in the dry-warm season than in the dry-cold one with the exception of Line 9 which presented similar values in both seasons, probably because it has a good ventilation system. In Azcapotzalco station, the temperature increases during the dry-warm season from 9 to 13 °C probably because there is no ventilation

system, and the presence of several open vents from the surface allows sunlight entry, thus warming up all the station. Wet bulb temperature ranged from 9 to 18.5 °C in the dry-cold season (13 °C on average) and increases in the dry-warm season with an average of 16 %.

The outdoor mean concentrations of airborne bacteria in Tacubaya station were 40 and 68 CFU m⁻³ for the dry-cold and dry-warm seasons, respectively, while in Azcapotzalco station, where vehicular and pedestrian's transit outside this station is limited, concentrations were lower with 1 and 6 CFU m⁻³ for dry-cold and dry-warm seasons, respectively. Airborne bacteria concentration during both dry-cold and dry-warm seasons ranged from 56 to 484 CFU m⁻³ in Tacubaya station and from 1 to 132 CFU m⁻³ in Azcapotzalco station (Fig. 2). Concentrations inside the subway varied according to season, site, and depth. The mean measured concentrations (CFU m⁻³) of airborne bacteria were in the dry-cold season 102 ± 45, 140 ± 119, 88 ± 63, and 67 ± 42 in the platform, concourses, corridors, and carriages, respectively, whereas the mean concentrations in the dry-warm presented higher CFU m⁻³ values: 219 ± 88, 235 ± 136, 182 ± 101, and 103 ± 31 in the platform,

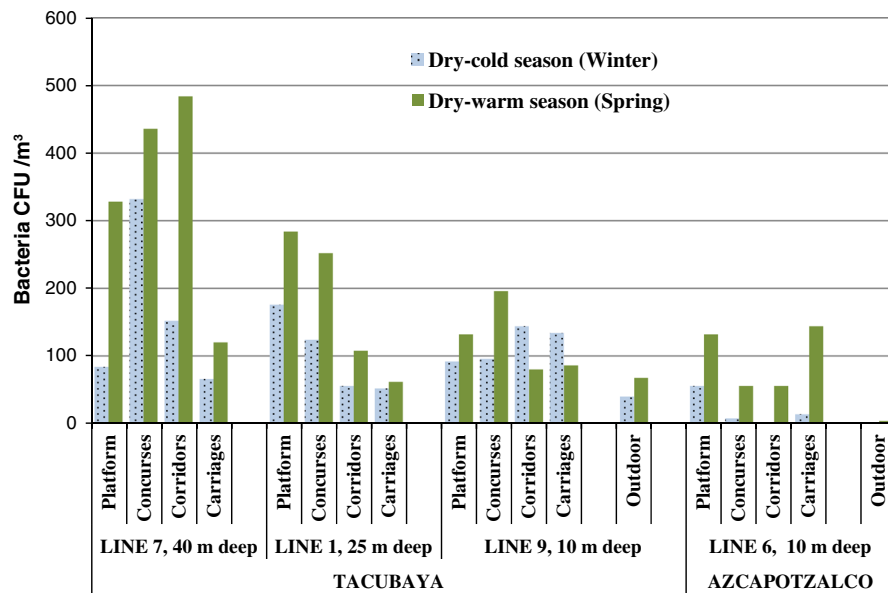


Fig. 2 Bacteria concentrations in subway sites

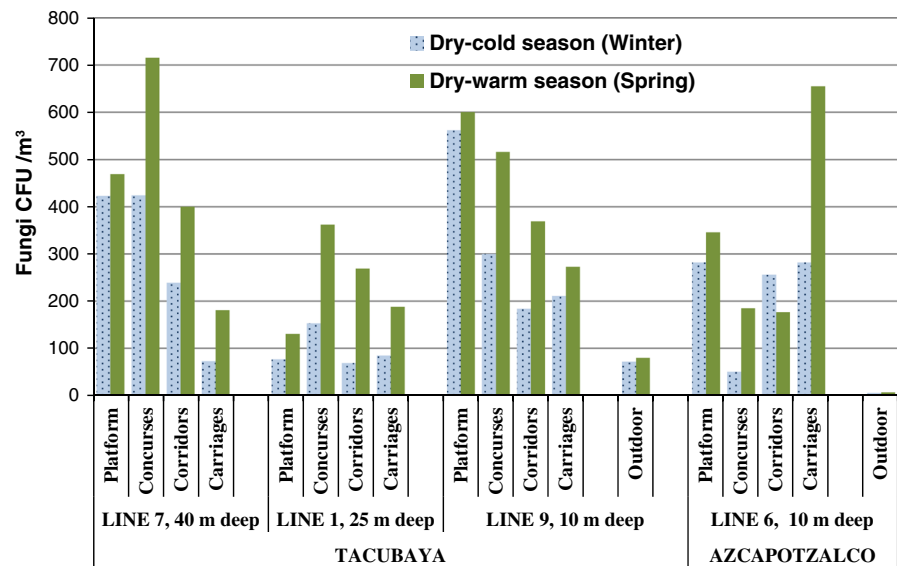
concourses, corridors, and carriages, respectively. Our findings showed that bacteria concentrations increased with increasing depth, where higher temperatures are found because of poor ventilation and confinement. Highest airborne bacteria concentrations were registered in Line 9, the most crowded line, putting in evidence the importance of people as vectors of microorganisms to indoor environments.

Although Azcapotzalco station (Line 6) and Line 9 in Tacubaya are at the same depth, they had different airborne bacteria concentration values, particularly during the dry-cold season when the bacteria concentrations in Azcapotzalco were very low. This could be explained because Azcapotzalco has no connection with other lines; then, the sites of this station have low passengers' transit. During the dry-warm season, the airborne bacteria concentrations are higher due to temperature increases, though still smaller than at Line 9. In general, carriages presented the lowest bacteria concentration due to the open windows and the constant motion. The exception was the carriages of Line 6 leaving Azcapotzalco station, which presented the highest value of airborne bacteria concentration. One possible explanation to this behavior is that Line 6 ends in a surface station, connecting there with another surface Line; therefore, bacteriological contamination in the terminal station could be introduced to the tunnel of that line.

Outdoor concentrations of airborne fungi were at Tacubaya station 72 and 80 CFU m⁻³ for the dry-cold and dry-warm seasons, respectively, while in Azcapotzalco stations, they were very low with 6 and 7 CFU m⁻³ for dry-cold and dry-warm seasons, respectively, as was the case with bacteria. Airborne fungi concentrations inside both stations station and during winter and spring ranged from 69 to 715 CFU m⁻³ in Tacubaya station and from 51 to 356 CFU m⁻³ in Azcapotzalco station, as can be observed in Fig. 3. A clear trend of increasing concentrations with depths and sites was not found. In general, there are higher concentrations during the dry-warm season than during the winter. On average, microorganisms' concentrations in the Tacubaya station were higher than in Azcapotzalco, with the exception of Line 1 which presented lower concentrations. It is interesting to note again, as for bacteria, the high concentration of fungi within the Line 6 carriages, which once more shows that important aerobiological pollution is present in that tunnel.

3.2 Statistic analysis

Pearson's correlation coefficients were estimated in order to determine the relationship between bacteria and fungi airborne concentrations and depth, temperature, and relative humidity percent (Tables 2, 3 for

Fig. 3 Fungi concentrations in subway sites**Table 2** Pearson's correlation among variables in dry-cold season

Variable	Bacteria concentration	Fungi concentration	Temperature	%H	Depth
Bacteria concentration	1.000				
Fungi concentration	0.285	1.000			
Temperature	0.531*	−0.318	1.000		
RH %	0.150	0.079	0.248	1.000	
Depth	0.551*	0.144	0.383	0.386	1.000

* Bold values signifies correlation which is significant at the 0.05 level (two tailed)

the dry-cold and dry-warm seasons, respectively). Temperature and relative humidity percent correlated highly and negatively in the dry-warm season but not in the dry-cold season. During the two seasons, there were positive and significant relationships between bacteria and depth, but only in the dry-cold season, temperature had a significant correlation with temperature. In the case of fungi, a negative correlation was found with temperature, whereas the correlation was positive with depth but no significant. The relationship with relative humidity percent was strong for bacteria and fungi in the dry-warm season; conversely, in the dry-cold season, bacteria and fungi levels had low correlation with relative humidity percent. As mentioned above, this could be because the conditions of each line and station are different.

Figures 2, 3 show that airborne microorganisms' concentrations during the dry-warm season were higher than in the dry-cold season, although these differences were not statistically significant when the

Mann–Whitney test was applied ($p \leq 0.05$). The Levene test showed the homogeneity of variance into the groups. A two-way ANOVA was carried out between lines and sites. The results showed that these two factors interact in the bacteria and fungi cases, with F equal to 4.18 and 9.55, respectively, at $p \leq 0.001$. Then, a one-way ANOVA was performed to analyze the difference in concentration levels of microorganisms in each site among lines, and post hoc tests using the Fisher LSD test were conducted to determine significant differences among each line (Table 4).

Significant differences were found among bacteria concentrations and depth of lines in the platforms, concourses, and corridors. Differences were also significant between Azcapotzalco and Tacubaya stations, especially with the deepest Line 7. Opposite to that, there was no significant difference between bacteria concentration in the carriages of different lines during their transit in the tunnels, which means

Table 3 Pearson's correlation among variables in dry-warm season

Variable	Bacteria concentration	Fungi concentration	Temperature	RH %	Depth
Bacteria concentration	1.0000				
Fungi concentration	0.459	1.0000			
Temperature	0.293	-0.749**	1.0000		
RH %	0.530*	0.499*	-0.711**	1.0000	
Depth	0.886**	0.313	-0.111	0.290	1.0000

* Bold values signifies correlation which is significant at the 0.05 level (two tailed)

** Bold values signifies correlation which is significant at the 0.01 level (two tailed)

that motion and air penetration due to open windows can have an important influence.

Regarding airborne fungi concentrations, significant differences were found in platform and concourses, but not in corridors, amidst different depth of lines, as well as in the reference station of Azcapotzalco at Line 6. Finally, in the case of carriages, significant differences were found for fungi concentrations among the three lines of Tacubaya station with Azcapotzalco station, but not among different depths, as was the case with bacteria (Table 4).

3.3 Characterization of microorganisms

In average, 45 % of bacteria and 76 % of fungi colonies inside the stations occurred in the small-particle fraction, isolated on the second stage, and could be considered respirable, meaning that these microorganisms can be inhaled by workers and passengers. No significant difference was found for any respirable microorganism between the two seasons. The sites inside the station with the lowest percentage of bacteria and fungi were the corridors, perhaps due to the constant passenger's transit that promotes large particles' resuspension. A lower concentration of respirable bacteria is observed in carriages, maybe because of the presence of larger particles from the tunnels (Table 5).

Sixty-one bacteria colonies were accounted by means of isolation and microscopic observation, most of them belonging to the genera *Bacillus*. Three prevailing types were found in two stations: Gram-positive sporulating short *Bacillus*, Gram-positive non-sporulating short *Bacillus*, and *Streptococcus* (Table 6). These bacteria indicate pollution which could be a health risk for users and workers, although Gram-negative coliform bacteria, especially *Escherichia coli*,

that is a fecal indicator and has been reported in outdoors environment in Mexico City (Rosas et al. 1994) was not found. Looking at fungi, 57 colonies were accounted for, and the genera identified at both stations and seasons were, in order of frequency, *Aspergillus* spp, *Penicillium* spp, and *Alternaria* spp.

4 Discussion

Health effects associated with poor indoor air quality have created a need for monitoring the microbiological content of indoor air, particularly in places with large concentrations of people. In this study, we investigated, for the first time in Mexico, the airborne concentrations of bacteria and fungi in the subway environment. Generally speaking, indoor microorganism's concentrations in such enclosed environments are several folds greater than outdoors. Statistically significant differences were found between the two stations Azcapotzalco and Tacubaya. The first one presented the lowest microorganisms' concentrations since it has low passenger transit and it is located nearest to ground level. Although it is generally accepted that a microorganisms' concentration in the indoor aerosols should be similar to outdoor values, meaning that the ratio indoor/outdoor (I/O) should be close to 1 (Strykowska-Sekulska et al. 2007), this is not the case in this research, particularly in deeper subway stations where indoor concentrations of bacteria and fungi were several times higher than outdoor concentrations. The values of I/O ratio inside Tacubaya station ranged from 1.9 to 8.3 (5.4 on average) for bacteria and from 1.6 to 8.9 (4.5 on average) for fungi. The largest ratios were found at the deepest Line 7. These high values are explained by station's overcrowding with high passenger's transit almost all its

Table 4 One-way ANOVA and post-hoc test using LSD in different sites (*p* values)

	Fungi					
	Bacteria			Fungi		
	Platform	Concourses	Corridors	Carriages	Platform	Concourses
ANOVA	F(3,8) = 1.61 <i>p</i> = 0.26	F(3,8) = 0.46 <i>p</i> = 0.71	F(3,8) = 2.5 <i>p</i> = 0.13	F(3,8) = 1.81 <i>p</i> = 0.22	F(3,8) = 0.19 <i>p</i> = 0.90	F(3,8) = 0.34 <i>p</i> = 0.79
Levene Test	F(3,8) = 1.61 <i>p</i> = 0.26	F(3,8) = 0.46 <i>p</i> = 0.71	F(3,8) = 2.5 <i>p</i> = 0.13	F(3,8) = 1.81 <i>p</i> = 0.22	F(3,8) = 0.19 <i>p</i> = 0.90	F(3,8) = 0.34 <i>p</i> = 0.79
L7–L1	≥0.05	0.001	0.01	≥0.05	0.0001	0.008
L7–L9	≥0.05	0.0001	0.02	≥0.05	0.0001	0.01
L7–L6	0.002	0.0001	0.003	≥0.05	0.0001	0.001
L1–L9	≥0.05	≥0.05	≥0.05	≥0.05	0.0001	≥0.05
L1–L6	≥0.05	0.005	≥0.05	≥0.05	0.0001	≥0.05
L9–L6	≥0.05	0.023	≥0.05	≥0.05	0.0001	0.12
						0.02

F degrees of freedom. Values in bold format show significant differences among lines

opening time. A strong relationship between occupant density, human activity, and microorganisms' concentration into the indoor air in university rooms has been reported previously (Stryjakowska-Sekulska et al. 2007). In contrast, the I/O values in carriages were lower, twice the amount found outside, with an average 1.7 and 2.2 for bacteria and fungi, respectively. Although carriages are overcrowded most of the time, this decrease is due to the constant ventilation in the carriages promoted by the open windows and the piston effect of the train.

Although there was no statistical significant difference between the microorganisms' concentrations and the seasons, in general, these were larger during the dry-warm season. Therefore, temperature is an important variable, which favors the presence of microorganisms into the stations, since it directly affects the microorganisms' metabolism and reproduction rate. Temperature increase is related mainly to the ventilation system efficiency in the stations. In the carriages, where the ventilation is effected only through the open windows, the elevated temperatures are most related to the passengers' number. These results clearly underline the importance to improve the ventilation systems not only in the stations but also within the carriages to maintain temperatures below or above 25 °C.

The largest microorganisms' concentrations were noticed in the most crowded Line 9 and the deepest Line 7. Our findings showed that bacteria concentrations increased with increasing depth, due to poor ventilation that favors confinement and higher temperatures.

The number of bacteria and fungi spores measured indoor was about two times those isolated in outdoor samples and showed no significant difference between seasons. Airborne bacteria concentrations found in the Mexican subway are similar to those measured in Tokyo, and during the day at a subway station in Norway (Seino et al. 2005; Dybwad et al. 2012), as well as those measured in several stations in Seoul (Hwang 2010), but they are lower by one and two orders of magnitude compared to bacteria concentrations, and between half and a third of fungi concentrations measured in El Cairo subway (Awad 2002). Nevertheless, it should be highlighted that different microorganisms sampling methods have been used in these studies, thus limiting a conclusive comparison with this research. Gram-positive nonsporulating

Table 5 Percentage of respirable microorganisms

	Bacteria		Fungi	
	Dry-cold season	Dry-warm season	Dry-cold season	Dry-warm season
Outdoor	29 ± 3	62 ± 4	90 ± 7	87 ± 8
Platform	51 ± 5	32 ± 12	91 ± 2	83 ± 9
Concourses	57 ± 13	46 ± 4	72 ± 18	73 ± 18
Corridors	34 ± 19	51 ± 12	57 ± 24	74 ± 3
Average inside the stations	47 ± 10	44 ± 6	73 ± 14	80 ± 5
Carriages	33 ± 4	35 ± 3	75 ± 17	67 ± 28

Table 6 Microscopic identification of isolated microorganisms

	Tunnel stations		Outdoor	
	n	%	n	%
<i>Fungi</i>				
<i>Aspergillus</i> spp.	85	47	43	51
<i>Penicillium</i> spp.	54	30	27	32
<i>Alternaria</i> spp.	41	23	15	17
Total isolated	180		85	
<i>Bacteria</i>				
Gram-positive nonsporulating short <i>bacillus</i>	152	85	55	61
Gram-positive <i>streptococcus</i>	23	15	35	39
Total isolated	175		90	

Bacillus was the most abundant bacteria, accounting for 85 % of all collected bacteria, followed by *Streptococcus* with 15 %. The outdoor presence of *Streptococcus* showed a higher contribution (39 %), which suggests that this genera is less resilient to the environment found within the stations. The presence of these Gram-positive bacteria is in agreement with the culture-based studies made in indoor environments. Hwang (2010) and Dybwad et al. (2012) also reported, for a Norwegian subway, that more than 80 % of the isolated spore-specific bacteria cultivations were classified into the bacterial genus *Bacillus*. Many *Streptococcus* species are nonpathogenic, but others represent known pathogens that can cause pharyngitis, pneumonia, or meningitis in children (Lee et al. 2007).

Aspergillus spp was the most abundant genera of the isolated fungi, accounting for 47 % of collected colonies, followed by *Penicillium* spp with 30 % and *Alternaria* spp with 23 %. These percentages are quite similar to those measured outdoor, suggesting that passengers play an important role in the introduction of microorganisms to the station. These three fungi genera were also reported by Awad (2002), *Aspergillus* being the most abundant genera at El Cairo's subway. On the other hand, Hoseini et al. (2012) reported *Penicillium* spp and *Alternaria* spp as the most abundant genera at Tehran's subway with 35 and 29 % of total airborne fungi, respectively, and Picco and Rodolfi (2000) reported the presence of *Penicillium* spp and *Aspergillus* spp in two subway stations in Milan. Airborne fungi have been identified as a causal factor of several human diseases and allergic reactions, particularly, the presence of *Alternaria* has been associated with rhinitis and asthma in children (Stark et al. 2005).

The potential hazard of airborne bioaerosols is related to the particle size, since respirable particles smaller than 5 µm can be deposited in the alveoli, while larger particles deposit in the upper respiratory tract (Thomas et al. 2008). The percentages of indoor respirable fraction for fungi colonies were greater than bacteria, ranging from 57 to 91 % as compared with 45 % on average for bacteria. This could represent an important health risk issue, since microorganisms can be deposited within the lungs, increasing the risk of illness.

The lack of standards related to indoor microorganisms' concentrations in public places, not only in Mexico but in other countries, makes it difficult to interpret the health significance of results obtained. The OSHA has established 1,000 CFU m⁻³ as the indoor maximum level for bacteria and fungi in working places (OSHA, Eagle Industrial Higiene Associates 2004). According to this standard, the concentrations measured in this study do not represent a risk for the workers of the subway. Nevertheless, in comparison with the limit proposed by the American Industrial Hygiene Association of 250 CFU m⁻³, in almost all the sampled sites, this standard was exceeded (Wonder Makers Environmental Inc 2001).

5 Conclusions

The results of this study showed that the indoor air quality in the Mexico City subway system is worse

than outdoors. Microorganisms' concentrations increased significantly in the most crowded places as well as with increasing depth. It is recommended the improvement of ventilation systems for deeper stations.

Workers and commuters are exposed to aeroallergen agents such as *bacillus* and invasive fungi (*Aspergillus*, *Penicillium*, and *Alternaria*) in the different places of the Mexico City subway although health implications are still uncertain; thus, further research should address the isolation and identification of the most dangerous species, as well as the impact of indoor bioaerosols on human health.

Considering the lack of guidelines related to indoor environments at sites poorly ventilated with a relatively large population concentration, our findings can contribute to the establishment of Mexican standards linked with indoor air quality in airports, buses, and subway stations, as well as inside public transport units.

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