J Occup Health 2002; 44: 112–118

Journal of Occupational Health

Field Study

Environmental Study in Subway Metro Stations in Cairo, Egypt

Abdel Hameed A. AWAD

Air Pollution Department, National Research Centre, Egypt

Abstract: Environmental Study in Subway Metro Stations in Cairo, Egypt: Abdel Hameed A. AWAD, Air Pollution Department, National Research Centre—Airborne viable and non-viable measurements were carried out in two different metro stations, one located in a tunnel and the other on the surface. The concentrations of airborne total viable bacteria (incubated at 37°C and 22°C), staphylococci, suspended dust and oxidants (ozone) were higher in the air of the tunnel station than those recorded at the surface station. In contrast, spore forming bacteria, *Candida spp*, fungi and actinomycetes were found at slightly higher levels in the surface station than in the tunnel station. A statistically significant difference ($p<0.01$) was found between the levels of suspended dust at both stations. *Cladosporium, Penicillium* and *Aspergillus* species were the dominant fungi isolates. *Fusarium, Aspergillus* and *Penicillium* are the most common fungi that produce toxins. Under certain circumstances (host susceptibility, infective dose and aerodynamic diameter) some of the airborne microorganisms e.g. actinomycetes and *Aspergillus* species and staphylococci may cause health problems in exposed persons based on toxic or allergic reactions. *(J Occup Health 2002; 44: 112–118)*

Key words: Air microorganisms, Suspended dust, Ozone, Metro stations

Many quantitative and qualitative studies have been conducted on airborne viable and non viable pollutants in various indoor and outdoor environments such as hospitals[1], schools[2], residential houses[3], cotton mills[4], farm buildings[5], barns housing swine[6], slaughter house[7] and caves[8]. Very limited studies and information exist on the occurrence of microorganisms in the air of subway metro environments, but London's underground railway was investigated by Andrew's[9] and Forbes[10] and the New York subway by Soper[11]. The levels of airborne microorganisms in three underground railway stations were studied in Budapest, Hungary by Szam *et al*[12]. The authors detected pathogenic and anthropogenic bacteria and they found a correlation between bio-contaminant levels and number of passengers and space size. Dust clouds containing high levels of airborne spores (fungi and bacteria) posed a potential exposure problem for persons with hypersensitivity. Data on the aerial concentrations of fungi and actinomycetes were sought during this preliminary survey because these microorganisms can incite allergic reactions in human health when inhaled[13–15].

The primary objective of the study was the comparison of airborne viable and non-viable pollutants at two metro stations with different numbers of passengers and ventilation systems. A secondary objective was to provide information about air quality and hygienic conditions in such an environment.

Materials and Methods

Sampling sites

Two metro stations differing in location, design, nature and number of passengers were selected for this survey. The first station, "Sadat" is located in the tunnel, at El Tahrir square. It is one of the most crowded metro stations in Cairo (about 17–20,000 passengers/h). It is characterized by a mechanical ventilation system (heating, ventilating and air conditioning, HVAC) and the ventilation rate ranges from 4 to 6 times per hour. This station is maintained under positive pressure by a flow of air filtered to remove large particles. The second station "Sayeda Zeinab" is a surface station, located about 1.5 km south of the first station. It is characterized by good natural ventilation, smaller area, fewer passengers (10–12,000 passengers /h) and is located near the Cairo University hospitals and bus station. Air samples were collected near the ticket offices, between 10 a.m and 1 p.m. One set of samples was collected on the same day, once a week, from both stations for 7 wk, during the summer season (May to July, 1997).

Microbial analysis

Air samples were collected with All Glass Impinger

Received July 16, 2001; Accepted Dec 28, 2001
Correspondence to: A.H.A. Awad, Air Pollution Department, National Research Centre, Dokki, Giza, Egypt

Table 1. The concentrations of airborne viable particles (10^3 cfu m^{-3}), total suspended particulate (μg m^{-3}) and ozone (ppb) in the tunnel and surface stations

Indicator	Tunnel station			Surface station		
	mean	SD	range	mean	SD	range
Bacteria, 37°C	2.94	1.83	0.66–6.18	2.81	1.35	1.25–4.92
Bacteria, 22°C	75.4	100	6.1–302	8.14	6.28	3.25–20
Spore forming bacteria	1.04	0.5	0.28–1.94	1.9	1.97	0.62–5.83
Staphylococci	0.194	0.27	0.0–0.69	0.11	0.1	0.0–0.28
Candida	0.06	0.06	0.0–0.14	0.12	0.154	0.0–0.42
Fungi	0.8	0.42	0.0–1.25	0.985	0.8	0.28–2.5
Actinomycetes	0.027	0.055	0.0–0.14	0.32	0.3	0.0–0.97
Total suspended particulate	938.3	124	793.6–1096	447.3	270	131.5–921
Ozone	181	61.7	93.1–267.5	147.4	105	69.3–341.4

n=7.

(AGI-30) samplers, containing 25 m*l* of sterile 0.1% peptone water (Difco, Detroit, MI), in the breathing zone, at a flow rate of 3 *l* air per min. The sampling time was 30 min in duration. A surface plate technique using selective media, Baird Parker agar, Candida selective agar (Merck, Darmstadt), malt extract agar, starch nitrate agar and standard plate agar (Difco, Detroit, MI) were used for counting staphylococci, candidas, fungi, actinomycetes (streptomycetes) and total viable bacteria, respectively. Five milliliters of each sample was heated in a water bath to 80°C for 15 min, to isolate (cultivated only) spore forming bacteria. A poured plate technique with two layers of standard plate agar medium was used for quantifying spore forming bacteria[16]. Two replicate plates were used for counting microbial indicators of each sample. Bacterial plates were incubated at 37°C and 22°C for 48 h whereas; candidas and fungi plates were incubated at 22°C for 3–7 d. Actinomycete plates were incubated at 28°C for 7–14 d. The resultant colonies were expressed in colony forming units per cubic meter of air (cfu/m^3).

Total suspended particulate

Total suspended particulate (cut size up to 35 μm) was collected on conditioned preweighed cellulose nitrate membrane filters (pore size 0.45 μm, diameter 25 mm). The dust samples were collected with an open face filter holder and sampling pump calibrated to draw 3.8 *l*/min, for one h. The filters were weighed after sampling and the amounts of dust were expressed in $\mu g/m^3$. The filters were eluted in 25 m*l*, 0.1% peptone water containing 0.01 (v/v) Tween 80. Samples were shaken well at room temperature for about 15–30 min and several dilutions were prepared. The levels of microbial indicators associated suspended dust were examined as mentioned previously and the resultant colonies were expressed in cfu per gram (cfu/g). The aerodynamic diameter (dae) of the dust particles was calculated from the density and physical diameter (physical diameter measured microscopically at 400X).

Oxidants (expressed as ozone)

Ozone was measured by using the alkaline potassium iodide method[17]. Ozone samples were collected with a pump calibrated to draw 1 *l*/min, for 1 h. The levels of ozone were measured and expressed in ppb.

Climatic parameters

During every sampling dry and wet temperatures were recorded with a psychometer (type 27576 Lürilh LAMPRECHT, Göttingen) and the relative humidity was computed from the psychometer chart.

Statistical analysis

The degree of significance of difference was calculated by using Student's t-test (p=0.01)[18]. Logarithmic transformation (log x+1) was used to normalize microbial data.

Results

A total of seven trials were conducted to evaluate the results of airborne particulate (both viable and non-viable) and ozone in two different metro stations (Table 1). The amounts of total viable bacteria (mean values of 2.94×10^3 cfu/m^3 and 7.54×10^4 cfu/m^3, incubated at 37°C and 22°C, respectively) and staphylococci (a mean value of 1.94×10^2 cfu/m^3) were slightly higher in the tunnel station than those recorded in the surface station (mean values of 2.81×10^3 cfu/m^3, 8.14×10^3 cfu/m^3 and 1.1×10^2 cfu/m^3 for corresponding indicators, respectively). Suspended dust levels in the tunnel station, averaging 793.6 to 1094 $\mu g/m^3$ (a mean value of 938.3 ± 124 $\mu g/m^3$) were higher than those recorded in the surface station averaging 131.5 to 921 $\mu g/m^3$ with a mean value of 447.3

Table 2. Maximum, minimum and mean levels of microbial indicators associated suspended dust (cfu/g)

Indicator	Tunnel station			Surface station		
	Min	Max	Mean	Min	Max	Mean
Tvc, 37°C	3.6×10^3	3.1×10^7	9.3×10^6	2.0×10^6	3.1×10^7	1.1×10^7
Tvc, 22°C	6.9×10^6	8.0×10^7	3.1×10^7	7.1×10^6	2.5×10^8	1.0×10^8
S.E.B	1.7×10^5	1.4×10^7	3.7×10^6	1.0×10^6	8.3×10^7	1.9×10^7
Fungi	0.00	6.8×10^5	3.3×10^5	7.7×10^5	8.3×10^6	2.8×10^6
Actino.	0.00	3.4×10^5	1.4×10^5	0.00	2.9×10^6	1.3×10^6

Tvc: total viable bacteria, S.F.B: spore forming bacteria, Actino: actinomycetes.

± 270 $\mu g/m^3$, and a statistically significant difference ($p<0.01$) was found between dust levels at the two stations. The aerodynamic diameter (dae) of suspended dust ranged from 1.2 to 28 μm. The highest frequency (50–55%) of the aerodynamic diameter distribution was 3.5 μm in the tunnel station, whereas the highest frequency (27–37%) was 10–14 μm in the surface station. In the present study, ozone was detected at a mean value of 181.5 ppb in the tunnel station, whereas it was only 147.4 ppb in the surface station (Table 1). Relative humidity ranged between 58 to 70% with a mean value of 65% in the tunnel station, whereas it varied from 35 to 48% with a mean value of 42% in the surface station. In contrast, the temperature averaged 30°C and 25°C in the surface and underground stations, respectively. Temperature and relative humidity are controlled by ventilation.

Airborne spore forming bacteria (a mean value of 1.9×10^3 cfu/m^3), candidas (a mean value of 1.2×10^2 cfu/m^3), fungi (a mean value of 9.85×10^2 cfu/m^3) and actinomycetes (a mean value of 3.2×10^2 cfu/m^3) were found at relatively higher levels in the surface station than in the tunnel station. Table 2 gives the average and mean concentrations of total viable bacteria, spore forming bacteria, total fungi and actinomycetes (streptomycetes) associated suspended dust (cfu/g). It is clear that the microbial indicators associated with suspended dust in the surface station recorded higher averages (10^6–10^8 cfu/g) than those found in the tunnel station (10^5–10^7 cfu/g).

The mycological examination of 272 isolates is summarized in Table 3. *Penicillium*, *Cladosporium* and *Aspergillus* species were the dominant fungi isolates at both stations. *Aspergillus fumigatus* comprised a small fraction (2.1–2.4%) of the total *Aspergillus species* detected at both stations. In contrast, *Fusidium*, *Gunninghamella*, *Helminthosporium*, *Mucor* and *Spicaria* were only detected in the surface station, whereas *Botrytis* and *Monilia* were only found in the tunnel station, but in low numbers.

Table 3. Microscopic identification of isolated fungal spores at both stations

Fungus	Tunnel station		Surface station	
	n	%	n	%
Alternaria	2	2.44	8	4.21
Aspergillus fumigatus	2	2.44	4	2.10
Aspergillus niger	10	12.2	30	15.79
Aspergillus species	10	12.2	68	35.78
Botrytis	2	2.44	0	0
Cladosporium	26	31.7	32	16.84
Fusarium	2	2.44	6	3.16
Fusidium	0	0	2	1.05
Gunninghamella	0	0	2	1.05
Helminthosporium	0	0	2	1.05
Monilia	4	4.87	0	0
Mucor	0	0	4	2.10
Penicillium	10	12.2	18	9.47
Spicaria	0	0	2	1.05
Sporotrichium	2	2.44	2	1.05
Yeasts	10	12.2	4	2.10
Non sporulating hyphae	2	2.44	6	3.16
Total isolates	82		190	

Discussion

This study can be considered to be the first one that deals with such an environment in Egypt. The two sampling locations differ in two major respects: one is human activity and the other is the nature of the indoor/outdoor environments. This environment is quite different from other environments investigated, so that some difficulties with interpretation of results are due to, insufficient data and the presence of numerous variables such as human activities and train piston effects. The liquid impinger sampler was used in the present study because it is efficient for sampling bacteria and fungi[6]. And collection in liquid provides some protection for the microorganisms from physical stresses[19]. On the other

hand, survival of vegetative cells decreases with increasing collection time[20]. Collection efficiency also drastically decreases due to insufficient impingement into the liquid, particle bounce and reaerosolization. The number of reaerosolized particles increases with sampling time, but it's less than 10% in the first hour of sampling[21]. The filtration method is efficient for collecting fungi and spore forming bacteria and the problem associated with filters is inactivation of vegetative cells due to stress and desiccation[22].

In the present study, airborne total viable bacterial counts varied from 10^2 to 10^4 cfu/m^3 and saprophytic bacteria incubated at 22°C were recorded in higher counts than those incubated at 37°C. These results were similar to those previously reported by Riley and O'Grady[23] who found airborne bacterial counts raised to about 1000 cfu/m^3 under any domestic activity. Lee *et al.*[24] found airborne bacteria in the range of 10^2–10^4 cfu/m^3 in a city. Moreover, Bovallius *et al.*[25] recorded bacteria at mean values of 4×10^3, 3×10^3 and 5.6×10^2 cfu/m^3 in the air at city, country and coastal stations, respectively. Gregory[26] found that concentrations of airborne bacteria vary greatly with the amount of mechanical and human activity. In addition, saprophytic microorganisms are the most prevalent in the indoor environment and are important in respect to health effects[27]. Moreover, staphylococci are mainly related to skin scales and respiratory secretions[28] and human activities[12]. It is suggested that the slightly higher counts of airborne staphylococci and total viable bacteria in the tunnel station are due to insufficiency of the air conditioning system and ventilation rate, passenger activities and numbers.

In contrast, spore forming bacteria were detected in higher counts in the surface station than those found in the tunnel station. This may be because spore forming bacteria are related to natural and larger dust particle size[29]. Air is exchanged with the outdoors which reduces levels of contaminant indoors. Therefore, mechanical ventilation in the tunnel station reduces and removes particles >5 μm whereas natural ventilation at the surface station possibly removes smaller particles and leaves larger ones suspended[30]. Brown[31] reported that 80% of the dust particles in the London underground averaged 2.5 μm in diameter. Moreover, Gram positive bacteria (*Staphylococcus* and *Micrococcus*) are normally found predominantly indoors[32], and the presence of Gram positive bacteria indicates overcrowding and inadequate ventilation[28], but some microorganisms survive better at higher relative humidity but other at lower relative humidity. Relative humidity is the most crucial factor for the survival of air microorganisms, but the effect of relative humidity on airborne bacteria is controversial. De Ome[33] found that the death rate of airborne bacteria is greater at high relative humidity, whereas Dunklin and Puck[34] reported that intermediate humidity levels are the most lethal. And the movement of water molecules of microorganisms dependent on humidity and temperature[35]. Mackenzie[36] found that *Candida spp* are affected by higher rather than lower humidity. This may cause the killing of candidas in the tunnel station, because logically, relative humidity is higher (65%) than that at the surface station (42%). Also Nevalainen[37] concluded that the amount of moisture indoors is the major factor affecting the survival of airborne microorganisms. In addition, candidas (dermatophytes) are derived from patients and infected skin, and can be disseminated into air from menstruum or intermediate resting places[30, 38]. The surface station is located near Cairo University hospitals and many of the patients and carriers of candidas pass through this station. It is suggested that this may cause increased dissemination of candidas into the air.

There is no statistically significant difference (P>0.01) between airborne fungi counts at both stations (Table 1). Fungal spores are always present in outdoor air and their numbers are affected by weather and geographical locations[15]. The slightly higher fungi counts recorded at the surface are due to the fact that this station is naturally ventilated, whereas the tunnel station is mechanically ventilated. Fungi spores from the external environment are therefore filtered out of the air supplied to the underground station and remain in the air of the surface station. *Penicillium*, *Cladosporium* and *Aspergillus* are common at both stations. *Penicillium*, *Cladosporium* and *Aspergillus* are common in both indoor and outdoor environments[39, 40]. Furthermore, *Aspergillus* and *Penicillium* are commonly found indoors, whereas *Cladosporium* and *Alternaria* are of outdoor origin[41]. Reponen[42] found *Cladosporium* and *yeasts* in higher counts outdoors than indoors. *Aspergillus* and *Penicillium* species survive better under dry air conditions whereas *Botrytis* and *Stachybotrys atra* very quickly decline in viability[43]. Moreover, HVAC systems have been associated with increased indoor airborne densities of *Penicillium and Aspergillus*[44]. Kozak *et al.*[3] found that, indoor mold spore levels reflected outdoor levels. Also, Pady[45] found *Fusarium*, *Gunninghamalla*, *Monilia*, *Helminthosporium* and yeasts in low numbers during summer. Some common microbial agents such as (1-3)-β-D-glucan and mycotoxins possess biological potency and cause diseases. *Fusarium*, *Cladosporium* and *Aspergillus* are the most common fungi producing toxins that cause mycotoxicosis[46]. In the present study, fungi levels were similar to those detected previously in a poultry house by Clark *et al.*[47] and a computer facility building by Hung and Terra[48]. Moreover, Abdel Hameed and Farag[49] found airborne mold at mean values of 456, 820 and 648 cfu/m^3 in livingrooms, bedrooms and outdoors, respectively, at homes in Cairo. Human exposure to airborne fungal spores may cause adverse health effects[50]. In the present study, airborne fungi levels

exceeded the acceptable level of fungi indoors, less than 150 cfu/m^3 for a mixture of species other than pathogens and 300 cfu/m^3 if the species is *Cladosporium*[41]. In the present study actinomycetes were recorded at higher counts in the surface station than in the tunnel station. Actinomycetes are commonly found in agricultural areas (outdoor). Lloyd[51] reported that streptomycetes are mainly transported on natural and soil dust particulates. The presence of actinomycetes indoors is an indication of bio-contamination, and several types of actinomycetes are associated with allergenic alveolitis[52]. In addition, streptomycetes species stimulate lung macrophage reactions which lead to inflammation and tissue injury[53].

The high dust levels in the tunnel station are attributed to the activities of passengers near the sampling sites, insufficient ventilation, effect of train piston pressure, airflow at the front of the platform and floor cleaning. The variation (not statistically significant, $p>0.01$) in the microbial indicator levels associated suspended dust (Table 2) may be attributed to the differences between particle sizes, nature, composition and sources of dust at the two stations. This conclusion is confirmed by many investigators such as Lighthart *et al.*[54] and Thorne *et al.*[6] who concluded that dust and its composition significantly affect the survival of attached microorganisms. Dust on surfaces (floors, walls and clothes) exists in different sizes and is periodically resuspended in air due to human activities[29]. Moreover, the aerosolization of fungal spores or other organisms may be sporadic and the air may not be perfectly mixed. Therefore, non detection of viable fraction was not proof of the absence of specific organisms[55]. Microorganisms associated dust may be disseminated into air which can be inhaled by workers and passengers. Particles less than 5 μm can penetrate into lung tissue. So the chance for particle penetration into worker's lungs is high in the tunnel station (the highest frequency, 50–55%, of aerodynamic diameter was 3.5 μm).

The high ozone concentrations in the tunnel station are attributed to the effects of electrical charge from trains, daytime lamps and insufficient ventilation. These, in addition to photocopying machines and electronic dust cleaners are ozone generators[56]. Our measurements of ozone were higher than indoor guidelines (120 ppb)[57]. These concentrations give rise to eye irritation[58] and inhibit the immune systems[56]. Ozone reacts with olefins (exhaust from cars) to form a germicidal pollutant, Open Air Factor (OAF)[59]. Microbial clusters (such as staphylococci) are more likely to survive than single cells[60]. OAF is extremely rapidly adsorbed onto the surface before reaching the inner cells of clusters[61] and the rapid adsorption of OAF onto any surface effectively removes ozone from the underground station. This is likely to have a major effect on the difference in survival of vegetative cells in the indoor and outdoor stations. It is suggested that ozone may preserve or kill normal microorganisms and it may cause genetic effects and modify an organism to be more environmentally resistant, pathogenic and infective.

Conclusion and Recommendations

There are no accepted standard guidelines for the levels of air bio-contamination indoors and outdoors. Because the concentration of microorganisms may be low but the predominant species dangerous. A low airborne microorganism levels do not indicate a clean and healthful environment[28]. The impact of airborne microorganisms on human health should be further investigated in subway metro station environments. Workers and passengers in metro stations may be exposed to unsafe levels and types of air microorganisms. The aeroallergen agents (mold, streptomycetes and bacilli species), infectious indoor microbes (candidas and staphylococci) and invasive fungal agents (*Aspergillus fumigatus*) were detected in both stations. Moreover, total suspended particulate and ozone were recorded at higher levels in the tunnel station. Therefore, the chance of causing pneumoconiosis, allergy and epidemiological diseases is present, but bioaerosol risk assessment is very difficult due to the complexity of bioaerosols, lack of guidelines and susceptibility of people. Ventilation systems, moisture and human activities are the main factors increasing air biocontaminants and mitigating or reducing air biocontamination indoors. It is recommended to prevent moisture, water incursion and to improve ventilation systems. Wiping and cleaning should be carried out during the night with water and disinfectants.

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