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Toward Understanding Prevalence of Airborne Microorganisms in a Hot-Arid Environment

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Abstract

This study aims to determine prevalence of microorganisms in the air state and those associated particulate matter (PM) in a hot arid environment (Makkah city, Saudi Arabia) in relation to time of the day, PM concentration and meteorological conditions during the period between July and September 2014. PM and black smoke samples were collected on cellulose nitrate membrane filters during the daytime (8.00 am - 20.00 pm) and the nighttime (20.00 pm - 8.00 am). PMs filters were eluted in buffer phosphate and aliquots were spread plated onto the surfaces of trypticase soya agar, malt extract agar, and starch casein agar media for counting bacteria, fungi and actinomycetes associated PM, respectively. Airborne microorganisms were collected using an Andersen two stage impactor sampler equipped with Petri plates containing the previously mentioned agar media. The Andersen two-stage viable cascade impactor sampler separates particles into coarse (≥8 μm) and fine (≤8 μm) size fractions. Airborne microorganisms were taken at three day time-scales: in the morning (8 am - 10 am), at the afternoon (13.00 pm - 16.00 pm) and in the evening (22.00 pm - 1.00 am). The average concentrations of PM (149.5 μg/m^3) and smoke (57.03 μg/m^3) were higher in the daytime and nighttime, respectively. The greatest concentrations of microorganisms associated PM were found in the daytime, however the peak concentration of airborne microorganisms was found in the evening time. Fine microbial fraction constituted ~60% - 75.9% of the total microbial concentrations. Positive correlations were found between bacteria with PM concentration in the daytime and meteorological conditions at the nighttime. Temperature and relative humidity positively affected survivability of microorganisms associated PM at the nighttime and airborne fungi as well. This study helps understand distribution pattern of microorganisms in the atmosphere of a hot-arid environment.

Keywords: microorganisms; air; size fraction; PM; time of the day; arid environment; meteorological conditions

1. Introduction

Arid regions/deserts are major source of PM (Meola *et al*., 2015). PM varies in its composition (biological or mineral), size and shape (Kulkarni *et al.,* 2011). Particles originate from various natural and anthropogenic sources (Fang *et al*., 2007). Biological particles are particles of biological origin suspended in the air such as: bacteria, fungi, viruses, microbial toxins, plant debris, pollen grains and enzymes (ACGIH, 1999). Biological particles estimated at ~ 25% of the total PM over the land surface (Jaenicke, 2005). Microorganisms are independently (freely) or not-independently (attached PM) suspended in the atmosphere (Shaffer and Lighthart, 1997; Burrows *et al*., 2009), and in turn their biological and physiological characters and prevalence pattern may be changed (Alghamdi *et al*., 2014).

Biological particles (mainly microorganisms) affect air quality, ecosystem and human health (Lin and Li, 2000; Huffman *et al*., 2013; Tao *et al*., 2014). Biological particles are efficient cloud condensation nuclei (Morris *et al*., 2013) and influence weather and climate system (Chen *et al*., 2012). Biological particles including "fungi and pollen grains" are potential aeroallergens (Sindt *et al*., 2016); and enhance asthma and other respiratory conditions (Dales *et al*., 2003). Particles with both biological and non-biological origins can be linked and transported together in the atmosphere (Alghamdi *et al*., 2014), enhancing human health responses (Ryan *et al*., 2009). The effect of dust storms on atmospheric microbiology (Griffin *et al*., 2003; Garrison *et al*., 2003) and their impact on human health were studied in the arid environment (Griffin and Kellogg, 2004).

Microbial concentrations vary over various time-scales (Lighthart, 1994) and microbial particle size determines its behavior (deposition/dispersion) in the atmosphere (Nicholson, 1988). Anthropogenic activities, environmental conditions and periodicity of sources may influence composition, abundance, behavior, and survivability of microorganisms in the atmosphere (Sippula *et al*., 2013). Many studies have been carried-out on concentrations and types of air pollutants inside and outside environments of the holy mosques, Saudi Arabia (Abdel Hameed and Habeeballah, 2013; Mashat, 2015). However less information is available on the prevalence of culturable microorganisms in relation to time of the day, particle size, PM concentration and meteorological conditions in the atmosphere of Makkah city, although many studies have been conducted worldwide (Levetin and Dorsey, 2006; Sadyś *et al*., 2016; Vélez-Pereira *et al*., 2016; Gao *et al*., 2016). The present study aims to understand the prevalence of culturable microorganisms suspended in the air either as individual organisms or attached to dust particles through: 1) evaluating airborne bacteria, fungi, and actinomycetes regarding their concentrations, size fractions, and temporal variations, 2) determining microbial community associated PM in the daytime and the nighttime, and 3) understanding the effects of meteorological conditions and PM concentration on microbial survivability.

2. Materials and Methods

2.1 Sampling site and strategy

Makkah (the holly) city (21^{o} 29 N, 39^{o} 45 E), Saudi Arabia, is located at an altitude of 277-m above the sea level and ~ 80 km inland, east of the red sea (Fig. 1). Makkah is an arid region characterized by severe geographical and environmental conditions. Mountains disperse along over the city, representing ~ 90% of the city area with no permanent plant cover, hot weather, frequent dust storms and rare rain events. Makkah city is always busy by people along over the year due to its religious importance for Muslim world.

The sampling site was chosen to be away from any direct anthropogenic activities. Sampling was taken at the roof of a ~ 13 m high building of the Custodian of the Two Holy Mosques Institute for Hajj and Umrah Research, located inside the main campus of Umm Al-Qura University, Aziziyah region (Fig. 1). Aziziyah region is a commercial-residential area (Auer, 1978), characterized by heavy traffic, parking, shops, hotels, and limited plant cover.

PM and black smoke samples were conducted during the daytime (8.00 am - 20.00 pm) and the nighttime (20.00 pm - 8.00 am). Airborne microorganisms were conducted at three time-scales: in the morning (8.00 am - 10 am), at the afternoon (13.00 pm - 16.00 pm), and in the evening (22.00 pm - 1.00 am) to determine their variations along over the daytime. The samples were monthly collected on 20th - 30th days, between July and November 2014, where weather is extremely hot and millions of people arrive Makkah to perform Umrah (Ramadan month) and Hajj (Hajj season).

2.2 Sampling of PM and black smoke

PM and black smoke samples were collected on pre-weighted sterilized cellulose nitrate membrane filters (0.45 μm pore size, 25 μm diameter) using open face holders and vacuum pumps calibrated to draw 12.5 *L*/min and 1 *L*/min, respectively. The filters of PM were weighted and along with sampling time and flow rate, the concentrations were calculated. The amount of light reflected from black stain was measured using a reflectometer (EEl-Model 43-Digital, UK), smoke concentration was determined from look up tables (available from AEA Technology Environment), and the equivalent smoke concentration was calculated using the volume of air sampled and the reading of the reflectometer. PM and smoke concentrations were calculated and expressed as microgram per cubic meter of air (μg/m^{3}).

2.3 Microorganisms associated PM

The filters of PM were eluted in 10 ml sterilized distilled water containing 0.01% Tween 80 (Sigma-Aldrich, USA), and shaken well for 30-60 min. Aliquots (0.5 ml) of the original sample were spread plated, in duplicate, onto the surface of Petri plates containing trypticase soya agar supplemented with 50 ppm cyclohexamide, malt extract agar supplemented with 50 ppm chloramephenicol, and starch casein agar media (BD, Sparks, USA) for counting of bacteria, fungi and actinomycetes associated PM, respectively.

2.4 Airborne microorganisms

Airborne microorganisms were collected by using an Andersen two stage viable cascade impactor sampler (TE-10-160, Tisch Environmental, OH, USA). It separates particles into two size ranges, fine (<8 μm) and coarse (>8 μm). The sampler was operated at a manufacturer recommended flow rate 28.3 *L*/min for 5 min. Petri plates containing the previously mentioned agar media were used to count bacteria, fungi and actinomycetes, respectively. Two consecutive air samples were taken during each sampling event (a total of 12 plates/event, 36 plates/day). The sampler was sterilized by using isopropyl alcohol between each sampling run.

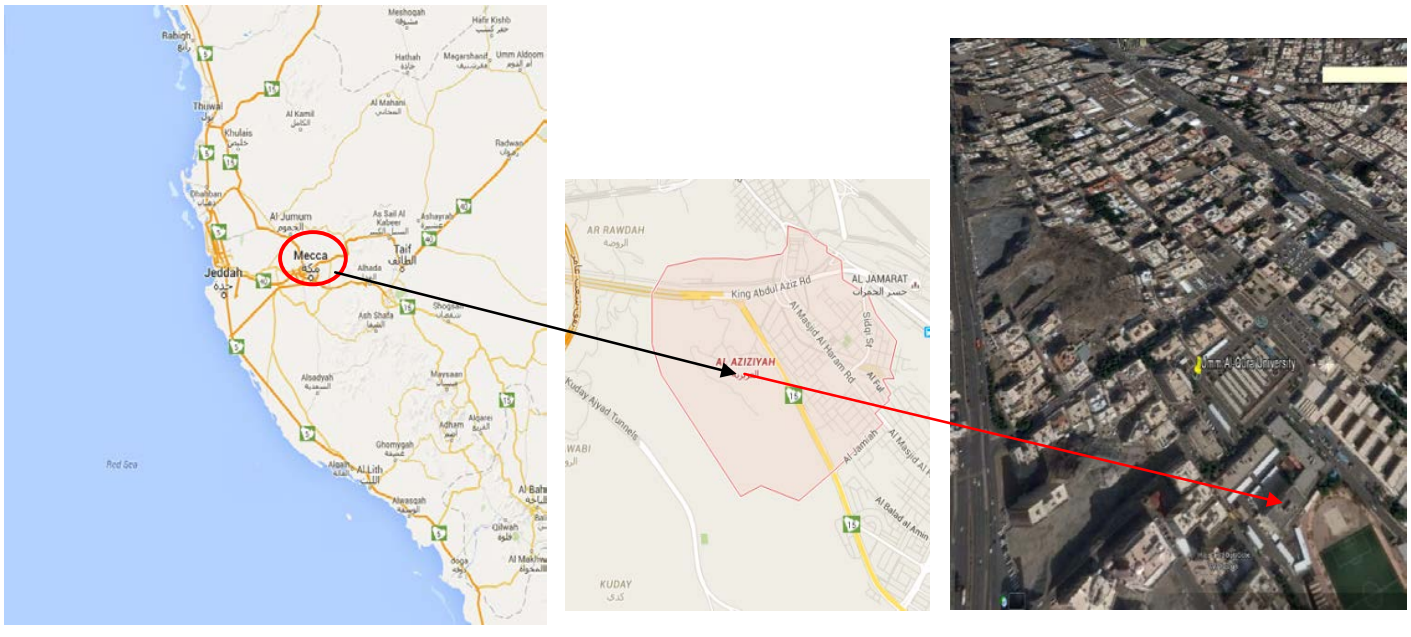

Figure 1. Map of Makkah city showing the sampling site

2.5 Microbiological analysis

Bacterial plates were incubated at 28°C for 2 days, while fungal and actinomycetes plates were incubated at 28°C for 5 - 7 and 7 - 15 days, respectively. In the case of using Andersen cascade sampler, the positive-hole correction was applied to the raw colony forming unit (CFU) recovered on each plate (Andersen, 1958). The resulting colonies were counted and the concentrations were calculated and expressed as CFU/m^3. Fungal colonies were identified to the genus level, except *Aspergillus* to the species level. Fungal isolates were identified using macroscopic and microscopic features according to literature (Raper and Fennel, 1977; Ellis, 1971; Barnett and Hunter, 1999).

2.6 Meteorological conditions

The hourly data of meteorological conditions were obtained from the Presidency of Meteorology and Environment (PME) Saudi Arabia. Meteorological conditions significantly differed during the times of the day. Table 1 shows the meteorological data of temperature (T°C), relative humidity (RH%), wind speed (WS) and wind direction (WD). Temperature, relative humidity, and wind speed ranged within 30 - 39°C, 17.6 - 67.3% and 0 - 10 mph, respectively. Temperature and wind speed achieved the greatest records at the afternoon time while relative humidity in the evening time. The daily prevailing wind directions were west-south-west (W-SW) and west-North West (W-NW) during the period of study.

2.7 Statistical analysis

The results were analyzed using descriptive statics, range, mean, standard deviation and percentiles. Spearman's rank correlation test was used to determine the relationships between PM and black smoke concentrations and meteorological conditions with airborne microorganisms. Mann Whitney U test was used to ascertain the significance of differences between PM, smoke, and airborne microbial concentrations in different time scales. A probability of ≤ 0.05 was considered significant. Non parametric static was used because environmental data were not normally distributed.

Table 1. The range and mean values of meteorological conditions during the period of study

Time	T °C	RH%	WS/ mph	Prevailing WD
Morning	(26-39) [34±2.87])	(10-78) [30.3±18.2]	(0-8) [3.2±2.6]	N-NW/S-SW
Afternoon	(35-46) [40.9±2.45]	(9-56) [18.7±11.4]	(0-20) [11.1±5.7]	W-NW/W
Evening	(29-37) [33.2±2.1]	(18-79) [47.9±14]	(0-5) [2.6±1.2]	N-NW/NW
Daily	(30-39) [35.9±2.1]	(17.6-67.3) [32±13.3]	(0-10.3) [5.6±2.6]	W-SW/ W-NW.

(Range), [mean±SD]

3. Results and Discussion

3.1 PM and smoke

PM and black smoke concentrations ranged between 62.2 - 298.4 µg/m^3 and 13.5 - 103 µg/m^3, respectively. The average concentration of PM was higher in the daytime (149.5 µg/m^3) while smoke in the nighttime (57.03 µg/m^3), (Fig. 2). Non-significant differences were found between concentrations of PM registered in the daytime and nighttime, however significant difference was found between black smoke concentrations ($P = 0.036$), higher concentration shifted toward the nighttime.

In the present study PM concentrations exceeded the World Health Organization limit value of 120 µg/m^3 (WHO, 2000), as no-limit value is available for Saudi Arabia. Generally Saudi Arabia has experienced large amount of suspended dust (AlHarbi *et al*., 2014) due to the nature of desert environment and frequent windblown dust storms (Griffin and Kellogg, 2004). Anthropogenic activities (traffic/ construction) significantly increase PM concentration in the daytime but in the nighttime weather stability conditions and relatively higher relative humidity accelerate settlement of coarse particles, leaving smoke particles airborne a longer period of time. Particle's size determines whether particle remains airborne or settles down (Fernstrom and Goldblatt, 2013).

3.2 Microorganisms associated PM

PM provides nutrition and protection to the attached microorganisms; however composition of PM may have toxic effects to microorganisms. Fig. 3 shows the mean concentrations of microorganisms associated PM in the daytime and nighttime. Microbial concentrations were higher in the daytime, with bacteria-associated PM were the common parameters in the daytime (420.2 CFU/m^3) and nighttime (330.7 CFU/m^3). Actinomycetes were found in very low concentrations, and fungi averaged 16.8 CFU/m^3 and 12.9 CFU/m^3 in the daytime and nighttime, respectively. In the present study, microbial community associated PM was low, achieving higher concentration in the daytime. In general PM is considered a poor medium for survival of the attached microorganisms in the arid environment, because PM is a poor nutrient medium; PM is mainly sand that may keep temperature high longer period of time. Higher microbial concentrations during the daytime may be attributed to higher PM concentrations due to high turbulences, re-suspension of street dust and human activities (Abdel Hameed *et al*., 2016). Bacteria are mainly correlated to anthropogenic activities and fungi to biotic sources (Bowers *et al*., 2011). Moreover fungi and actinomycetes are typically autochthonous organisms (Alghamdi *et al*., 2014), and ubiquitous in biosolid and organic soil; and such conditions are less present in Makkah's environment.

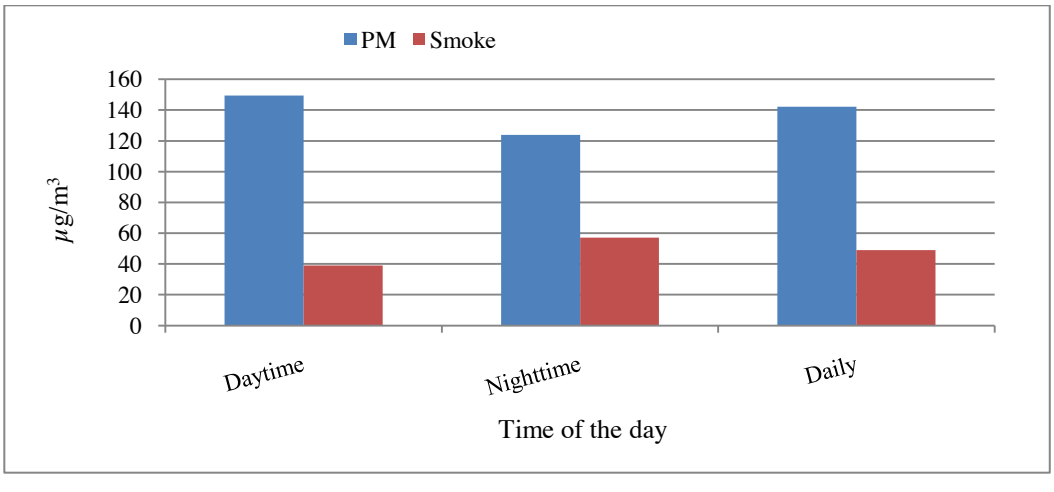

Figure 2. The mean concentrations of PM and black smoke during the period of study

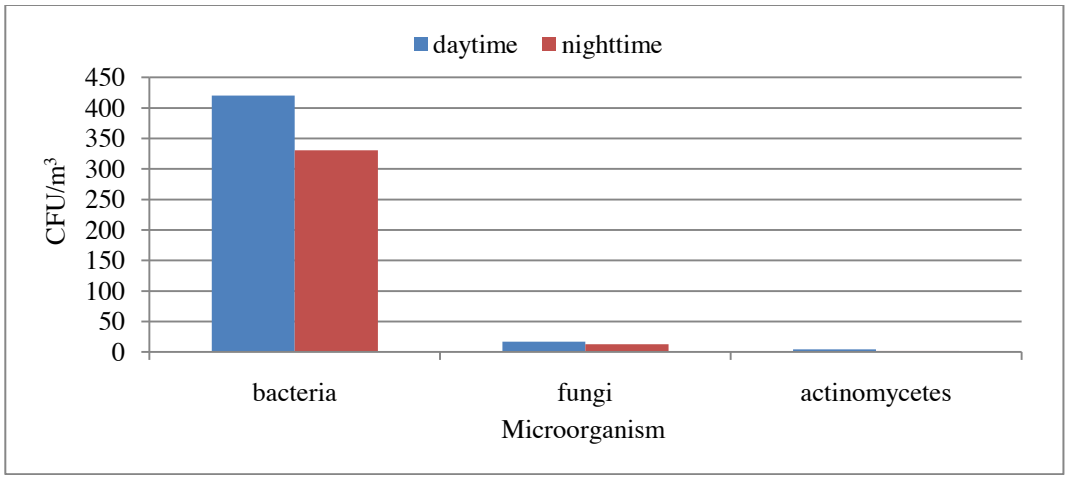

Figure 3. Concentrations of microorganisms associated PM during the daytime and nighttime

Table 2 shows Spearman's rank correlations between microorganisms associated PM with variables including PM, black smoke and meteorological conditions. Positive correlations were found between bacteria and PM concentration as well as fungi and smoke concentration. Actinomycetes showed positive correlation with smoke at the nighttime and negative correlations with PM, black smoke and meteorological conditions in the daytime. It is suggested that the dominant actinomycete sources may be changed between the daytime and nighttime according to the prevailing wind directions. The nature and composition of PM effectively influence microbial survivability than PM mass concentration.

PM, meteorological conditions, depth of the mixing layer, particle size and time of the day may synergistically affect microbial survivability in the atmosphere. Bacteria showed positive correlation with PM in the daytime, and with meteorological conditions in the nighttime (Table 2). In general hot weather conditions detrimentally affect microbial survivability. Significant differences were found between fungal concentrations registered in the daytime and nighttime in Taiwan (Lin and Li, 1996). Hot weather increases mycelia growth and reduces spore production (Damialis *et al*., 2015). Fungi were found in low concentrations in summer months (Sindt *et al*., 2016).

3.3 Airborne microorganisms

3.3.1 Overall culturable microbial concentrations

Airborne microorganisms varied over various time of the day (Fig.4). The maximum microbial concentration was found in the evening time and the minimum at the afternoon time. Significant differences ($p \leq 0.032$) were found between total airborne microbial concentrations recovered in the evening time and afternoon time. Breza-Boruta and Paluszak (2007) classified the daily bacterial concentrations as moderately contaminated (>1000-3000 CFU/m^3) and uncontaminated (<1000 CFU/m^3).The WHO suggested 500 CFU/m^3 as an acceptable limit value of airborne fungal concentration (WHO, 1990). In the present study airborne microbial concentrations were considered low, bacteria and fungi did not exceed 3000 CFU/m^3 and 500 CFU/m^3, respectively.

Table 2. Spearman's rank correlations between microorganisms-associated-PM with PM, smoke and meteorological conditions

Variable	Daytime			Nighttime		
	Bacteria	Fungi	Actinomycetes	Bacteria	Fungi	Actinomycetes
PM	0.390*	0.104	-0.290	0.143	0.164	0.222
Smoke	0.017	0.154	-0.133	0.123	0.156	0.340
T °C	0.180	0.080	-0.200	0.580*	0.470*	0.146
RH%	-0.070	0.056	-0.133	0.540*	-0.050	-0.150
WS	-0.020	-0.450*	-0.006	-0.759*	-0.210	0.130

*$P \leq 0.05$

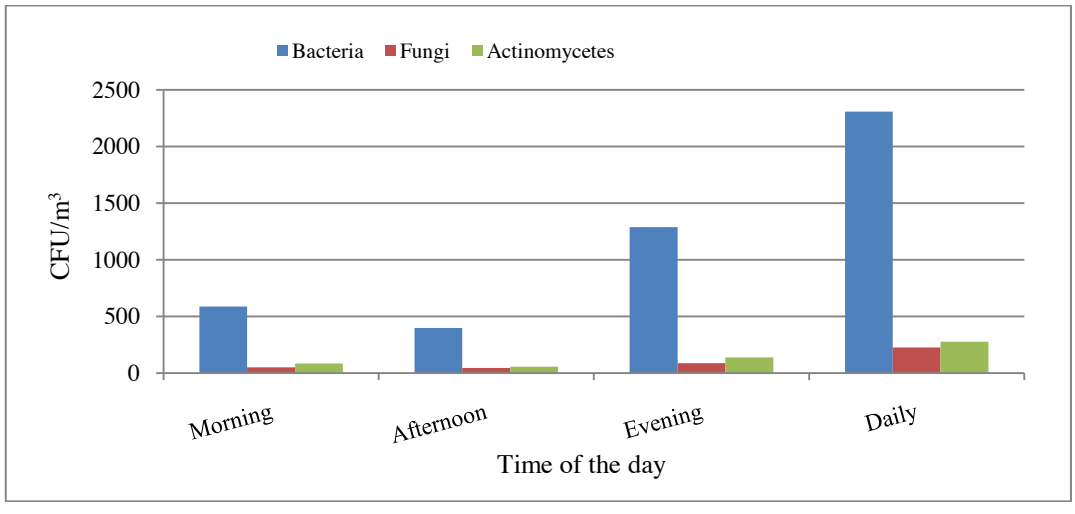

Figure 4. Variations of total culturable airborne microorganism regarding time of the day

Temporal variation of airborne microorganisms is important to understand their prevalence and distribution pattern. In the present study airborne microbial concentrations were relatively higher in the morning time compared to the afternoon time, and achieved their maximum peak in the evening time. Worldwide, the distribution patterns of airborne microorganisms are similar, showing two main peaks, one in the morning and the other in the evening (Burch and Levetin, 2002; Abdel Hameed *et al*., 2009; Fang *et al*., 2007). Generally microorganisms are released into the air under the effects of electrostatic and surface tension forces; human activities and environmental conditions (Jones and Harrison, 2004; Rossi *et al*., 2005). Turbulences help release of fungal spores in the morning time and vertical mixing reduces their concentrations at the afternoon time, while stability conditions in the nighttime allow spores to settle down, achieving a peak concentration in the evening time (Rich and Waggoner, 1962). Solar radiation and lower relative humidity may have detrimental effects on microbial survivability, particularly at noon time (Royes, 1987). Desiccation (loss of water) of microorganisms is found to be the greatest during the midday (Levetin, 1995), and in desert location the minimum bacterial concentrations were found at noon time (Tong and Lighthart, 1997).

Fig. 5 shows the monthly variations of airborne microorganisms. The greatest concentrations of airborne bacteria (1797.9 CFU/m^3) and actinomycetes (222.2 CFU/m^3) were found in month of September (Hajj season) and in the evening daytime. This is due to large numbers of people visit Makkah to perform Hajj "Hajj is an annual Islamic pilgrimage to Makkah and a mandatory religious duty for Muslims that must be carried out at least once in their lifetime" (Matthew, 2011). On the other hand airborne fungi achieved another trend, the maximum concentration (169.2 CFU/m^3) was found in the month of August and in the morning daytime. The morning peak of fungi confirms that fungal spores are actively released due to changes of bonding and surface tension forces, under the effects of sun heat and loss of water in the morning time.

Table 3. Concentrations of fine and coarse size fractions of airborne microorganisms

Daytime	CFU/m^3					
	Bacteria		Fungi		Actinomycetes	
	<8 µm	>8 µm	<8 µm	>8 µm	<8 µm	>8 µm
Morning	(60.6-1092) [347.2±283.3]	(45.5-984.8) [278±242.7]	(0-390.9) [77.1±83.6]	(0-122.7) [18.1±24]	(0-169.7) [51.4±41.7]	(0-169.7) [30.9±37.6]
Afternoon	(30.3-637.8) [222.7±164.6]	(15.1-342.4) [171.4±108]	(0-122.7) [33.45±36.6]	(0-60.6) [11.4±18]	(0-107.6) [41±61.9]	(0-60.6) [14.3±19.6]
Evening	(153-3172.7) [821.4±604.3]	(60.6-1295.4) [466.6±269]	(0-184.8) [60.6±44.4]	(0-90.9) [25.3±22]	(0-375.7) [106±101.6]	(0-90.9) [31.7±24.4]
Daily	(383.3-2936.6) [1391.8±807]	(289-2013.6) [919.2±433]	(30.3-451.5) [171±121.9]	(0-198.5) [54.3±48]	(15.15-589.3) [194.7±146]	(0-245) [80.2±59.7]

(Range), [mean ± SD]

3.3.2 Microbial size fraction

Airborne microorganisms were detected in two size fractions (Table 3). The percentage of fine size fraction (≤ 8 µm) was higher than the coarse fraction (≥ 8 µm). The fine fractions represented ~60%, 75.9% and 70.8% of the total bacteria, fungi and actinomycetes concentrations, respectively. The greatest percentages of fine fraction of bacteria (63%) and actinomtycets (77%) were found in the evening time, while fungi (80%) in the morning time. Coarse particles are settled down faster than fine particles, and long range transport of microorganisms increases fine particle fraction. In the present study sampling point was located at ~ 13 m height building in the main campus of the University, the height of building influences particle size distribution due to sedimentation effect (Clauß, 2015). Moreover larger particles are mainly of local origin, because particle ≤ 8 µm has a sedimentation velocity in still air ~ 0.2 cms^{-1} and a 3.3 µm particle has a sedimentation velocity of 0.002 cms^{-1} (Lighthart and Stetzenbach, 1994), indicating that sources of bacteria and fungi are far away from the sampling site.

Fine particles are linked to microorganisms (Huffman *et al*., 2013; Saari *et al*., 2015), and up to 80% of bacteria are particles ≥ 3.1 µm (Jones and Harrison, 2004). In addition high temperature reduces microbial sizes (Kauserud *et al*., 2012) due to loss of cell wall water (Cole and Cook, 1998), but loss of water may detrimentally affect microbial survivability. Particle size determines deposition and dispersion of microbial particle in the atmosphere and human respiratory system (Chen *et al*., 2012; Sadyś *et al*., 2016). Particles ≥ 6 µm tend to deposit in the upper respiratory tract and particles ≤ 2 µm deposit in alveoli (Darquenne, 2012). Particles ≤10 µm can be penetrated deeper into the respiratory tract and particles ≥ 10 µm deposited on upper respiratory tract (Nicas *et al*., 2005). In the present study the presence of fine fraction in higher percentages raises a question about its health effects.

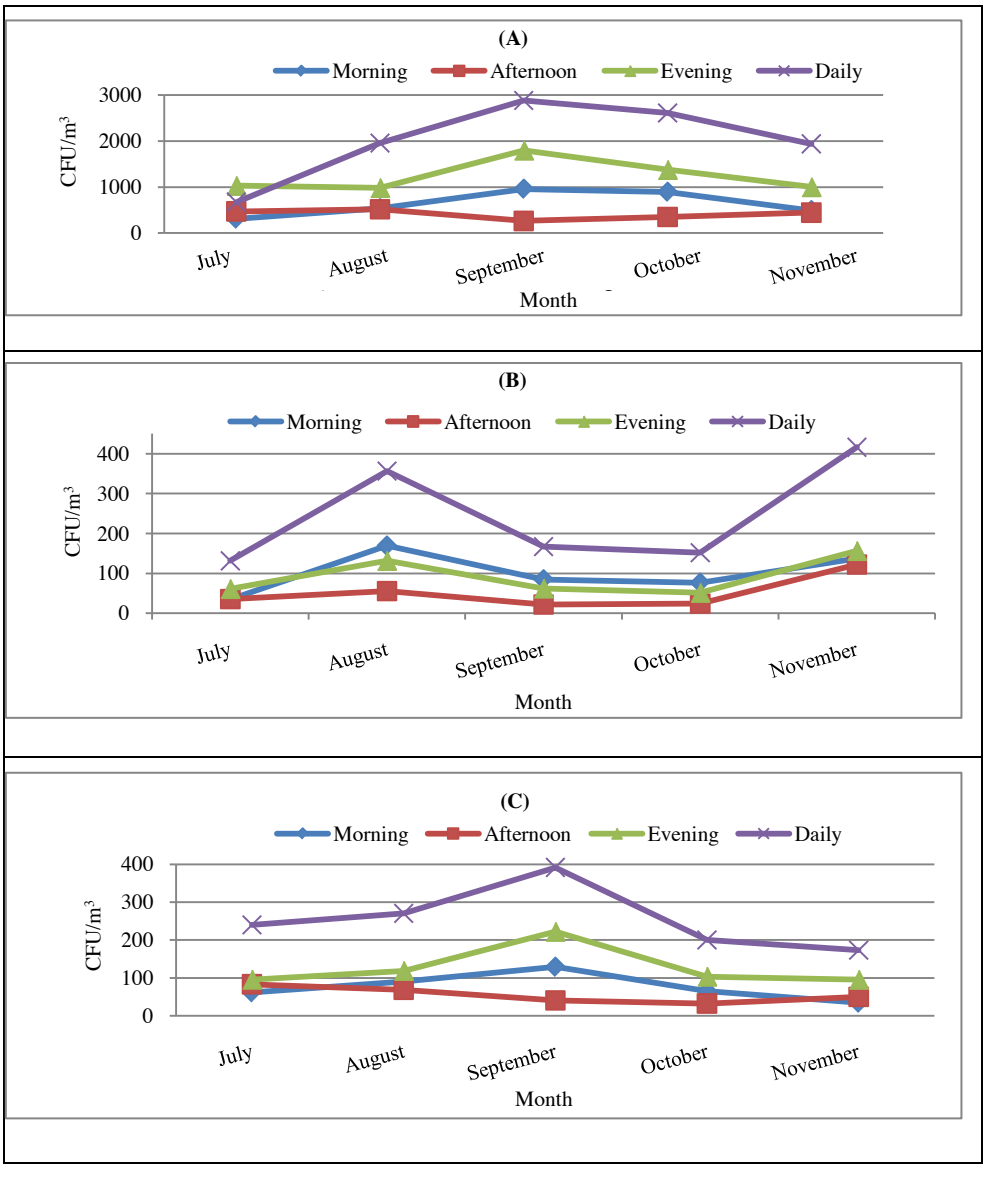

Figure 5. Monthly mean-concentrations of airborne bacteria (A), fungi (B) and actinomycetes (C)

Table 4. Spearman's rank correlations between airborne microorganisms and meteorological conditions

Variable	Bacteria				Fungi				Actinomycetes			
	Mo	Aft	Ev	Daily	Mo	Af	Ev	Daily	Mo	Af	Ev	Daily
T°C	0.016	0.24	0.27	0.17	-0.08	0.01	-0.06	-0.03	0.025	0.33	0.47*	0.36
RH%	0.131	-0.04	-0.014	0.17	0.45*	0.39*	0.32	0.48*	-0.08	0.14	0.123	-0.08
WS/mph	-0.41*	-0.01	-0.03	-0.20	-0.4*	-0.1	0.02	-0.39*	-0.11	0.06	-0.07	0.04
WD	-0.03	-0.11	0.20	0.123	0.147	-0.21	-0.14	-0.13	0.06	0.13	0.47*	0.31

Mo: morning; Aft: afternoon; Ev: evening, *$P \leq 0.05$*

3.3.3 Correlations between airborne microorganisms and meteorological conditions

Table 4 shows Spearman's rank correlations between airborne microbial parameters and meteorological conditions. The correlations varied depending on microbial type and sampling time. A moderate significant correlations were found between fungi and relative humidity ($r = 0.48$), and actinomycetes and temperature ($r = 0.47$). Wind direction moderately influenced actinomycetes concentrations in the daytime ($r = 0.31$) and in the evening time ($r = 0.47$). No significant correlations were found between airborne bacteria and meteorological conditions; however wind speed showed a significant negative correlation ($r = -0.41$) with bacteria in the morning time, as bacteria may be released into the atmosphere under convection effect (effect of heat) in the morning time. In general, wind speed is a dilution factor and helps transport of microorganisms (Smith, 1966). In the present study wind speed ranged between 0 - 20 mph, hence microorganisms may take longer time to transfer from their sources to sampling point, and consequently the age and decay rate of microorganisms may be increased. Lin and Li (2000) concluded that wind velocity was a dilution factor of bioaerosols at ≤ 5 m/s and was a release factor at ≥ 5 m/s.

It is suggested that meteorological conditions synergistically affected microbial survivability, moreover human activities, geographical factors and timing of microbial growth may mask the effects of meteorological conditions on microbial survivability (Adams *et al*., 1986); and the extremes of these factors differ from place to place. Interactions of these factors/variables may interpret the complex (unclear) correlations between microorganisms and meteorological conditions in the present study.

Table 5. Identification of airborne fungal isolates

Fungus	%					
	Airborne fungi				Fungi associated-PM	
	Morning	Afternoon	Evening	Daily	Daytime	Nighttime
Aspergillus	68.28	55.3	57.04	61.45	73.64	67.87
Asp. fumigatus	1.83	2.12	2.48	2.11	7.11	1.08
Asp. versicolor	9.75	13.83	4.96	9.23	-	-
Asp. flavus	16.46	2.12	4.96	9.23	8.37	7.94
Asp. niger	23.78	27.66	37.2	29.01	30.96	32.5
Asp. nidulanus	12.2	6.38	7.44	9.23	-	-
Other Asp. species	4.26	3.19	-	2.64	27.2	26.35
Alternaria	1.83	3.19	4.13	2.9	0.42	-
Aureobasidium	1.83	4.25	-	1.84	1.67	3.97
Cladosporium	6.1	10.63	8.26	7.915	-	-
Curvularia	-	1.06	1.65	0.8	-	-
Emericella	6.7	7.44	6.61	6.86	10.04	10.47
Eurotium	5.49	5.32	5.78	5.54	3.76	2.12
Fusarium	1.22	1.06	0.82	1.05	2.93	0.36
Geotrichum	0.6	-	0.82	0.53	-	-
Paecilomyces	-	-	2.48	0.79	-	-
Penicillium	1.83	-	-	0.79	-	0.72
Phoma	0.6	2.12	1.65	1.32	1.67	0.72
Rhizomucor	2.44	2.12	0.82	1.84	-	-
Rhizopus	-	-	-	-	0.42	1.81
Scopularopsis	-	-	0.82	0.26	-	-
Sterile hyphae	3.05	6.38	5.78	4.75	5.44	11.91
Unkown	-	1.06	3.3	1.32	-	-
Total isolates	164	94	121	379	239	277

- Not detected

3.3.4 Identification of fungi

Fungi are common biological particles and their concentrations and types varied from place to place, depending on microenvironment conditions. Fungal types are used as indicator of microenvironment characters. Fungi cause allergic symptoms even if their counts are low (Grinn-Gofroń and Bosiacka, 2015). In the present study fungal biodiversity was low with *Aspergillus* and its telemorphs (*Emericella* and *Eurotium*) were the common genera (Table 5). *Asp. fumigatus, A. flavus, Asp. niger* and sterile hyphae were frequently found in the collected samples. *Penicillium* was detected in the morning time while *Paecilomyces* and *Scopularopsis* at the nighttime and *Rhizopus* was exclusively found associated PM.

The worldwide abundance fungi e.g., *Alternaria*, *Cladosporium*, *Fusarium* and *Penicillium* were found in low incidence. Hot weather and barren region are not suitable environments for fungal growth (Cventić and Pepeljnjak, 1997). *Penicillium* and *Cladosporium* are sensitive to temperature (Pyrri and Kapsanaki-Gotsi, 2007). A negative trend was found between increase air temperature and *Cladosporium* counts in Greece (Damialis *et al*., 2015). *Alternaria* proliferates in the presence of suitable humidity (80 - 90%) and temperature, and presence of vegetation debris (Humpherson-Jones and Phelps, 1989), these conditions are less present in Makkah city. The results in the present study agree with those have been reported in Makkah city (Abdel Hameed and Habeeballah, 2013) and Jeddah city (Alghamdi *et al*., 2014) who found fungi in low biodiversity with *Aspergillus* was the common genus.

4. Conclusion

Concentrations of microorganisms in the air state and/or associated PM were low. Airborne microorganisms showed different prevalence patterns achieving a significant peak concentration in the evening time. Microorganisms associated PM achieved higher concentrations in the daytime than the nighttime. The greatest concentrations of airborne bacteria and actinomycetes were found in the evening time while fungi in the morning time, they had different mode of release. Fine particles constituted ~ 60 - 75.9% of total microbial concentrations. Bacteria positively correlated with PM in the daytime and meteorological conditions in the nighttime. Temperature was the crucial factor affecting survivability of microorganisms associated PM; and relative humidity was the main factor affecting survivability of airborne fungi. Low microbial community in Makkah's atmosphere is due to harsh environmental conditions that preselected microbial taxa to survive. Long term study on distribution patterns of airborne microorganisms in relation to geographical location, human activity and dominant sources should be studied in the future.

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