

## Air Microbial Contamination at the Holy Mosque, Makkah, Saudi Arabia

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### ABSTRACT

Airborne microbial contamination was collected from the main directions of the holy mosque (Al-Haram mosque), Makkah city, by using the gravitational method. Bacteria, fungi and actinomycete concentrations ranged between 1470 - 21800 CFU/m<sup>3</sup>; 44 - 572 CFU/m<sup>3</sup>, and 0.0 - 264 CFU/m<sup>3</sup>, respectively at all directions. Bacterial concentrations significantly differed between directions, and Gram positive bacteria constituted ~ 90-100% of the total bacterial isolates. Gammaproteo bacteria were the common Gram negative bacteria, and *Aspergillus* was the predominant fungal genera. Mesophilic bacteria negatively related ( $P < 0.05$ ) with relative humidity. Human activities had more effective influence on the microbial concentrations than the meteorological factors.

**Key words:** Air, Biocontamination, Directions, Biodiversity, The holy mosque, Makkah.

### INTRODUCTION

Atmospheric particles of biological origin are diverse group of microorganisms and their excretions. Airborne microorganisms originate from many sources: animal, human, and vegetation. The number and composition of airborne microorganisms have increased in the urban and rural areas<sup>1</sup>. The presence of microorganisms depends on seasonality, geographical conditions, meteorological factors, type of sources<sup>2,3,4</sup>, and air pollution<sup>5</sup>. Airborne microorganisms may contribute ~ 20%, 22% and 10% of the total particulate matters in the remote continental, populated-continental, and remote maritime environments, respectively<sup>6</sup>. The concentrations and composition of airborne microorganisms undergoes daily, weekly and seasonally changes<sup>7</sup>.

Millions of people arrive to the Holy City, Makkah city, particularly in the Ramadan month, the fasting month, every year. Microbial contamination represents a dangerous risk factor in many human activities areas<sup>8</sup>, as airborne microorganisms cause allergy, infections, and toxicity<sup>9,10</sup>. Some studies

have been carried out on microbial contamination in the atmosphere of Saudi Arabia cities: such as Riyadh<sup>11</sup>, Hofuf<sup>12</sup>, Makkah<sup>13</sup>, and Taief<sup>14</sup>. However few studies have been performed on the airborne microorganisms in the area near and around the holy mosque. The results showed that microbial concentrations differed depending on methodology and sampling location<sup>15, 16</sup>.

Until now no complementary studies have dealt with airborne microbial community at the main directions of the holy mosque that may be differed in their human activities and topographical features. The evaluation of microbial contamination, in places at risk, is considered a basic step toward prevention and control. The present study aims to gain more insight into the variability of airborne bacteria, fungi, and actinomycetes at the main directions of the holy mosque, in order to determine its microbial air quality and give site specific information.

### MATERIALS AND METHODS

#### Site description

The Holy City, Makkah, is located at an

altitude of 277 m, 21°29' N 39°45' E, and ~ 80 km inland from the Red Sea, with a population of ~2,000, 000<sup>17</sup>. The area around the holy mosque comprises the old city; which is characterized by heavy traffic, parking, hotels, and hospitals with no predominant plant cover. Four sampling sites were selected to carry out the present study. Air samples were collected at the main directions (North, South, West and East) of the holy mosque (Fig. 1), in order to represent various geographical features and human activities. A brief summary of the general human activities and environmental characteristics at each direction are shown in Table 1.

During every sampling event, temperature and relative humidity were measured using a portable Psychrometer (Extech -model 42270, China), adjusted to record data at every 10 min intervals. Temperatures ranged between 27– 35°C with mean values of 30.3 °C, 29.6 °C, 31.4°C and 30.8 °C at the North, South, East, and West, respectively. Relative humidity ranged between 32– 63% with mean values of 38.7%, 43%, 34% and 42% at the corresponding directions, respectively. Wind speed records were obtained from The Presidency of Meteorology and Environment, Makkah. The wind speed ranged between 0.5 to 1.5 m/s, with north-east was the prevailing wind direction.

### Sampling strategy

A total of 16 samples were collected between 19<sup>th</sup> to 27<sup>th</sup> days of Ramadan (7-15, August, 2012). Two consecutive samples were collected every sampling event. Air samples were collected at ~1 - 3 m height above the ground level, between 19.00 PM and 24.00 AM, the night time. This time was chosen because it is the time of the overcrowding in the holy mosque during the Ramadan month. The gravitational method was used to collect airborne culturable microorganisms. The Petri plates, in triplicate, containing trypticase soya agar, czapek's dox agar, and starch casein agar (Hi-Media Laboratories, Mumbai, India) media were used to collect bacteria, fungi and actinomycetes, respectively. The sampling time varied within 10-20-min. The exposed plates were incubated for 5– 7 days at 28°C for fungi; 48 hrs at 22 and 37 °C for psychophilic, and mesophilic bacteria; respectively, and 10 days at 25 °C for actinomycetes.

The resultant colonies were counted and converted into colony forming unit per cubic meter of air (CFU/m<sup>3</sup>) using Omeliansky formula<sup>18</sup>:

$$N=5a \times 10^4 (bt)^{-1}$$

Where: N=colony forming unit per cubic meter of air (CFU/m<sup>3</sup>) a= number of colonies per Petri dish, b=dish square centimeter, t= exposure time (min).

All the grown fungal colonies, and five to ten bacterial colonies which appear in more than 5% of trypticase soya agar were purified and identified to the genus level. *Aspergillus* was identified to the species level. The bacterial isolates were identified on the basis of Gram stain, oxidase, and catalase tests<sup>19</sup>. Fungal isolates were identified using macroscopic and microscopic features<sup>20-23</sup>.

### Statistical analysis

Data was analyzed using the non parametric statics. The kruskal Wallis test was used to compare microbial contamination at the different directions ( $p < 0.05$ ), followed by post hoc test (Student t-test). The assumption for this test is that the samples from different groups are randomly independent. Spearman's rank correlation test ( $r_s$ ) was used to determine the relationships between microbial concentrations and meteorological factors.

## RESULTS

The range and mean concentrations of airborne culturable microorganisms at the main directions of the holy mosque are shown in Table 2. The bacterial concentrations ranged between 1470- 21800 CFU/m<sup>3</sup>, with mesophilic bacterial concentrations exceeded psychophilic concentrations. The highest bacterial concentration was found at the north direction, and the lowest at the East. Fungal concentrations ranged between 44 - 572 CFU/m<sup>3</sup>, with the highest concentrations were found at the North and West directions. Airborne actinomycete concentrations were found in the range of 0-264 CFU/m<sup>3</sup> (Table 2). Mesophilic and psychophilic bacterial concentrations significantly differed between the all directions ( $P < 0.05$ ), however fungi and actinomycete

concentrations did not show any significant differences.

A total of 502 bacterial isolates belonging to 11 genera were identified (Fig. 2). Gram positive and negative bacteria constituted 90-100% and 2.4

- 9.4%, respectively of the total isolates. Staphylococci (4.2-26%), and *Bacillus* (5.5- 30.9%) were the dominant Gram positive bacteria. *Streptococci* and spore-formers were only detected at the north and south directions, respectively. Gammaproteobacteria (*Pseudomonas*) constituted

**Table 1: A brief description of the environmental features around the holy mosque**

Direction	Denomination	General environmental characteristics
North	Shamiah	Constructions, demolition, unpaved roads, small workshops, dusty environment, light vehicles (motorcycle)
South	Ajeadh	Considered closed area, tall buildings, steady air, high Worshipers density, spray humidifiers.
East	Ghaza	Considered open area, good natural air ventilation, library, near to bus parking.
West	Shobaikah	Similar to south direction, high Worshipers density, fair natural ventilation, spray humidifiers.

**Table 2: The range and mean concentrations of airborne microorganisms at the main directions of the holy mosque**

Indicator	CFU/m <sup>3</sup>			
	North	South	East	West
Bacteria	(8.76x10 <sup>3</sup> - 1.74x10 <sup>4</sup> )	(2.38x10 <sup>3</sup> -1.58x10 <sup>4</sup> )	(1.47x10 <sup>3</sup> -3.21x10 <sup>3</sup> )	(1.54x10 <sup>3</sup> -4.76x10 <sup>3</sup> )
22°C	[1.13x10 <sup>4</sup> ±4.09x10 <sup>3</sup> ]	[6.65x10 <sup>3</sup> ±6.16x10 <sup>3</sup> ]	[2.29x10 <sup>3</sup> ±7.38x10 <sup>2</sup> ]	[3.25x10 <sup>3</sup> ±1.56x10 <sup>3</sup> ]
Bacteria	(1.10x10 <sup>4</sup> - 2.18x10 <sup>4</sup> )	(2.25x10 <sup>3</sup> - 1.76x10 <sup>4</sup> )	(1.85x10 <sup>3</sup> -3.66x10 <sup>3</sup> )	(1.94x10 <sup>3</sup> -6.22x10 <sup>3</sup> )
37°C	[1.41x10 <sup>4</sup> ±5.13x10 <sup>3</sup> ]	[8.60x10 <sup>3</sup> ±6.63x10 <sup>3</sup> ]	[2.86x10 <sup>3</sup> ±7.66x10 <sup>2</sup> ]	4.11x10 <sup>3</sup> ±2.02x10 <sup>3</sup>
Fungi	(1.17x10 <sup>2</sup> - 4.40x10 <sup>2</sup> )	(1.17x10 <sup>2</sup> - 5.72x10 <sup>2</sup> )	(4.4x10 <sup>1</sup> -2.34x10 <sup>2</sup> )	(4.4x10 <sup>1</sup> - 3.08x10 <sup>2</sup> )
	[2.60x10 <sup>2</sup> ±1.61x10 <sup>2</sup> ]	[2.60x10 <sup>2</sup> ±2.12x10 <sup>2</sup> ]	[1.461x10 <sup>2</sup> ±8.0x10 <sup>1</sup> ]	[1.98x10 <sup>2</sup> ±1.54x10 <sup>2</sup> ]
Actinomycetes	(8.8x10 <sup>1</sup> - 2.64x10 <sup>2</sup> )	(8.8x10 <sup>1</sup> - 1.76x10 <sup>2</sup> )	(5.9x10 <sup>1</sup> -1.76x10 <sup>2</sup> )	(0.00 - 1.32x10 <sup>2</sup> )
	[1.54x10 <sup>2</sup> ±8.4x10 <sup>1</sup> ]	[1.28x10 <sup>2</sup> ±3.6x10 <sup>1</sup> ]	[1.03x10 <sup>2</sup> ±5.1x10 <sup>1</sup> ]	[7.7x10 <sup>1</sup> ±5.5x10 <sup>1</sup> ]

(Range), [mean ± standard deviation]

**Table 3: Similarity triangle depicting agreement ratios among microbial isolates at the different directions of the holy mosque**

Direction	Bacteria				Fungi			
	N	S	E	W	N	S	E	W
N	1.0	0.84	0.94	0.88	1.0	0.28	0.65	0.46
S		1.0	0.7	0.94		1.0	0.38	0.11
E			1.0	0.82			1.0	0.46
W				1.0				1.0

N: North, S: South, E: East, W: West

8.75% of the total isolates at the south direction (Fig. 2). A total of 112 fungal isolates belonging to 19 genera were identified (Fig. 3). *Aspergillus* represented by *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus*. *Aspergillus niger* (14.27% - 46.9%) and *Fusarium* (6.90% - 13.62%) were the common fungal isolates. The highest fungal diversity was found at the south direction, among

which *Epicoccum*, *Mucor*, *Trichophyton*, *Chaetomium*, *Cladosporium*, *Alternaria*, *Emericella* were detected.

Table 3 shows the agreement ratios between the identified microbial isolates between directions. The agreement ratio is used to compare the identified microorganisms among sampling



Fig. 1: A diagram of the holy mosque showing sampling points

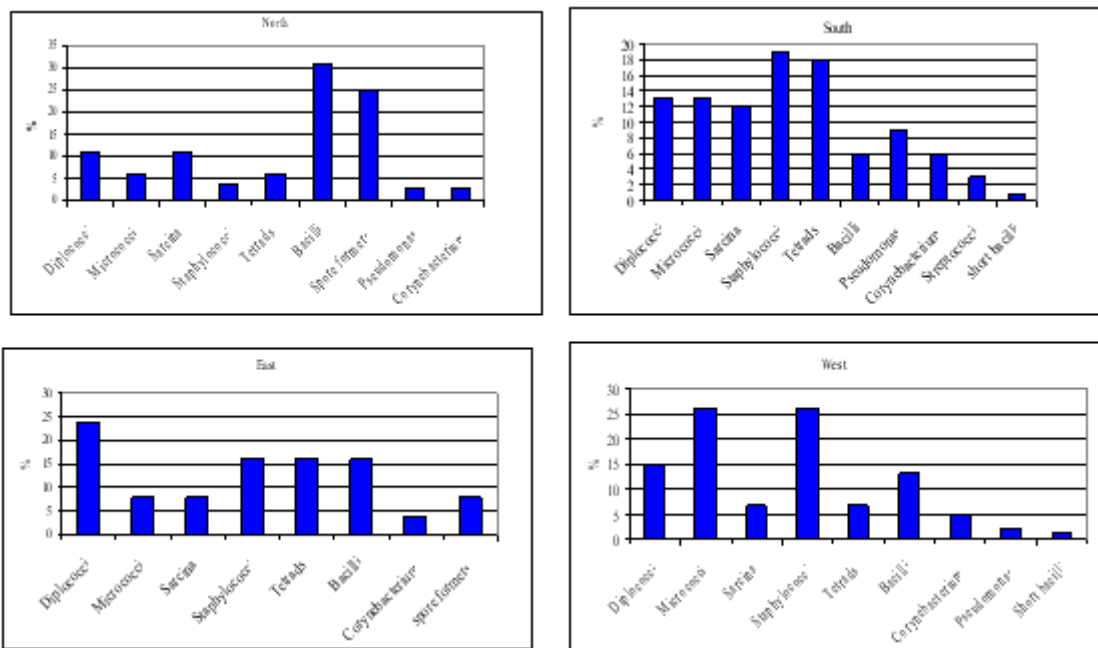
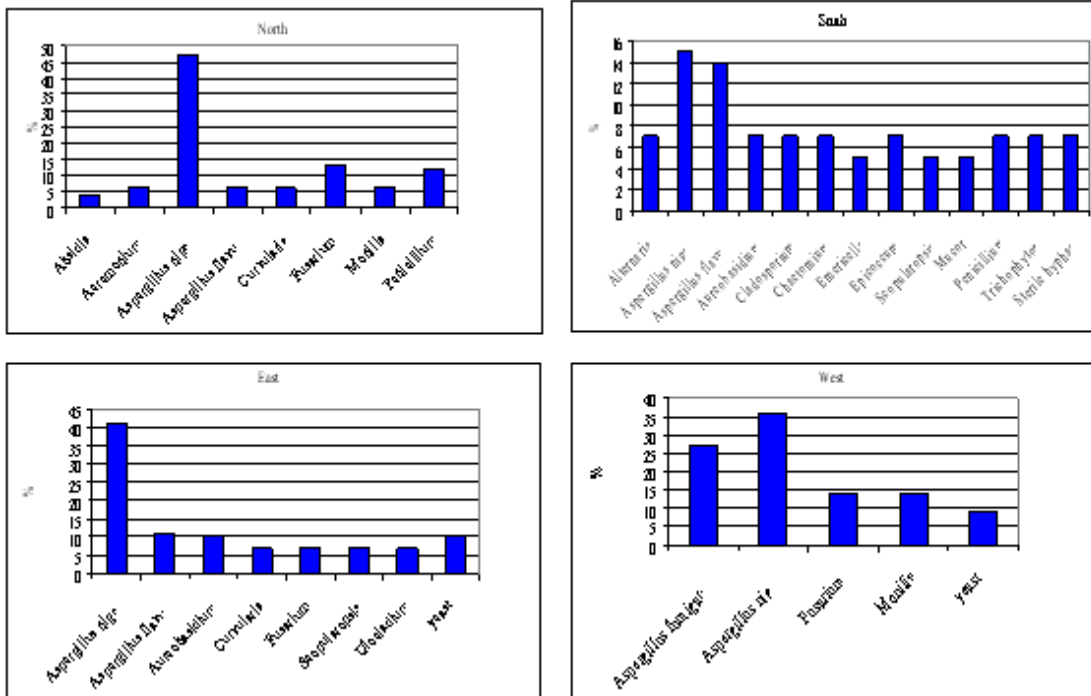


Fig. 2: Identification of airborne bacterial genera at the different directions of the holy mosque



**Fig. 3: Identification of airborne fungal genera at the different directions of the holy mosque**

sites, as it reflects the number of shared genera at the different sites in relation to the total number of genera<sup>24</sup>. High agreement ratios were found between bacterial genera at the all directions. The agreement ratios for fungi were relatively lower than bacteria, the highest ratio (0.65) was found between the North and East directions.

No clear relationships were found between the meteorological parameters and airborne microorganisms. The relationships differ according to type of bio-contaminant and direction. However, relative humidity was considered the main factor affecting survivability of mesophilic bacteria ( $r = 0.4 - 0.8$ ). Actinomycetes negatively related with temperature, however fungi negatively and positively related with relative humidity, and temperature, respectively. Wind speed show weak negative and positive relationships with microbial concentrations.

**DISCUSSION**

Airborne microbial contamination is a significant issue in the crowded centers and cities,

as air serves as a transmission vehicle for pathogens that have linked with adverse health effects ranging from infectious diseases to allergies and cancer<sup>25</sup>. Airborne microorganisms affect individuals who are at risk, immune-compromised, elders and children, particularly in overcrowded areas, however, human responses depend on type of microorganism, and individual's immune system<sup>26</sup>. Various sampling methods are used to collect airborne microorganisms including, impaction, impingement, filtration and gravity deposition<sup>27</sup>. Passive and active sampling methods are based on different principles. The passive samplers yielded data with the lowest standard deviation in comparison with the active samplers<sup>28</sup>. In the present study, the gravitational method was used because it is a simple, cheap, and many places can be checked at the same time. The gravitational method is a non-quantitative method<sup>29</sup>, as it is affected by the size and shape of the particles, and motion of the surrounding atmosphere<sup>30</sup>. Omeliansky formula is not a universal conversion formula; however it is used to allow comparing our results with other results that were obtained by using the active samplers.

Airborne microbial concentrations ranging from 4,500–10,000 CFU/m<sup>3</sup> have been suggested as the upper limit for ubiquitous bacterial aerosols<sup>31</sup>, and concentration < 100 cfu/m<sup>3</sup> may be unhealthy to immunosuppressed people<sup>32</sup>. In the present study bacterial concentrations exceeded 10<sup>4</sup>CFU/m<sup>3</sup> at the North and South directions. However, fungal concentrations did not exceed the allowable guideline limit value of 500 CFU/m<sup>3</sup><sup>33</sup>. Actinomycetes exceeded 100 CFU/m<sup>3</sup>, strongly contaminated air, and 10– 100 CFU/m<sup>3</sup>, moderately contaminated air at some directions as suggested by Polish Standards<sup>34</sup>.

Airborne bacterial concentrations exceed 1x10<sup>4</sup> CFU/m<sup>3</sup> over land<sup>35</sup>, as bacteria may be suspended as individual cells or attached to other particles such as soil and leaf<sup>36</sup>. Airborne fungal concentrations generally ranged from 10<sup>0</sup>-10<sup>3</sup> CFU/m<sup>3</sup><sup>37</sup>, and averaged ~10<sup>4</sup> CFU/m<sup>3</sup> in the urban air<sup>1</sup>. Airborne actinomycetes averaged 10<sup>2</sup> CFU/m<sup>3</sup> in the Cairo city centre<sup>38</sup>, as actinomycetes are highly dependent on the amount of dust in the air<sup>39</sup>.

Airborne microbial contamination is affected by multiple variables. These variables continuously changed, such as anthropogenic influence, human activity, topography, micro-environmental conditions, type of sources, and seasonality<sup>1</sup>. In the present study traffic flow disturbed dust particles at the north direction that might be the reason for increasing of microbial concentration. The lowest microbial concentration was found at the East direction. This is attributed to the absence of direct human disturbances, and good natural ventilation. Generally microbial concentrations in the urban environment are influenced by human activities<sup>40</sup>, and their composition varies in different cities<sup>41</sup>. In the present study there were unclear correlations found between airborne microorganisms and meteorological factors. This is attributed to the influence of irregular disturbances and human activities, which may have more influence on microbial concentrations than meteorological factors near the holy mosque. The lack of significant relationships does not mean that meteorological factors have no effects on microbial survivability, because in un-standardized conditions severe microbial variations are expected.

In the present study Gram positive bacteria were common. This could be explained on the bases of their cell wall structure and continuous sources. The low frequent detection of Gram negative bacteria is attributed to their sensitivity to the air environment<sup>41</sup>, and the initial shock due to desiccation after aerosolization<sup>42</sup>. Firmicutes, Proteobacteria and Actinobacteria are common bacterial types in the urban environment, where Gammaproteobacteria and Betaproteobacteria have been regularly identified<sup>1</sup>. Mouli et al.<sup>43</sup> found airborne Gram positive bacteria in the range of 60% - 90% of the total bacterial population, in Tirupati, India; however *Bacillus* constituted 47.62% and *Acinetobacteria* 14.27% in the atmosphere of El Taief, Saudi Arabia<sup>44</sup>. *Bacillus*, *Micrococcus* and *Staphylococci* differed from place to place depending on the micro-environment<sup>45</sup>. In the present study the frequent detection of *Pseudomonas* species at the south direction is of a particular interest as the spray humidifiers, and water reservoirs are suspected to be their sources.

Airborne fungi is likely to be similar in most parts of the world, however the dominating genera may differ from area to another, depending on the density of plant cover, geographical location, and human activity<sup>46</sup>. In the present study, *Alternaria*, *Penicillium* and *Cladosporium* were found in low counts because *Penicillium* and *Cladosporium* favor low temperature conditions, and sensitive to solar radiation<sup>47</sup>. *Alternaria* proliferates in the presence of suitable humidity, temperature, and vegetation debris<sup>48</sup>, these conditions are not available in the Saudi Arabia, as low humidity, high temperature and no permanent plant cover are the main characters of Makkah city. *Fusarium* can grow in water pipes, fresh water and humidifiers<sup>49</sup>, this confirms the presence of *Fusarium* in the air around the holy mosque. In the present study phyloplane and water indicators fungi<sup>50</sup> were detected in lower counts than soil fungi.

Actinomycetes may induce respiratory symptoms<sup>10</sup>, streptomycetes have been implicated in many diseases and several species stimulate lung macrophage reactions that lead to inflammation of the lung<sup>9</sup>. *Aspergillus*, *Eurotium*, *Penicillium* and *Trichoderma* species have been known as a cause of allergenic alveolitis<sup>51</sup>.

*Aspergillus flavus* and *Aspergillus fumigatus* cause aspergillosis<sup>52</sup>. Gram negative bacteria are an important source of endotoxins, which are the main pulmonary immunotoxicants<sup>53</sup>. Gram-positive bacteria should also be regarded as potential immunotoxicants<sup>54</sup>. *Bacillus* species have been found to be associated with allergic alveolitis<sup>55</sup>. The potential for extensive transmission of airborne infectious agents is present in large public settings; however the duration of exposure may be short. At the holy mosque, particularly in the Ramadan month, the duration of exposure to microbial contamination may be extended to be >10 hour/day and the probability of infection may be present. Therefore a program for surveillance, prevention and control of airborne microbial contamination should be established at the holy mosque.

## CONCLUSION

Airborne microbial concentrations not significantly varied between the main directions of the holy mosque. Airborne bacteria were the main microbial contaminants. The biodiversity of bacterial and fungal genera were higher at the south direction; however the north had the largest microbial concentrations. The anthropogenic activities are the main variables controlling air bio-contamination around the holy mosque. The spray humidifiers are suspected to be the main emission source of *Pseudomonas* bacteria. People may be exposed to infectious agents at the holy mosque; therefore the microbial contents should be included in the air quality standards and reports.

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