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Microbial Air Pollution of Muna Area, Makkah Region, Saudi Arabia

Abdullah M. K. Al-Falih
Department of Science (Biology), Teachers College in Riyadh, P. O. Box 4341. Riyadh 11491, Saudi Arabia

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Abstract. The air-borne fungal and bacterial flora at Muna, Saudi Arabia during Al-Hajj season 1419 H were screened. The screened areas included a food market, King Khaled bridge, Jamarat bridge, King Fahad tunnel, muasim tunnel and the central area. A total of thirty eight species of fungi and nineteen bacterial species belonging to six genera were isolated. The commonest fungi isolated were *Cladosporium herbarum* and *Penicillium citrinum* while the commonest bacterium was *Bacillus subtilis*. In all the localities, the highest number of fungal and bacterial colonies were obtained from air in the central area, followed by Jamarat bridge and the food market. The number of fungal and bacterial colonies was in general higher in densely populated than in less populated areas.

Introduction

During the second half of the twentieth century, pollution problems have become more acute than ever before. Pollution and its control have ceased to be the concerns of only sanitary engineers; they have become slogans for mass movements and the focal topic of scientific, public and legislative debates. The current environmental predicament of our planet has two recognizable components: conventional pollution, which now occurs on a vastly increased scale; and novel pollution problems, largely unknown before World War II. The human population explosion, combined with an international trend toward urbanization, has increased the intensity of conventional pollution to a high level. To stave off unacceptable deterioration of the environment, industrialized societies are being forced to pay heavily for the abatement of wastes and pollutants. The scientific background of these conventional pollution problems is well understood and effective control is largely a question of technology and finances. In contrast, many novel pollution problems, caused by chemicals to which the environment had not been exposed previously, have caught both scientists and lay people unprepared. The majority of the novel pollution phenomena involve radionuclides and organic chemicals called xenobiotics, chemicals synthesized by humans that have no close natural counterparts.

These xenobiotic chemicals include pesticides, plastics, and other synthetics, many of which persist because transport mechanisms and catabolic pathways for them have not evolved [1]. All living organisms carry out chemical transformations that influence their surrounding environment.

In general there are many types of pollutants which include oil, isotopes, minerals, sewage, fertilizers and microbes. Human activities are responsible for introducing many of these materials into the ecosphere at problem levels, while overall the state of health of pilgrims has a direct effect on microbial air pollution [2].

The microbial flora of air has been surveyed in many countries all over the world. [3-21]. Variations in the total numbers as well as the individual trapped genera and species were noticed. Seasonal variations in the air bacterial and fungal flora are also evident.

About two million pilgrims arrive in Makkah every year to perform Al-Hajj, Muna area which becomes very crowded increasing waste materials which provide a most suitable environment for microbial pollution.

The present survey was conducted to study the air-borne microbial flora at Muna, Saudi Arabia during Al-Hajj season 1419 H. The investigation includes, isolation, total count and identification of both bacteria and fungi. In addition this study offers some suggestions to reduce microbial pollution during Al-Hajj.

Materials and Methods

Samples were collected from a food market, King Khaled bridge, Jamarat bridge, King Fahad tunnel, muasim tunnel and the central area, with five air samples taken at each site (total 30 samples). The method described by Al-Sarrani and Al-Turk [2] was used for sampling air-borne spores of fungi and bacteria. Samples were then taken to a laboratory for fungal and bacterial cultivation. For the quantitative estimation of fungi and bacteria the aliquots of a suspension in sterilized water was used. The method is based on a Vacuum Pressure Pump and a Flo-Meter to estimate the volume of air samples. Each liter of air samples collected was treated with 100 ml of sodium chloride solution (0.85% w/v in 250 ml Erlenmeyer flasks). Samples of the resulting suspension were then spread on the surface of the Czapek-Dox agar and plate count agar medium for the growth and isolation of fungi and bacteria respectively. In each case the total count was then determined as colony-forming units (CFU) per liter of air. Incubation periods were 10 days at 30°C for total fungi and 3 days at 37°C for total bacteria.

Fungal genera and species were identified according to Johnson and Curl [22], Raper and Fennell [23], Gilman [24], Pitt [25], Ramirez [26], Nelson *et al* [27]. Bacterial genera and species were identified using Bergey's Manual of Systematic Bacteriology [28].

Standard statistical procedures were applied and a Minitab-for-Windows program used in analysis of frequency and standard deviation.

Results and Discussion

Monthly mean temperature and relative humidity was 28.7^{O}C and 40.3 % respectively, which is very low compared with many years ago [29].

The total count of bacteria and fungi collected from the different sites of Muna area are given in (Fig 1). Bacterial total count ranged between 31 to 140 colony-forming units (CFU) per liter of air. Similar results were reported earlier from other places in Saudi Arabia [2; 20; 30]. The highest count of bacteria population (140 CFU / liter air) was recorded in the central area of Muna followed by Jamarat bridge with (102 CFU / liter air). The lowest bacterial population was observed in the King Fahad tunnel with 31 CFU / liter air. King Fahad tunnel is located in the highest position of Muna area. This tunnel is for cars and pedestrians are not allowed.

The central area of Muna is the main place where pilgrims live. This site is crowded and rich in waste materials that accumulate with time; a most suitable environment for microbial pollution. In addition the central area and Jamarat bridge suffer from leaching of sewage water since they are located in the lowest position of the Muna area. As a result the central area and Jamarat bridge recorded the highest bacterial counts.

According to [Detrie, 5, p. 68] that the total count of bacteria in an underground tunnel ranged from 600 to 800 CFU / liter air; while the total count of bacteria in France ranged between 200 - 500 CFU / liter air depending on the density of people. As a result it appears that bacterial total count is highly correlated with people population. Environmental factors such as a temperature and relative humidity have a direct effect on the population and activities of microorganisms. Since the temperature and relative humidity in the atmosphere of Muna area during the time of sampling were very low, so the total count of bacteria was less than those reported from other countries.

Al-Sarrani and Al-Turk [2] reported that the total count of air-borne bacteria in Prophet's Mosque in Madinah region, Saudi Arabia, ranged between 5-130 CFU / liter air, while the recent study reported that the total count of bacteria in air samples collected from some natural environments of Saudi Arabia ranged from 12- 150 CFU / liter air depending on the density of people [2; 20; 30].

Fungal total count ranged between 20 to 122 colony-forming units (CFU) per liter of air (Fig.). This result is similar to earlier findings by other workers in Saudi Arabia [2; 19; 20; 21; 31, 35, 36]. The highest count of fungal numbers (122 CFU / liter air) was observed in the central area of Muna followed by the food market with 100 CFU /

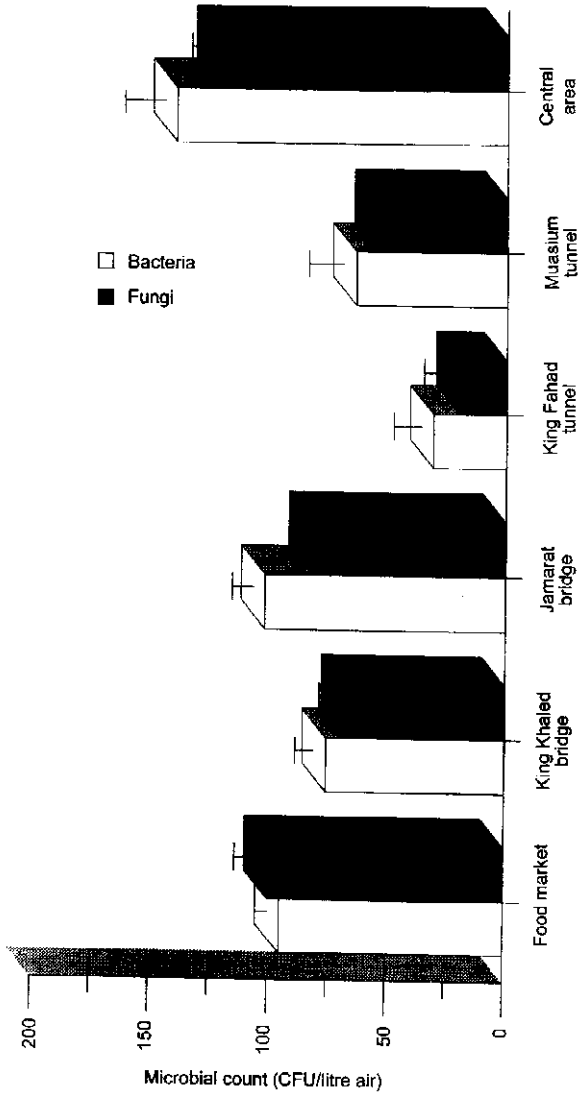

Fig. The total count of air-borne fungal and bacterial flora at Muna, Saudi Arabia.

liter air; while the King Fahad tunnel had the minimum fungal count with 20 CFU / liter of air. The total count of fungi in a survey of air-borne mould flora at Riyadh and Jeddah, Saudi Arabia ranged from 34 to 150 CFU / liter air [19, 31, 35, 36].

In fact all of these fungal and bacterial species were also isolated from different places of Saudi Arabia by other workers [2; 19; 20; 21; 31, 32, 33, 34, 35, 36, 37, 38]. There are several environmental factors led to abundance of microbes in atmosphere, such as rain precipitation, winds flaw, dust and suspended particles that caused by movement of cars and pedestrian pilgrims. So the Higher Organisation of Al-Hajj is requested to reduce the sandy roads by construction with asphalt and remove the waste of building materials before Al-Hajj season. This will decline the microbial air pollution and avoid the distribution of infectious diseases.

Table (1) shows the frequency of bacteria species isolated from different localities in Muna. The frequency percentage means number of sites which exhibited microbial species out of total places studied (six localities). A total number of nineteen bacterial species belonging to six genera were isolated; *Bacillus* was the most common genus and was represented by five species. The highest frequency was recorded in the case of *Bacillus subtilis* with 100 %, so it was present in all places, this was followed by *Bacillus anthracis, Bacillus megeterium, Micrococcus leteus, Neisseria elongata, Neisseria subflava, Pseudomonas pseudomallei, Pseudomonas stutzeri, Shigella boydii, Staphylococcus aureus and Staphylococcus capitis* all with a frequency 83 %. The species *Bacillus alvei* exhibited the minimum frequency with 50 % (present in 3 places). In the present study all bacteria species isolated were present in the food market and Muasim tunnel.

The population and frequency of fungi species isolated from different localities in Muna are given in Table (2).Thirty eight species of air-borne fungi belonging to six genera were isolated from the six localities.

The predominant fungal species were *Cladosporium herbarum* and *Penicillium citrinum* with frequency 100 %, followed by *Alternaria humicola, Alternaria phragmospora, Aspergillus fumigatus, Fusarium solani, Fusarium coeruleum, Penicillium lanosum, Penicillium chrysogenum* and *Penicillium islandicum* with frequency 83 %, while the frequency of *Curvularia* spp was 50 %. Al-Sarrani and Al-Turk [2] observed that *Penicillium citrinum* was the predominant fungi in the atmosphere of the Prophet's Mosque in Madinah region, Saudi Arabia, while in a survey of air-borne mould flora at Riyadh and Taif, Saudi Arabia *Cladosporium herbarum* predominated [18; 20 21, 36].

The frequency of *Cladosporium herbarum* and *Penicillium citrinum* was almost similar to earlier findings by other workers in Saudi Arabia [19, 20, 31]. *Cladosporium*

herbarum and *Penicillium citrinum* were the predominant fungi in all the localities searched (Table 2).

Table 1. Bacterial isolates from different localities in Muna. (colony-forming units (CFU) per liter of air)

Bacteria species	Locality						
	Food market	King Khaled bridge	Jamarat bridge	King Fahad tunnel	Muasim tunnel	Central area	Frequency %
Bacillus Total count	26	12	15	3	10	7	
B. alvei Cheshire and Cheyne	5	-	3		1	-	50
B. anthracis Cohn	7	7	6	-	1	2	83
B. circulans Jordan	9	1	-	-	3	3	66
B. megeterium de Bary	4	2	4	1	2	-	83
B. subtilis (Ehrenberg) Cohn	1	2	2	2	3	2	100
Micrococcus Total count	8	34	1	5	17	8	
M. leteus (Schroeter) Cohn	4	14	-	3	5	8	83
M. roseus Flugge	2	8	-	2	7	-	66
M. varians Migula	2	12	1	-	5	-	66
Neisseria Total count	5	8	12	6	23	46	
N. elongata Bevre and Holten	3	-	5	6	9	36	83
N. subflava (Flugge) Trevisan	2	8	7	-	14	10	83
Pseudomonas Total count	30	4	25	9	6	23	
P. aeruginosa (Schroeter) Migula	11	-	7	2		8	66
P. alcoligenes Monias	6	-	4	2	2	-	66
P. pseudomallei (Whitmore) Haynes	3	3	9	5	2	-	83
P. stutzeri (Lehmann and Neumann) Sijderius	10	1	5	-	2	15	83
Shigella Total count	17	15	23	1	4	-	
S. boydii Ewing	8	7	14	1	1	-	83
S. sonnei (Levine) Weldin	9	8	9	-	3	-	66
Staphylococcus Total count	9	3	26	7	4	56	
S. aureus Rosenbach	3	-	17	3	2	24	83
S. capitis Kloos and Schleifer	4	3	9	-	1	15	83
S. hominis Kloos and Schleifer	2	-	-	4	1	17	66

The present study also indicates that *Aspergillus niger, Aspergillus raperi* and *Penicillium expansum* exhibited the minimum frequency with 33 %. No isolates of *Curvularia* or *Alternaria* were obtained in samples taken from King Fahad tunnel. In the present findings, interestingly most fungi species were found in the food market of Muna. The central area exhibited the maximum population of air-borne fungi with 122 CFU / liter of air.

In conclusion, abundance of fungal and bacterial flora of Muna was less than recorded at other studies possibly attributed to levels of temperature and relative humidity during time of sampling. The highest number of fungal and bacterial colonies were obtained from air in the central area, a place with higher density of people and waste materials, followed by Jamarat bridge and the food market. King Fahad tunnel

exhibited the minimum population of air-borne fungi and bacteria. The number of fungal or bacterial colonies was in general higher in densely populated than in less populated areas. The commonest fungi isolated were *Cladosporium herbarum* and *Penicillium citrinum* while the commonest bacteria was *Bacillus subtilis*.

Table 2. Fungal isolates from different localities in Muna. (colony-forming units (CFU) per liter of air)

Fungi species	Locality						
	Food market	King Khaled bridge	Jamarat bridge	King Fahad tunnel	Muasim tunnel	Central area	Frequency %
Alternaria Total count	20	7	24	-	4	10	
A. alternata (Fr.) Keissler	7	-	9	-	-	2	50
A. chlamydospora Mouchacca	2	-	1	-	-	4	50
A. humicola Oudem	4	2	5	-	1	3	83
A. phragmospora van Emden	3	5	4	-	1	1	83
A. tenuissima (Fr.) Wiltshire	4	-	5	-	2	-	50
Aspergillus Total count	25	13	10	5	11	21	
A. apica Mehrotra & Basu	3	4	1	1	-	-	66
A. candidus Link ex Fries	1	1	-	3	-	6	66
A. clavatus Desm.	5	-	4	-	1	-	50
A. flavus Link ex Fries	6	2	-	1	2	-	66
A. fumigatus Fres.	1	1	2	-	5	2	83
A. nidulans (Eidam) Winter	-	-	2	-	1	3	50
A. niger van Teighem	3	-	-	-	1	-	33
A. ochraceous Withelm	1	1	-	-	-	1	50
A. oryzea (Ahlburg) Cohn	-	1	1	-	1	7	66
A. raperi Stolk & Meyer	4	2	-	-	-	-	33
A. terreus Thom	1	1	-	-	-	2	50
Cladosporium Total count	14	5	6	1	5	8	
C. cladosporioides (Fres.)de Vries	8	-	2	-	2	3	66
C. herbarum (Pers.: Fr.) Link	6	5	4	1	3	5	100
Curvularia Total count	9	4	2	-	7	-	
C. intermedia Boedijin	7	4	1	-	-	-	50
C. tuberculata Jain	2	-	1	-	7	-	50
Fusarium Total count	13	16	9	5	11	27	
F. solani (Mart.) Sacc.	4	12	-	2	4	2	83
F. oxysporum Schlecht.	-	1	6	-	7	15	66
F. coeruleum (Lib.) Sacc.	6	3	2	1	-	4	83
F. ciliatum Link	3	-	1	2	-	6	66
Penicillium Total count	19	23	31	9	17	56	
P. lanosum Westl.	3	3	9	-	4	11	83
P. notatum Westl.	-	5	-	2	8	3	66
P. citrinum Thom	2	6	5	1	1	9	100
P. nigricans (Bain.) Thom	9	-	7	1	-	10	66
P. expansum Link	-	-	8		-	4	33
P.frequentans Westl.	3	7	1	-	-	7	66
P. chrysogenum Thom	2	1	-	1	2	3	83
P. islandicum Sopp	-	1	1	4	2	9	83

The recommendations:

1- Medical care of the pilgrims before and during Al-Hajj season.

2- Some restrictions for water uses to avoid leaching of sewage water.

3- All foods provided to pilgrims must be covered.
4- Inform the pilgrims to get rid of all waste materials in a proper places.
5- Daily collection of all wastes in a special containers to be taken very far of Makkah city.
6- There should be a law organizing pilgrims accommodation to be in a suitable tents and preventing them to live everywhere such as under bridges, streets etc .

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التلوث الميكروبي للهواء الجوي في منطقة منى بمكة المكرمة بالمملكة العربية السعودية

عبدالله مساعد خلف الفالح

قسم العلوم (الأحياء)، كلية المعلمين بالرياض ، ص.ب ٤٣٤١ ، الرياض ١١٤٩١ ، المملكة العربية السعودية

(قدم للنشر في ١٤٢٠/٨/٢ هـ و قبل للنشر في ١٤٢١/٢/٢٣هـ)

ملخص البحث. تم حصر أعداد الفطريات والبكتريا الهوائية لكل لتر هواء جوي في منطقة منى بمكة المكرمة خلال موسم الحج ١٤١٩هـ . وشملت الدراسة محلات بيع الأغذية، جسر الملك فهد، جسر الجمرات، نفق المعيصم ووسط منى.

وفي هذا البحث، تم عزل ثمانية وثلاثين نوعا من الفطريات وتسعة عشر نوعا من البكتريا التابعة لستة أجناس. وجد في هذه الدراسة أن كلا من الفطرة *Clladosporium herbarum* والفطرة *Penicillium eitrinum* من أكثر الأنواع الفطرية تواجدا في جميع المواقع. بينما كانت البكتريا *Bacillus subtilis* هي السائدة انتشارا في منطقة منى.

أوضحت نتائج التحليل الميكروبي للهواء الجوي في منطقة منى أن أعلى معدل للمستعمرات البكتيرية والفطرية في وسط المنطقة يليها جسر الجمرات ثم محلات بيع الأغذية. وتشير الدراسة إلى تزايد معدل التلوث الميكروبي في الأماكن المزدحمة بالحجاج عنه في الأماكن الأقل إزدحاما.