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Bacterial and Chemical Contamination Associated Carpet Dust in the Holy Mosque, Makkah Al-Mukarramah

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Abstract. Carpets are widely used as surface covering in the holy mosque (Almasjed Alharam) settings, and they are prone to contamination with infectious agents and chemical components. This study aims to evaluate the concentrations of bacteria and chemical pollutants associated carpet dust in the holy mosque, as well as bacterial concentrations in the air state. The dust samples were collected from eighty carpets during month of Ramadan 2011, using a cord portable vacuum cleaner. Airborne bacteria were collected using Airport MD8 gelatin filter sampler. Bacterial concentrations varied depending on location of carpets inside the mosque, with no significant differences between locations ($P \geq 0.05$). The highest mean bacterial concentrations associated dust (256.42×10^6 CFU/m^2) and in the air state (1.12×10^5 CFU/m^3) were found at the ground floor of 1st expansion location. The highest re-suspension factor (RF) was found at the ground floor of 1st expansion, and the lowest at ground floor of 2nd expansion. Bacillus (25%), Pseudomonas (13%) and Micrococcus (12%) were the dominated bacterial genera. Chloride and ammonium concentrations associated dust were higher at the basement and the ground floor locations. The results did not indicate the presence of any apparent seriousness of the current situation of the carpets in the holy mosque. The data can be treated as a first step in determining the acceptable levels for microorganisms associated carpets in the common indoor environments.

Keywords: the holy mosque, carpet, dust, bacteria, chemical components, risk assessment.

Introduction

Indoor environment is a unique and is characterized by its own sources. The holy mosque is furnished with the finest carpets, and their number is ~30,000 with various sizes. The carpet is considered a good environment for the growth of microorganisms, if appropriate temperature and humidity are available. The carpet works on absorption and book dust. Simply by walking across a floor, carpet or other flooring materials, microbes can become airborne, which can cause non-infectious airway diseases, allergies, asthma, or serious infectious (Miller and Colemans, 2015).

Studies have demonstrated that, in addition to particles suspended in outdoor air, material resuspended from surfaces, as a result of human activities, is an important source of indoor airborne particles. Relationships have been found between indoor airborne microbial contents and those-associated carpets (Bholah and Subratty, 2002; Gehring, *et al*, 2002, Ferro, *et al*., 2004; Bouillard, *et al*, 2005). Carpets and sofas represented a huge reservoir of mites, and allergens (Custovic, *et al*., 2002), however other investigators found no relationships between carpets and microbial contents (Dotterud, *et al*., 1995; Hargreaves, *et al*., 2003).

Microorganisms usually enter buildings through outdoors or intake of air conditioning systems, human activities, and contaminated building materials (Hansen, 1999; Bowers, *et al*., 2012; Kembel, *et al*., 2012). Fungi can colonize walls, windows, frames, dump furniture, and carpets (Stepalska, *et al*., 1999; Khan and Karuppayil, 2012). Comparing bio-contamination of tiled floor and carpeted one, the carpeted surfaces being strong sinks and had higher surface loading of microorganisms, and airborne microbial levels were higher over the tiled floor (Foarde and Berry, 2004). In contrast fungal and bacterial concentrations in the air above carpets were consistently higher than those over-non carpeted floors (Gravesen, *et al*., 1983). Fungal and bacterial infections are transmitted by direct contact with infected persons, soils, skin and hair (Dahdah and Scher, 2008). Dermatophytes were isolated from house dust of patients, residence (Sugimoto *et al*., 1995), socks (Katoh, 2006), slippers (Goksugur, *et al*., 2006), communal ablution areas and prayer carpets of the mosques (Raboobee, *et al*., 1998, Yenisehirli, *et al*., 2012).

Millions of musilm people visit Makkah every year and they prefer performing all prayers (five times each day) in Almasjed Alharam. The floor of Almasjed Alharam is entirely covered with carpets to cushion the knees and heads during worship. People take-off their shoes before entering the Al-Haram and directly step on the carpets. The carpets may be possibly contaminated with normal and infectious microorganisms. Little research works have been done on the presence of microorganisms associated carpets. Therefore, the present study aims to determine bacterial and chemical component ($SO4^{2-}$, $PO4^{3-}$, $NO3^{-}$, Cl^{-}, $CO3^{2-}$) concentrations associated the carpet dust, and bacterial concentrations in the air at different locations in the holy mosque, as well as to discuss some solutions of reducing contamination of the carpet.

Materials and Methods

Sampling locations and strategy

A total of 240 dust associated carpet, and 80 airborne bacterial samples were collected at six locations inside and outside the holy mosque (Table 1). The sampling locations represented the different micro-environment and human activity inside the holy mosque. Air samples were collected at a height of 1.5 m, the breathing zone, above the floor level. The both dust associated carpet and air samples were taken between 4.00 p.m and 9.00 p.m, during the month of Ramadan 1432 H (1-29 August- 2011).

Chemical components associated settled dust sampling

Dust associated carpets were collected using cord portable vacuum cleaner ((Model Sanyo, 2000W, Japan), by sampling the carpet area of 1m2 during 3 min. After sampling, 100 mg of the collected dust was dissolved in 100 ml distilled water, and shaken well for ~30 -60 min at the room temperature. The solution was filtrated through Whatman filter paper No-42, and the concentrations of $SO4^{2-}$, $PO4^{3-}$, NO^{3-}, Cl^{-}, $NH4^{+}$ were determined in the water fraction (Harrison and Perry, 1986). The concentrations of chemical components were expressed as µg/g of dust.

Airborne bacteria and bacteria associated settled dust sampling

The bacteria associated carpet dust was collected by using a vacuum pump, and sampling an area of 100 cm^2 of the carpet, during 1 min (Fig 1). The samples were collected from three parts of the carpet: 1) the place of prostration (upper part), 2) the center of the carpet, and 3) the lower part (place of feet). The dust particles were separately aspirated into a washing bottle containing 25 ml sterilized buffer phosphate .

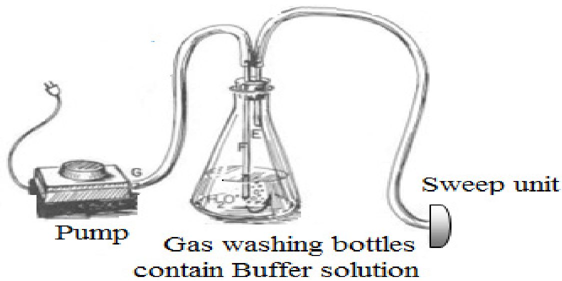

Fig.1. Illustrated device is used in microbiological accumulating samples from carpets.

Airborne bacteria were sampled using Airport MD8 gelatin filter sampler (Sartorius AG, Göttingen, Germany). The sampler was equipped with a flexible plastic hose connected with a filter head containing an 80-mm diameter gelatin membrane filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany). The air sampler was operated at a flow rate of 50 l/min, for 30 min. After sampling, filters were dissolved in 25 ml of phosphate-buffered saline, and shaken well in the room temperature, for 10 min.

Serial dilutions up to 105 were prepared and aliquots (0.5 ml) of the buffer phosphate and its serial dilutions were spread-plated onto the surface of nutrient agar medium to determine bacterial concentration. The bacterial plates were incubated at 37 °C for 48 h. The growing colonies were counted and the mean count was calculated. The concentrations were expressed as colony forming units per m^2 (CFU/m^2), and colony forming unit per cubic meter of air (CFU/m^3) for bacteria associated carpet dust and airborne bacteria, respectively.

Re-suspension factor (RF)

The airborne bacterial concentration of a finely particle contaminant can be related to its removable surface concentration via the resuspension factor equation:

$$RF\ (m^{-1}) = \text{airborne concentration } CFU/m^3 / \text{Surface concentration } CFU/m^2$$

The resuspension factor depends on the nature of the contaminant, the environment, and the activity taking place. The resuspension factor of air bacterial contamination is necessary for estimating the degree of contamination due to resuspension of surface/ carpet associated bacteria during maintenance and cleaning activities inside the holy mosque.

Identification of bacteria

Three to five bacterial isolates differed in morphology which appear in more than 5% of the nutrient agar medium were picked up, purified and subcultured for further identification. The bacterial isolates were identified using Gram stain, oxidation fermentation, oxidase and catalase tests as described in the Bergey's Manual of Systematic Bacteriology (Sneath, *et al*., 2000).

Risk assessment

Programs and mathematical equations were used in order to calculate the risks resulting from exposure to these compounds, and also the daily and age dose calculation, risk of cancer and as well as risk coefficient (Hassanien, *et al*., 2009).

Statistical Analysis

Data were statistically analyzed by one way analysis of variance test (One Way ANOVA) to find the difference between the means of bacterial counts associated carpets and their sampling sites inside the holy Mosque. $P \leq 0.05$ was considered as significant.

Results and Discussion

Microorganisms associated carpets are considered one source of bio-pollution in the closed spaces. Different studies have tended to identify the materials and factors that affecting bio-pollution and evaluating the control methods that can be efficiently reduced or eliminated bio-pollutants.

The range and the mean concentrations of bacteria associated carpets and in the air state are shown in Table 1. The concentrations of bacteria associated carpets ranged between $76x10^4$ - $572x10^6$ CFU/m^2 at the roof surface and $96x10^4$- $649.6x10^6$ CFU/m^2 at different sampling locations inside Al haram. The highest mean bacterial concentration ($256.42x10^6$ CFU/m^2) was found at the ground floor of 1st expansion, however the lowest (910 $x10^5$ CFU/m^2) was found at the roof surface, as roof surfaces' carpets are exposed sun ray's heat and natural wind. Inside Al Haram, the lowest bacterial concentration associated carpets was found in the basement location, because lower number of people perform worship in the basement place. In the present study, significant differences ($P \leq 0.05$) were found between bacterial concentrations associated carpets at the roof surface and basement with those associated carpets inside the mosque. On the other hand no significant differences ($P \geq 0.05$) were found between bacterial concentrations associated carpets at the ground floor and the first floor.

The bacterial concentrations vary regarding location. This may be related to the number of people and their activities, and ventilation rate. Microbial concentrations depend on many variables such as anthropogenic influence, human activity, topography, micro-environmental conditions, and type of sources (Després, *et al*., 2012). The influence of irregular disturbances and human activities lead to high microbial variations. Microorganisms are found /deposited on the floor surfaces through a variety of methods including foot traffic, food and drink spills, and bodily fluid deposition. Some microorganisms found on floors and in carpet can be re-suspended again into the air, and transported and settled on other locations in the closed spaces (Leonas, 2003).

In the present study, airborne bacterial concentrations ranged between $9x10^2$-$1.32x10^5$ CFU/m^3 (Table 1). The highest mean airborne bacterial concentration ($1.12x10^5$ CFU/m^3) was found at the ground floor of 1st expansion and the lowest ($1.31x10^3$ CFU/m^3) at the ground floor of 2nd expansion. This confirms the presence of a relationship between airborne microbial contents and those associated carpet dust, because carpets are one of the microbial sources in indoor air environment (Bouillard, *et al*., 2005; Ramachandran, *et al*., 2005).

Carpet can absorbs and reserves dust, and this phenomenon is growing in the holy mosque, due to the extensive demolition and renovation nearby the mosque. The carpet itself is a good environment for the growth of microbes, if suitable conditions are available" temperature, humidity and moisture". Almost 15% of bacterial types in the indoor air are carried and transmitted from the contaminated floors through human activities" walking, wiping and cleaning (Hambraeus, *et al.*, 1978). The carpet is considered one source of indoor air pollution in schools and hospitals, however the quality of the materials and cleaning help reduce the probability of indoor air pollution (Meckel, 1982). Anderson, *et al*. (1982) reported that the microbial content in patients' rooms furnished carpeted was higher than the microbial content of the unfurnished carpeted rooms.

In the present study, the highest resuspension factor (RF) was found at the ground floor of 1st expansion, and the lowest at ground floor of 2nd expansion (Table 1). The term resuspension is mainly used to describe the process by which any aerial deposited materials might become airborne. The resuspension can be of concern because the possibility of spreading human diseases and contamination in indoor environment (Aylor, 1976; Nicholson, 1995). In the present study, it is clear that inhalation dose may be higher at the ground floor of 1st expansion, which may be uncomfortable area for people inside the holy mosque.

Table 1. The range and mean concentrations of bacteria-associated carpet dust and airborne bacteria regarding location at Al Haram

Location	Bacteria associated carpet dust		Airborne bacteria		FR (m^{-1})
	No.	CFU/m^2 x10^4	No.	CFU/m^3 x10^3	
Basement	60	(96-49550) [13831.1]	15	(48-58) [53.2]	0.00038
Ground floor of 2nd expansion	30	(1159-64865) [21485.2]	15	(0.9-2) [1.31]	0.0000061
Ground floor of 1st expansion	30	(578-60060) [25642.2]	15	(96-132) [112.4]	0.00044
1st floor of 2nd expansion	30	(531-59676) [18890.4]	15	(63-72) [67.8]	0.00036
1st floor of 1st expansion	27	(570-55255) [22556.6]	15	(35-58) [46.3]	0.00021
The roof surface of Al-Haram	63	(76-57200) [9104.6]	15	(76-97) [84.6]	0.00093

No: number of collected samples, RF (m^{-1}): resuspension factor.

Figure 2 shows the mean concentrations of bacteria-associated dust regarding the part of the carpet. The concentrations ranged between 96 x 10^4 - 64865x10^4 CFU/m^2. The highest concentration (18.459 x10^7cfu/ m^2) was found in the lower part of the carpet (place of feet), with no significant differences with concentrations in the other parts of the carpet. This is attributed to during prayer; feet contact the carpets more longer time than other parts of the body. A carpet has some similarity to an air filter that the dirt particles can become mechanically trapped, and not easily dislodged during cleaning, and the particles adhesion is increased if the carpet is moist and if both particle and the carpet fiber are wetted (Pope, *et al*., 1993).

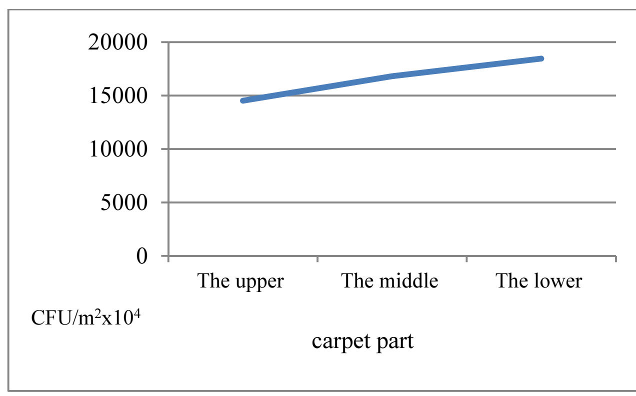

Fig. 2. The mean concentrations of bacteria associated dust regarding the part of the carpet.

In the present study, *Bacillus sp*, *B. subtilis*, *B. circulans*, *Escherichia coli*, *Klebsiella sp.*, *K. pneumonia*, *Micrococcus sp.*, *M. roseus*, *Peptostreptococcus sp.*, *Pseudomonas sp.*, *P. aeruginosa*, *Proteus sp.*, *Ruminococcus sp.*, *Staphylococcus sp.*, *S. aureus*, *Streptococcus sp.* were isolated. *Bacillus* (25%), *Pseudomonas* (13%) and *Micrococcus* (12%) were the dominated genera associated carpets. The genera associated carpet samples were similar with those genera isolated from the indoor air of the Holy Mosque (Nasrallah, *et al*., 2007). This confirms the existence of a relationship between bacterial content of the internal air and those in the carpet, and the resuspension has a significant role in the airborne bacterial content.

The concentrations of chemical components of the dust associated carpets at different sampling sites are shown in figure 3. The chemical component concentrations varied regarding sampling site. Sulfates, phosphates and nitrates concentrations were lower at the roof surface and the ground floor than those found in the basement and the first floor. This is attributed to the amount of outdoor dust particles infiltrate to indoors. The outdoor dust particles are mainly emitted from traffic and renovation activities around the holly mosque. On the other hand, chloride and ammonium concentrations were higher at the basement and the ground floor due to the use of detergents and lack of air ventilation.

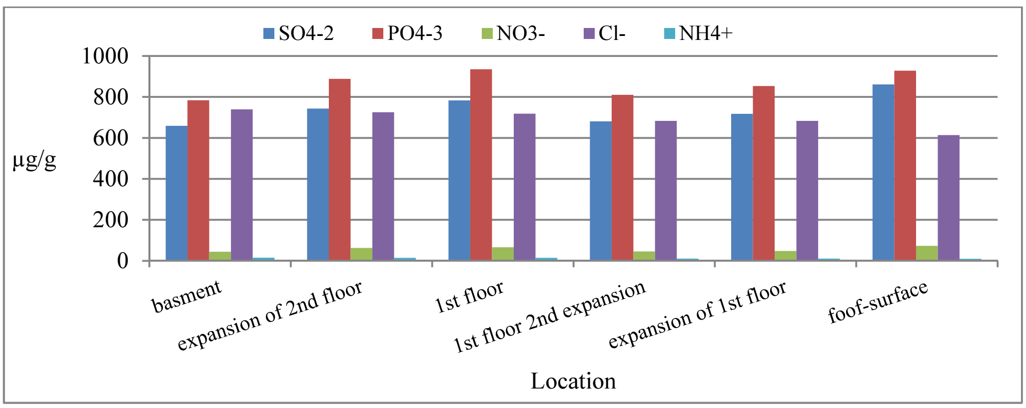

Fig.3. The mean concentrations of chemical components of dust associated carpets

The present study focuses on calculating risk coefficient that is occurred as a result of human exposure to chemical components, regarding daily and age dose, danger of cancer, coefficient of Hazard Quotient (H.Q), and the contribution of these chemical concentrations in the air (Table 2). The results showed that there was no cancer risk, and the H.Q represented little value (~ $4x10^{-6}$), "if H.Q ≤1, it means that no human risk due to exposure to the carpets' dust and its chemical components'(Hassanein, *et al*., 2009).

The visitors of the holy mosque who exposed to such chemical concentrations did not represent any unexpected dangerous under any other expectations. Due to the lack of specifications/or limits that are recognized by the quality and quantity of microbial and chemical contents associated carpets, the obtained results did not indicate the presence of any apparent seriousness of the current situation of the carpets in the holy mosque.

Table 2. The expected chemical component concentrations in the air, daily and age dose, risk coefficient and danger of cancer.

Substance	Daily dose		Age dose		Expected conc. in the air ($\mu g/m^3$)
	Exposure (mg/m^3)	Swallowing (mg/kg/day)	Exposure (mg/m^3)	Swallowing (mg/kg/day)	
Nitrate	$2 x 10^{-6}$	$7 x 10^{-6}$	$2 x 10^{-7}$	10^{-8}	$2 x 10^{-6}$
Sulphate	$3 x 10^{-5}$	$8 x 10^{-5}$	$2 x 10^{-6}$	10^{-6}	$3 x 10^{-5}$
Chloride	$2 x 10^{-5}$	$7 x 10^{-5}$	$2 x 10^{-6}$	10^{-6}	$2 x 10^{-5}$
Ammonium	$4 x 10^{-7}$	$1 x 10^{-6}$	$2 x 10^{-8}$	$2 x 10^{-8}$	$4 x 10^{-7}$
Sum of risk coefficient of all components = $4 x 10^{-6}$					

Decontamination of the carpets

The carpet cleaning machine reduced the number of *Salmonella enterica* but could not completely get rid of this strain (Rice *et al.*, 2003). Hydrogen peroxide gas reduced concentrations of *B. anthracis*, *B. subtilis,* and *Geobacillus stearothermophilus* associated carpets (Rogers *et al.*, 2007). Malik *et al.* (2006) got rid of *Feline calicivirus* in the contaminated carpet and upholstery by using disinfectant of Metricide and Microbac-II within a minute to 10 minutes, but the eliminated rate of the virus decreased in furnishings manufactured of 100% polyester. Recently some carpet manufacturers have treated their products with fungicidal and/or bactericidal chemicals that help reduce the overall numbers of bacteria or fungi and their use do not preclude the routine care and maintenance of the carpeting (CRI, 2013).

Conclusions

Carpets are considered one of the important indoor sources of microorganisms. Some infectious bacteria were isolated from the dust associated carpets. Bacillus, Pseudomonas and Micrococcus were the dominated bacterial genera. The resuspension factor has an important role in the airborne bacterial load. Regular cleaning with antifungal and antibacterial-carpet shampoo and using disposable slippers may control numbers of microorganisms. It is recommended to study organic and inorganic compounds, especially during urban activity in the Holy Mosque. Integrated risk assessment of the indoor environment of the Holy Mosque should be studied in the future

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التلوث البكتيري والكيميائي المرتبط بغبار السجاد في المسجد الحرام بمكة المكرمة

بسام مشاط

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المستخلص. يستخدم السجاد على نطاق واسع لتغطية الأسطح المختلفة في المسجد الحرام، وهو دائما ما يكون عرضة للتلوث الميكروبي والكيميائي. وتهدف هذه الدراسة إلى تقييم تركيزات البكتيريا والمركبات الكيميائية المرتبطة بغبار السجاد في المسجد الحرام. تم تجميع عينات الغبار من عدد ثمانين سجادة موزعة بكامل المسجد الحرام وذلك خلال شهر رمضان ١٤٣٢هـ، باستخدام مكنسة كهربائية. أيضًا تم تجميع عينات البكتيريا المحمولة بالهواء باستخدام جهاز MD8 المحتوى على مرشح الجيلاتين. أظهرت النتائج تفاوت تركيزات البكتيريا المرتبطة بغبار السجاد وذلك اعتمادا على المكان (الموقع) داخل المسجد الحرام، مع عدم وجود فروق ذات دلالة إحصائية بين التركيزات عند المواقع المختلفة . تم رصد أعلى تركيزات للبكتيريا المرتبطة بغبار السجاد (٢٥٦،٤٣X ١٠^{٦} مستعمرة/م^{٢}) وفي الهواء (١،١٢ x ١٠^{٥} مستعمرة/م٢) في الطابق الأرضي من موقع التوسعة رقم ١. كما تم رصد اعلى عامل اعادة تعليق(RF) في الطابق الأرضي من التوسعة رقم ١ والأقل عند الطابق الأرضي من التوسعة رقم ٢، مما يدل على دور غبار السجاد فى الحمل البكتيرى بالهواء. كانت البكتيريا العصوية (٢٥٪)، والباذيدوموناس (١٣٪) والميكروكوكس (١٢٪) هى الأجناس البكتيرية الاكثر انتشارا المرتبطة بغبار السجاد، اما بالنسبة للمكونات الكيميائية كانت املاح الكلوريد والأمونيوم هى الأعلى تركيزا فى غبار السجاد فى البدروم والطابق الأرضي. اظهرت النتائج عدم وجود أي خطورة من المحتوى الكيميائي الراهن المرتبط بغبار السجاد في المسجد الحرام، وتعتبر هذه البيانات خطوة أولى في تحديد المستويات المقبولة للملوثات الميكروبية المرتبطة بالسجاد في البيئات العامة المغلقة.

الكلمات الدالة: المسجد الحرام، السجاد، الأتربة، البكتيريا، التلوث الكيميائي، تقييم المخاطر.