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Estimation and Identification of Airborne Bacteria and Fungi in the Outdoor Atmosphere of Al-Mafraq Area, Jordan

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Abstract

Airborne bacteria and fungi were analyzed during November, 2013. Morbidity due to respiratory diseases was also reported. The studied zones include Al-Mafraq downtown, Al al-Bayt University, Al-Zaatari refugee camp and the open desert. A total of sixty air samples were collected by a microbiological air sampler on nutrient and tryptic soy agars as cultivation media for bacteria. Potato dextrose, Sabouraud dextrose and malt extract agars were used as cultivation media for fungi. Statistical analysis revealed that there was a significant difference between almost all studied zones ($P<0.05$). The highest bacterial level was detected in Al-Mafraq downtown with 2055 CFU m^{-3}, whereas the lowest level was detected in the open desert with 23 CFU m^{-3}. The highest level of fungi was detected in Al-Zaatari refugee camp (405 $CFUm^{-3}$), whereas the lowest level of fungi was observed in the open desert zone (13 $CFUm^{-3}$). Bacteria and fungi levels were within the suggested threshold value limits for culturable bacteria and fungi. Eleven different bacterial species and four fungal species were isolated from these zones and identified by biochemical and molecular techniques. Fungi were examined macroscopically and microscopically and compared to the morphology of published fungal species. The identified bacterial species were *Bacillus cereus*, *Bacillus aerius*, *Bacillus safensis*, *Bacillus subtilis*, *Bacillus axarquiensis*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus methylotrophicus*, *Bhargavae acecembensis*, and *Cellulomonas* sp. The isolated bacteria were all aerobic, Gram-positive, endospore-forming bacteria and catalase positive. The identified fungi were *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp. and *Fusarium* sp. In respect to respiratory diseases in the studied area, the most frequent lung diseases in the studied area was bronchitis (42%), followed by chest infection (25%), pneumonia (21%), and chronic obstructive pulmonary diseases (12%).In conclusion, the isolated microbial species may appear to originate from the dusts of human and animal.

Keywords: Airborne Bacteria, Air quality, Desert, Environment, Respiratory Diseases.

1. Introduction

It is well known that the outdoor air quality significantly affects the human health and ecosystem. At the global level, a sharp rise in outdoor air pollution was observed during the past decades (Ostro, 2004; Mandal and Brand, 2011; IARC, 2013). Outdoor air pollutants include various chemical compounds as well as several biological pollutants, especially airborne bacteria and fungi. It has been well documented that outdoor airborne bacteria and fungi as well as their spores are public health problem that affects the health of millions of people around the world (Ostro, 2004; Qudiesat *et al*., 2009; Menteşe *et al*., 2009; Mandal and Brand, 2011; Ko and Hui, 2012).

Several investigations have reported that soil, water, plants, animals and human are the main sources of outdoor air borne bacteria and fungi (Swan *et al.*,2002; Ostro, 2004; Menteşe *et al*., 2009; Abdul Hameed *et al*., 2009; Yassin and Almouqatea, 2010; Bowers *et al*., 2011; Hospodsky *et al*., 2012; Muhsin and Adlan, 2012; Ghosh *et al*., 2013).Based on these investigations, the diversity, distribution, and abundance of outdoor airborne bacteria and fungi were studied in several regions of the world and found to be diverse between any two cities, towns or villages around the world. These variations might be due differences in population size and density, and the type of activities within the examined areas. These mentioned variations can be very difficult to control.

Globally, the rising in the levels of outdoor airborne pathogens in highly populated areas is essentially a human ecological and health problem due to placing populations at risk of high burdens of respiratory diseases and other infectious diseases (Ostro, 2004; Qudiesat *et al*., 2009; Menteşe *et al*., 2009; Ko and Hui, 2012). For instance, outdoor airborne bacteria and fungi can cause several types of respiratory illnesses or conditions such as asthma, bronchitis, pneumonia, chronic obstructive pulmonary disease (COPD), seasonal allergies, and others (Yassin and Almouatea, 2010; Bowers *et al*., 2011). In addition, certain airborne bacteria and fungi or their endotoxins are known to induce infectious diseases, acute toxic effects, allergies and eye irritation in some individuals (Menteşe *et al*., 2009; Hospodsky *et al*., 2012). Outdoor airborne bacteria and fungi spores can

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easily travel through the air. Consequently, inhaling these microorganisms can impair human wellbeing and health and cause various respiratory diseases (Ostro, 2004; Qudiesat*et al*., 2009). Based on international reports, diseases associated with outdoor airborne bacteria and fungi can causes significant mortality and morbidity every year, particularly among children due to respiratory diseases (Ostro, 2004).

The causal links between the outdoor air quality and the levels of airborne bacteria and fungi, in any environment, are complex because they are often indirect, displaced in space and time, and dependent on a number of modifying factors, including the source types and the ambient physicochemical conditions (Ostro, 2004; Abdul Hameed *et al*., 2009; Dong and Yao, 2010; Bowers *et al*., 2011). More importantly, the survival and spreading of airborne bacteria and fungi is largely dependent on certain physicochemical factors like temperature and humidity (Lighthart and Stetzenbach, 1994; Brodie *et al*., 2007; Fierer*et al*., 2008). The airborne microorganisms usually thrive and circulate in damp and humid air conditions. Crowded conditions and poor air circulation can increase the spreading and the survival rate of these microorganisms and also increase the chance of people at risk of contagious respiratory diseases (Lighthart and Stetzenbach, 1994; Goyer *et al*., 2001; Ostro, 2004; Tsai and Macher, 2005; Brodie*et al*., 2007; Muhsin and Adlan,2012).

The levels of outdoor airborne bacteria and fungi were determined for different cities and countries around the world (Yassin and Almouqatea, 2010; Hospodsky *et al*., 2012; Muhsin and Adlan, 2012; Ghosh *et al*., 2013) and the threshold value limits for culturable bacteria and fungi were also suggested(Dong and Yao, 2010). The published data reveal that outdoor airborne bacteria and fungi were studied at the regional level (Yassin and Almouqatea, 2010; Muhsin and Adlan, 2012). However, at the local level, i.e., in Jordan, few studies were carried out to investigate the presence of the outdoor airborne bacteria and fungi. For instance, one study was conducted in northern Jordan (Saadoun *et al*., 2008) and another one was carried out in Zarqa city (Qudiesat*et al*., 2009) to analyze airborne microorganisms in hospitals.

The levels of outdoor airborne bacteria and fungi in Jordan are widely unexplored especially in the main cities, including Al-Mafraq governorate. This governorate is the second largest governorate in Jordan with respect to area but it is one of the lowest with respect to population (306,900 at the end of 2013) and population density (11.6 at the end of 2013) [DOS, 2015]. However, the population density and human activities are concentrated in certain areas in the governorate. It is also worth mentioning that the number of population is increasing due to the increase in the refugee's numbers after the Syrian crisis. For instance, the United Nations data indicated that the total persons of concern in Al-Zaatari refugeecamp are more than 84,000 at the beginning of 2015 (UNHCR, 2015). This number reflects an increase by about 30% in population size. Therefore, the present study was carried out to quantify and identify airborne bacteria and fungi in Al-Mafraq governorate in northern Jordan to establish standards for future reference. The levels of airborne bacteria and fungi were determined in the outdoor air of four selected zones in this governorate: Al-Mafraq downtown, Al al-Bayt University, Al-Zaatary refugee camp, and the open desert using different types of cultivation media. Furthermore, to examine if there is a link between inhalation or exposure to these airborne fungi and bacteria and development of respiratory symptoms or diseases, the morbidity rate, associated with respiratory diseases related to airborne bacteria and fungi,was also reported in the present study.

2. Materials and Methods

2.1. Description of Sampling Sites

In the present study, four different zones located in Al-Mafraq governorate were chosen for the collection of airborne bacteria and fungi. These zones were AL-Mafraq downtown (Zone A), Al al-Bayt University (Zone B), Al-Zaatari refugee camp (Zone C) and the open desert (Zone D) [Figure 1]. These selected sites differ basically in the population density, the type and the intensity of anthropogenic activities. Zone A is the commercial center of the city characterized by various types of human activities. Zone B, the only university in Al-Mafraq governorate, includes about 18500 persons. Zone C hosts more than 80,000 people (UNHCR, 2015). The last zone, Zone D is an open desert area, non-populated and has no human activities. Generally, Al-Mafraq governorate is semi-desert area in its nature. During sampling in November, 2013, the temperature was at daytime between 18-22°C and the humidity at that time was 34-38%.

2.2. Sampling and Cultivation of Airborne Bacteria and Fungi

A total of sixty air samples were collected in this study using microbial air sampler (M.A.Q.S.II-90, OXOID, UK). This device can hold 90 mm Petri dish containing media within an aluminum head of 380 holes. The air sampler device was set at an air sampling rate of 100 $Lmin^{-1}$ per sample and at one meter tall. At the end of the sampling, the plates were removed and aerobically incubated at 37°C for 3 days in case of bacteria and at 25° C for 7 days in case of fungi. The aluminum head of the air sampler was sterilized by 70% alcohol between sample collections.

Five different types of microbiological media were used during the present study. Tryptic Soy Agar (TSA) and Nutrient Agar (NA) (HiMedia Laboratories Pvt. Mumbai, India) were used to cultivate airborne bacteria. Sabouraud dextrose agar (SDA), potato dextrose agar (PDA) and malt extract agar (MEA) (HiMedia Laboratories Pvt. Mumbai, India) were used to cultivate fungi and chloramphenicol was added to fungi media as a bacterial growth inhibitor.

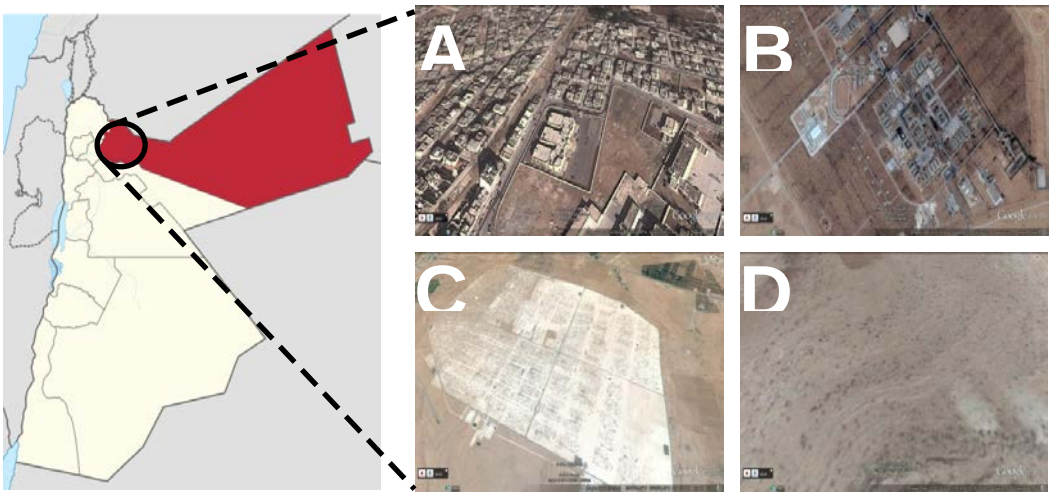

Figure 1. Map of the sampling sites: To the left, Jordan map with Al-Mafraq Governorate colored with red and a circle around the sampling area. To the right, aerial view of the sampling zones: (A) Al-Mafraq downtown (Zone A), (B) Al al-Bayt University (Zone B), (C) Al-Zaatari refugee camp (Zone C), and (D) the open desert (Zone D). Map and aerial views were retrieved from Google and Google Earth.

2.3. Enumeration and Isolation of Airborne Bacteria and Fungi

After the incubation of airborne bacteria and fungi as mentioned earlier, the developed colonies were counted and expressed as colony forming units per cubic meter ($CFUm^{-3}$).

The cultivated bacteria were compared with respect to colonial morphology, including shape, pigmentation, elevation, texture and other characteristics. Morphologically different colonies were transferred to new media and isolated as a pure culture. Additionally, 30% glycerol stock cultures were prepared from each isolate and stored at -20ºC (Jacob and Irshaid, 2012).The isolated fungi were subjected to macroscopic and microscopic examinations to observe their growth behavior, the nature of their mycelium and hyphae structure (Watanabe, 2002). Pure cultures of fungi were stored as tube slants at 4°C.

2.4. Identification of Airborne Bacteria

The bacterial isolates were first identified based on their reaction with Gram stain. Gram staining is essential to determine the further steps in identification to the species level. The bacterial isolates were then subjected to further identification using biochemical techniques.

First, the catalase test was conducted as a pre-requirement for the identification of bacteria by RapID CB® plus system. This test was performed by adding a few drops of the catalase test reagent (hydrogen peroxide) on a viable culture. The positive test leads to bubbles (oxygen) formation. This reaction can be seen with the naked eye.

Second, the RapID CB® Plus System (Remel, Lenexa, KS, USA) was used to identify the isolated strains to the species level. This system is mainly used for identification of Gram-positive bacteria. The system panel consists of four tests for utilization of carbohydrate and fourteen tests for single-substrate enzyme. Preparation of bacteria suspension of each isolate, inoculation, incubation times and temperatures, interpretation of reactions, and quality control were performed according to the manufacturer's recommendations for RapID CB® Plus system. Electronic RapID Compendium(ERIC) software was used to identify the isolated strains to the species level.

To identify those bacterial isolates that could not be identified by the biochemical method described above, the 16S rRNA gene sequencing was performed. In this method, DNA was extracted as previously mentioned (Jacob and Irshaid, 2012). Briefly, genomic DNA was extracted and purified from pure bacterial culture from each isolate using the EZ-10 Spin Column Genomic DNA (Biobasic, Ontario, Canada) following the instructions of the manufacturer.

Pure DNA samples were then subjected to 16S rRNA gene sequencing by GENEWIZ, Inc., USA.The16S rRNA gene sequences of the new isolates were deposited in GenBank® database. The resulting 16S rDNA sequences were analyzed to identify these strains by comparison with the complete nucleotide collections obtained from GenBank® database using Web BLAST Service (http://blast.ncbi.nlm.nih.gov /blast/Blast.cgi).

2.5. Fungal Identification

The colonial morphology of fungi was examined using a stereomicroscope (Meiji Techno Co., Ltd, Japan) and then wet mounts in lacto-phenol cotton blue were prepared for microscopic examination. The observations were then evaluated and compared to what is documented in the literature (Watanabe, 2002).

2.6. Statistics of Morbidity

The present study is also interested in investigating the relationship between outdoor airborne bacteria and fungi and respiratory illnesses or conditions especially as bronchitis, chest infection, pneumonia, asthma and COPD. Thus, data about respiratory diseases were collected from Al-Mafraq governmental hospital. The data represent all the admitted respiratory diseases cases in this hospital during2013.

2.7. Statistical Analysis

A statistical analysis was carried out using SPSS 19.0 software. Data of the bacterial counts underwent one-way ANOVA test and data of fungal counts underwent two-way ANOVA test. The means were compared using Duncan's multiple range tests at significance level of 5%. Microsoft Excel 2010 was used to calculate the standard deviation (SD) and standard error of the mean (SEM) and preparing the graphs.

3. Results

The present work was undertaken to quantify and identify the outdoor airborne bacteria and fungi in four different sites in Al-Mafraq governorate. The studied sites were Al-Mafraq downtown, Al al-Bayt University campus, Al-Zaatari refugee camp and the open desert. A total of sixty samples were collected and studied from these sites. The temperature values in the selected sites ranged from 18-22 °C during the sampling time, whereas the relative humidity was within the range of 34-38%.

3.1. Levels of Airborne Bacteria and Fungi

The quantitative analysis indicated that the level of bacteria in all the four studied zones range from 50 to 2055 CFUm^{-3} when NA was used as the cultivation medium. The highest bacterial level was detected in Al-Mafraq downtown zone, whereas the lowest level was detected in the open desert zone (Figure2). The statistical analysis indicated that there was a significant difference ($P<0.05$) in bacterial levels between all zones ($P<0.05$). However, when TSA was used as the cultivation medium, the levels of bacteria was found to range from 23 to 1263 CFU m^{-3}. The highest level of bacteria was detected in Al-Zaatari refugee camp, whereas the lowest level of bacteria was detected in the open desert, when TSA was utilized for growth (Figure 1). Statistical analysis indicated that there wasa significant difference ($P<0.05$) in the bacterial levels between the four studied zones when TSA was used as the cultivation medium, except between zone A and C ($P>0.05$).

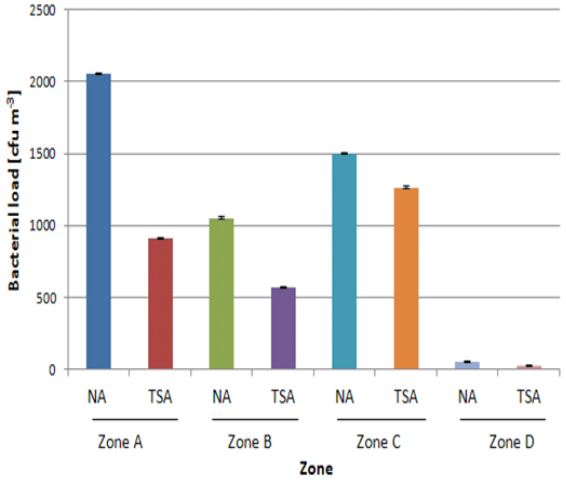

Figure 2. Bacterial levels (CFUm^{-3}) in outdoor air of the studied zones (A, B, C, and D) according to the type of medium used for their cultivation. CFU: colony forming unit; TSA: tryptic soy agar; NA: nutrient agar. Data represent the mean of three separate measurements.

In respect to the levels of airborne fungi, the statistical analysis indicated that there was a significant difference in the fungal levels between the four zones ($P<0.05$). The level of fungi ranged from 13 to405 CFU m^{-3}, when MEA was used as the cultivation medium. The highest level of fungi was detected in Al-Zaatari refugee camp zone, whereas the lowest level of fungi was observed in the open desert zone. When SDA was used for fungal growth, the level of fungi decreases and ranged from 14 to 295 CFUm^{-3}. The highest number of fungal count was found in Al-Mafraq downtown, whereas the lowest number of fungi was detected in the open desert. Moreover, when PDA was used as the cultivation medium, the level generally decreased and ranged from 16 to 191 CFU m^{-3}. The highest level of fungal colonies was detected in Al-Zaatari refugee camp zone, whereas, the lowest level of fungal colonies was found in the open desert zone (Figure 3).

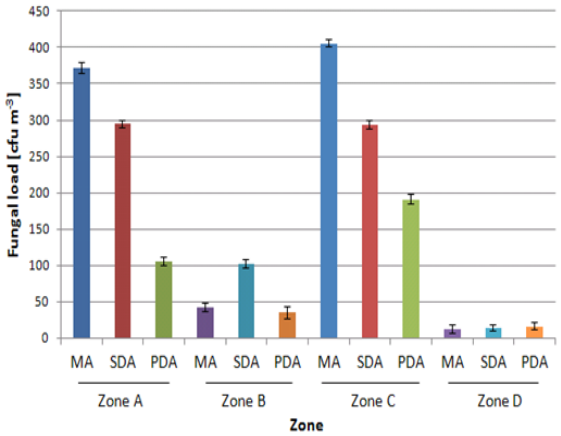

Figure 3. Fungal levels (CFU m^{-3}) in the outdoor air of the studied zones (A, B, C, and D) according to the type of media. CFU: colony forming unit; MEA: malt extract agar; PDA: potato dextrose agar, SDA: Sabouraud dextrose agar.

3.2. Identification of Airborne Bacteria and Fungi

A total of seventy two isolates of bacteria and fungi were isolated. The isolates were found to represent eleven different bacterial species and four fungal species. Eleven morphologically different bacterial isolates were selected from these examined zones. These eleven isolates were designated as TSA1.3A, TSA1.3C, TSA1.4C, TSA2.3D, NA3.1A, NA2.5B, NA2.4B, NA3.4C, NA3.5IC, NA3.2C and NA3.5IIC. The TSA or NA in the beginning of isolate code refers to TSA or NA medium used for their cultivations, whereas the letter at end of the isolate code (A, B, C or D) refers to zone from which the isolate was obtained. Six of these eleven isolates were recovered from Al-Zaatari refugee camp samples (zone C). Two isolates were recovered from each of Al-Mafraq downtown (zone A) and Al-al-Bayt University campus(zone B) samples. Only one isolate was obtained from sample collected from the open desert (zone D).

Morphologically, all the recovered isolates were shown to be Gram-positive aerobic bacteria. These isolates were also subjected to biochemical identification by RapID CB® plus system and ERIC software. The biochemical properties of the isolated bacterium are shown in Table 1. All the tested isolates gave a positive

reaction with catalase test as well as positive for utilization of glucose and potassium nitrate. Furthermore, all examined isolates were able to hydrolyze p-nitrophenyl-β,D-glucoside, p-nitrophenyl-glycoside and the fatty acid ester. Based on the data generated from these biochemical analyses and using ERIC software, only one bacterial isolate (TSA1.3A), out of eleven, could be identified by this method. TSA1.3A isolate was identified as *Cellulomonas* sp. with 99% probability.

Table 1. Qualitative biochemical tests of the isolated strains and their identification results using ERIC software.

Test	Result
GLU*	+
SUC	+
RIB	-
MAL	-
αGLU	-
βGLU	+
NAG	-
GLY1	+
ONPG	-
PHS	-
EST	+
PRO	-
TRY	-
PYR	-
LGLY	-
LEY	-
NIT	+
CAT	+
PIG	-

***Abbreviations of chemical tests**: +: Positive reaction; -: Negative reaction. GLU: Utilization of Glucose, SUC: Utilization of Sucrose, RIB: Utilization of Ribose, MAL: Utilization of Maltose, αGLU: Hydrolysis of p-Nitrophenyl-α,D-glucoside, βGLU: Hydrolysis of p-Nitrophenyl-β,D-glucoside, NAG: Hydrolysis of p-Nitrophenyl-n-acetyl-β,D-glucosaminide, GLY1: Hydrolysis of p-Nitrophenyl-glycoside, ONPG: Hydrolysis of o-Nitrophenyl-β, D-galactoside, PHS: Hydrolysis of p-Nitrophenyl phosphate, EST: Hydrolysis of the fatty acid ester , PRO: Hydrolysis of Proline-β-naphthylamide, TRY: Hydrolysis of Tryptophan-β-naphthylamide, PYR: Hydrolysis of Pyrrolidine-β-naphthylamide, LGLY: Hydrolysis of Leucyl-glycine-β-naphthylamide, LEU: Hydrolysis of Leucine-β-naphthylamide, URE: Hydrolysis of Urea, NIT: Utilization of Potassium nitrate, CAT: Catalase test, and PIG: Yellow Pigmentation.

Biochemical tests were not enough to identify most of the isolated strains. Therefore, for the remaining unknown isolates, DNA was isolated and subjected to molecular analysis, namely, 16S rRNA gene sequencing and analysis. The unknown isolates TSA2.3D, TSA1.4C, NA3.1A, NA2.5B, NA2.4B, NA3.4C, NA3.5C, NA3.2C, TSA1.3C and NA3.5IIC have 97% identity or more with *Bacillus methylotrophicus*, *Bacillus axarquiensis*, *Bacillus cereus*, *Bhargavaea cecembensis*, *Bacillus safensis*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus aerius* and *Bacillus subtilis*, respectively. All 16S rRNA sequences were deposited in GenBank® database. All isolates and their identifications as well as their GenBank database accession numbers are listed in Table 2.

Table 2. The closest relatives of the isolated airborne bacteria based on 16S rRNA gene sequence as well as the GenBank® accession number (GBAN) of the sequences of the isolated airborne bacterial species.

Isolate code	Identification	Identity (%)	GBAN
TSA2.3D	*Bacillus methylotrophicus*	97	KP297807
TSA1.4C	*Bacillus axarquiensis*	99	KP297808
NA3.1A	*Bacillus cereus*	97	KP297809
NA2.5B	*Bhargavaeacecembensis*	99	KP297810
NA2.4B	*Bacillus safensis*	98	KP297811
NA3.4C	*Bacillus pumilus*	98	KP297812
NA3.5C	*Bacillus amyloliquefaciens*	99	KP297813
NA3.2C	*Bacillus licheniformis*	99	KP297814
TSA1.3C	*Bacillus aerius*	97	KP297815
NA3.5IIC	*Bacillus subtilis*	98	KP297816

3.3. Identification of Airborne Fungi

Four morphologically different fungal isolates were isolated from the four selected zones using three different media (SDA, PDA and MEA). These fungal isolates were designed as SDA1.1D, PDA3.1A, PDA1.2C and MEA2.2A. Based on macroscopic and microscopic examinations, the SDA1.1D, PDA3.1A, PDA1.2C and MEA2.2A were classified as *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp. and *Fusarium* sp., respectively. *Aspergillus fumigatus* and *Fusarium* sp. were isolated from the samples taken from Al-Mafraq downtown zone, whereas *Penicillium* sp. and *Fusarium* sp. were recovered from the samples collected from Al-Zaatari refugee camp zone and the open dessert zone, respectively.

3.4. Annual Morbidity Due to Respiratory Diseases in the Studied Area

The data collected from Al-Mafraq governmental hospital for the year 2013 indicated that the highest percentage of respiratory diseases was bronchitis (42%, 100 cases). This was followed by chest infection (25%, 60 cases), pneumonia (21%, 50 cases) and COPD (12%, 30 cases) [Figure 4].

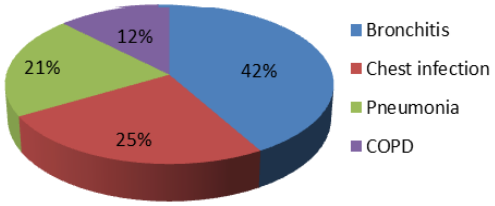

Figure 4. Annual rate of respiratory diseases among admissions to Al-Mafraq governmental hospital in the year 2013.

4. Discussion

In the present study, experiments were conducted to determine the levels of microorganisms (mainly bacteria and fungi) in four selected zones in Al-Mafraq governorate and characterize themto species level. In addition, the present study tends to examine the link between the presence of these airborne bacteria and fungi and development of respiratory diseases. Thus, the respiratory diseases in the same area were also reported.

The results of the present study indicated that there were significant differences in the number of bacteria and fungi among the tested zones. The levels of bacteria and fungi were found to be related to the population density as well as to the human activities and traffic in the studied zones. This conclusion is true when the crowded zones (Al-Mafraq downtown, Al al-Bayt University, and Al-Zaatari refugee camp) were compared to the zone of low or no human activity (the open desert). Human activities seem to be the main generator of outdoor bioaerosols as indicated by many reports (Menteşe *et al*., 2009; Ostro, 2004). Different human activities can contribute in generating or increasing the bioaerosol levels. These include shedding of skin cells, talking, coughing, and sneezing. Sneezing is one of the most vigorous mechanisms of spreading airborne microorganisms by generating as many as two millions of droplets per sneeze (Krishna, 2004). The presence of such droplets or particulates in air adds protection to bacterial cells and result in enhanced survival of the airborne microorganisms. Additionally, both humans and animals release small skin fragments from the body containing different bacterial species. Humans walking will generate up to 5,000 bacteria per minute to the surrounding air (Smith, 2006).

The highest level of bacteria was detected in the atmosphere of Al Mafraq downtown with 2055 $CFUm^{-3}$ when NA was used as the cultivation medium and 1263 $CFUm^{-3}$ when TSA was used. The threshold value limit for the culturable bacteria was suggested as 5000-10000 CFU m^{-3} (Dong and Yao, 2010). The levels of bacteria in all zones and the use of different media did not exceed this suggested threshold. However, caution should be taken when the results of different studies are compared due to differences in the geographic zone, season and time of sampling, media of cultivation, type and intensity of human activity, growth cycle of organisms, and meteorological factors (Abdel Hameed *et al*., 2009; Dong and Yao, 2010). For comparison, higher number of bacteria (12,639 CFU m^{-3}) was observed in the train stations and subway system in Beijing (Dong and Yao, 2010). The Beijing environment is characterized by being among the highest density and intensity of human activity compared to our selected region. In the open commercial streets of Beijing, the number was much lower than those reported for the train stations and subway system, supporting the role of human density and activities in the obtained results.

Furthermore, eleven bacterial species and four fungal species were isolated from the outdoor air environment of the four selected zone areas in this governorate. The isolated bacteria were found to belong to the following species: *Cellulomonas* sp., *Bacillus methylotrophicus*, *Bacillus axarquiensis*, *Bacillus cereus*, *Bhargavaea cecembensis*, *Bacillus safensis*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus aerius* and *Bacillus subtilis*. The isolated bacteria are mostly typical airborne bacteria and possess characteristics that provide them with resistance to harsh environmental conditions and dispersal ability. These isolated bacterial species were all Gram-positive and endospore-forming. Gram-positive bacteria are in general more resistant to drying than Gram-negative bacteria because of their thick and rigid cell wall (Madigan *et al*., 2009). Furthermore, the endospores of endospore-forming bacteria are extremely resistant to drying and promote survival in air (Goyer*et al*., 2001). It is also clear that most of the isolated genera belong to the genus *Bacillus* which is a typical spore-forming bacterium. The spores of this genus are characterized by their resistance to dryness and UV radiation. These suitable characteristics will ultimately increase the chance of these species to grow and thrive on these examined areas.

It has been reported that Gram-positive bacteria seem to predominate in dusts of animal and human origin, whereas Gram-negative bacteria predominate in dusts of plant origin (Swan *et al*., 2002). Because all of our isolates were Gram-positive, this supports the aforementioned assumption that the detected species and levels of bacteria are mainly due to human presence and its activities and density. Similarly, certain studies in Europe have demonstrated that Gram-positive bacteria are the most commonly found bacteria in indoor air environment (Gorny and Dutkiewicz, 2002). In addition, other study on US office buildings revealed that Gram-positive cocci are the most prevalent in both indoor and outdoor air (Tsai and Macher, 2005).

In respect to the levels of airborne fungi, the statistical analysis indicated that there was a significant difference in fungal levels between the four zones ($P<0.05$). The levels of fungi ranged from 13 to 405 CFU m^{-3}, when malt extract agar was used as cultivation medium. When SDA was used as cultivation media, the levels of fungi decreased, ranging from 14 to 295 CFU m^{-3}. When PDA was used as cultivation media, the levels generally decreased and ranged from 16 to 191 CFU m^{-3}. The threshold value limit for the culturable fungi was suggested as 5000-10000 CFU m^{-3} (Dong and Yao, 2010). These data revealed that the reported level of fungi in the examined areas are generally far below the suggested threshold value limit. In other parts of the world, fungi level was found to be 1528 and 1806 in train stations and the subway system in Beijing respectively, whereas, in open commercial streets of Beijing, the calculated level was very low (Dong and Yao, 2010). Based on our data, the values of certain physicochemical factors are closely similar, including the temperature and humidity. Taken together, these findings indicated that the levels of fungi might be due to human presence and its activities and density.

The data generated from this study also revealed that the *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp., and *Fusarium*sp. were only identified fungi species in atmosphere of these examined areas. *Aspergillus niger*

and *Aspergillus fumigates* are usually associated with two respiratory diseases in humans, which are known as allergic and invasive aspergillosis. These species seem to be the most frequently isolated airborne species of fungi in other regions. For instance, the common genera of fungi frequently isolated from the air of hospitals at the United Arab Emirates were *Aspergillus* and *Penicillium* (Jaffal *et al*., 1997). Similarly, *Aspergillus* and *Penicillium* were also among the frequently detected species in the industrial town of Helwan, Egypt (Abdel Hameed *et al*., 2009). Recent study also revealed thatthe genera *Aspergillus*, *Penicillium*, and *Fuzarium*were isolated from the outdoor air of the Basrah city of Iraq (Muhsin and Adlan, 2012).Thus, these findings are in agreement with our findings. In addition, these findings and our findings suggest that the potential source of these fungi species is likely to be the similar. However, a closer inspection of the aforementioned areas revealed that some of the environmental factors among these areas are not necessarily alike or the same.

It is worth mentioning that the level and distribution of these airborne bacteria and fungi species among the tested zone areas were not uniform or similar; rather, each species appeared to be associated with a certain zone. This is in consistence with the previous studies which showed that the cell concentrations of airborne bacteria and fungi species can be affected by various environmental factors (Ostro, 2004; Menteşeet al., 2009; Yassin and Almouqatea, 2010; Mandal and Brand, 2011; Muhsin and Adlan, 2012). These factors include temperature, humidity, air dust, soil dirt, sanitary conditions as well as human presence, activities and density. Type of cultivation medium, sampling location and height from which these samples were collected can also influence the level and distribution of these airborne microorganisms. A closer look at these four examined locations revealed that there were high similarities regarding these factors, with exception human presence, density and type of activities as well as the sanitary conditions. Therefore, the observed variations in levels and distribution of these airborne species are more likely due to human density and activities as well as due to the sanitary conditions of the tested areas.

Based on the data generated from the present study, only a small number of outdoor airborne fungal and bacterial species were identified in our selected areas by using cultivation-dependent techniques. Therefore, it is possible to speculate that the exact number of the species of fungi and bacteria in our air samples are likely to be underestimated or overlooked by the cultivation-dependent methods. Hence, the full extents of the outdoor airborne bacterial and fungal diversities in the examined zones remain poorly characterized and understood.

Data about respiratory diseases were also collected exclusively from Al-Mafraq governmental hospital for the year 2013 during this study. The highest percentage of these diseases was bronchitis (42%, 100 cases). Bronchitis is the inflammation of bronchial tubes. The main causes of bronchitis are viral; however, airborne bacteria may cause bronchitis, especially in people underlying health problems (Warrel, 2008). Chest infection was the second most frequent respiratory disease (25%, 60 cases) that was recorded by Al-Mafraq governmental hospital. Chest infection is the infection of lungs or airways and it has two main types: bronchitis and pneumonia. Pneumonia represents 21% of respiratory diseases. It is an inflammation of lungs that is usually caused by an infection. One type of pneumonia occurs when aerosols are inhaled into lungs (called aspiration pneumonia). Bacteria are among the common cause of pneumonia in adults (Meterske*t al*., 2012). However, the detected species are not among the main causes of pneumonia. A COPD is the least frequent respiratory disease. Causes of COPD are almost smoking and/or air pollution. A previous study also reported that outdoor air pollution was associated with the development of COPD (Ko and Hui, 2012). Taken together, it appears that the isolated species do not correspond directly to the reported cases of respiratory diseases in this governorate during the year 2013. Nonetheless, the resident of the examined areas may face some health problems due to the continuous inhalation of or the exposure to the isolated airborne pathogens. It is also worth mentioning that it is not definite that the admitted cases of respiratory diseases by the studied hospital belong exclusively to the studied zones. Therefore, precise correlations could not be made between the reported cases of respiratory diseases and the isolated microbial species during this study.

5. Conclusion

Eleven bacterial species and four fungal species were isolated and identified from the outdoor air environment of the four selected zone areas in Al-Mafraq governorate, Jordan. The levels of outdoor airborne bacteria and fungi in the open desert zone were found to be considerably lower than those found in Al-Mafraq downtown, Al al-Bayt University and Al-Zaatari refugee camp. It was also found that the isolated species does not correspond directly to the reported cases of respiratory diseases in this governorate. However, inhalation of or exposure to some of the isolated microorganisms might cause some human respiratory diseases. Therefore, implementation of better strategies for reducing the number of outdoor airborne bacteria and fungi would have benefits for human health.

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