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Atmospheric Movement of Bacteria and Fungi in Clouds of Dust in Erbil City, Iraq

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Abstract: In this study monthly aerosol samples collected at Erbil city, Iraq, throughout 2008-2009 yielded significant concentration of viable (culture forming) bacteria and fungi only when the dust was present. The results indicated the concentration of total bacteria was ranged from 3×10^4 to 36×10^6 CFU/g, and concentration of fungi varied a wide range from 6×10^3 to 36×10^3 CFU/g. The dominant group of bacteria isolated from the dust was *Pseudomonas* 25.6%, including *P. corrugate*, *P. diminuta*, *P. marginata* and *P. agaramicus*, followed by *Zanthomonas* 17.9%, including *Z. orgzae*, *Staphylococcus* 12.8%, including *S. xylosus*, *S. epidermidis*, *S. homonis* and *S. cohini*, *Bacillus* 10.2%, including *B. subtilis* and *B. licheniformis*, *Curtobacterium*, *Micrococcus* and *Streptococcus* 5.1%, *Ctrubacterium*, *Rhodococcus* and *Agrobacterium* 3.4%. Our data also confirms the existence of *Aspergillus* which comprised 23.4% of fungi colonies, followed by *Penicillium* 12.8%, *Emerciella* sp. 10.62%, *Tetracoccosporium*, *Eurotium* sp. 8.51%, *Rhizopus* sp., *Sterile fungus*, *Trichocladium* sp. 6.4%, *Alternaria* sp., *Phoma* sp., *Yeast*, *Curvularia* sp., *Ascochyta* sp., *Taeniolella* sp., *Trichophyton*, *Fusarium* sp., 2.12% of total isolated fungi.

Key words: Bacteria, dust, Erbil, fungi, Iraq, microbial transport

INTRODUCTION

Winds serve as a vector that enables the transport of microorganisms among widely dispersed habitats (Isard and Gage, 2001). These include organisms pathogenic to human, plants and animals (Brown and Hovm¨ller, 2002). The large deserts on the planet, which include Rub-Al khali, Al Jazerah of khalig Al-Araby, Gobi, Takla Makan, and Badain Jaran deserts of Asia, Sahara and Sahel region of North Africa, are the primary source of mobilized desert top soil that move great distances through the atmosphere each year, it is believed that the deserts of south Africa have been the dominant sources of dust in the atmosphere of Iraq (USGS, 2003), there has been increased dust activity over the last 10 years that has been attributed to climate change and desertification, and desert topsoil is laden with viable and diverse prokaryote communities (Caimi and Eisenstark, 1986; Kuske *et al*., 1997; Carlton *et al*., 2001; Dose *et al*., 2001; Papova *et al*., 2002) Globally, a gram of topsoil contains 10^7 to 10^9 prokaryote (Whitman *et al*., 1998), these populations are believed to consist of 10^3 bacteria 99.9% of diversity (Torsvik *et al*., 1990; Torsvik *et al*., 2002; Gans *et al*., 2005). Studies that have examined the number of cultivatable prokaryotes in desert soil have reported concentrations ranged from 0 to 10^7 per g (Kwaasi *et al*., 1998; Navarro-Gonzalez *et al*., 2003; Maier *et al*., 2004), and the total No. of fungal typically found in a gram of topsoil is approximately 10^6 (Tate, 2000). In this study we examine the monthly temporal record of bacteria and fungi concentration and genera, only when the dust was present over the period November 2008-Juli 2009 in Erbil city, Iraq.

MATERIALS AND METHODS

Dust samples were collected passively by allowing sedimentation monthly, using Sampling bulk or wet design collectors. The bulk sampling system typically consist of bucket or funnel and bottle configuration that is opened to the atmosphere during precipitation events and dry period (Durst *et al.*, 1991), stored in sterile container until analysis.

Microbial count: The concentration of cultivable bacteria and fungi per gram of dust (CFU/g) was estimated using standard plate count (Ellringer *et al*., 2000).

Identification of fungi: Traditional microbial detection method are based on culturing method underestimate the total amount of microbes present in the sample, one gram of dust sample was placed into a screw cup vial which

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Table 1: Concentration and species of cultivable bacteria in dust during 2008-2009 in Erbil city

Month	Isolated bacterial species	No. of colonies (CFU/g)
November, 2008	*Pseudomonas corrugate, Pseudomonas diminuta, Bacillus subtilis, Microbacterrium* spp	36×10^6
December, 2008	*Pseudomonas marginata, Pseudomonas diminuta, Curtobacterium* spp.	3×10^6
January, 2009	*Pseudomonas* spp. *Xanthomonas* spp., *gl, Micrococcus* spp.	3×10^7
February, 2009	*Pseudomonas testosteroni, Pseudomonas diminuta, Staphylococcus xylosus, staphylococcus epidermidis, Streptococcus* spp.	3×10^6
March, 2009	*Microbacterium* spp., *Staphylococcus homonis, Xanthomonas orgzae*	3×10^6
April, 2009	*Microbacterium* spp., *Staphylococcus cohinil, Xanthomonas oryzae*	3×10^4
May, 2009	*Ctrubacterium* spp., *Streptococcus epidermidis, Xanthomonas oryzae*	3×10^4
Jun, 2009	*Staphylococcus xyolsus, Rhodococcus fasians, Xanthomonas oryzae*	3×10^6
July, 2009	*Micrococcus* spp., *Microbactrium* spp., *Xanthomonas oryzae*	3×10^6
Augest, 2009	*Microbacterium* spp., *Bacillus lichenformis, Bacillus subtilis, Agrobacterium* spp., *Pseudomonas agaramicus*	3×10^6
September, 2009	*Bacillus subtilis, Bacillus licheniformis, Streptococcus epidermidis, Pseudomonas* spp., *pseudomonas corrugate, Xanthomonas* spp.,	3×10^6

Table 2: Concentration and isolated species of cultivable fungi from outdoor dust during 2008-2009 in Erbil city

Month	Isolated fungi species	No. of colonies (CFU/g)
November, 2008	*Aspergillus* spp., *Trichocladim* spp., *Phoma* sp, *yeast, Penicillium* spp., *Tetracoccosporium* spp.	27×10^3
December, 2008	*Aspergillus* spp., *Rhizopus, Eurotium* spp., *Taeniolella* spp., *Tetracoccosporium* spp. *aspergillus niger*	19×10^3
January, 2009	*Rhizopus* sp. *Emericella* sp., *Tetracoccosporium* spp., *Sterile fungum, Aspergillus niger,*	1×10^4
February, 2009	*Aspergillus niger, Aspergillus tamari, Ascochyta* sp., *Alternaria* sp., *Emerciella* sp., sterile fungus, *Tetracoccosporium* spp.	29×10^3
March, 2009	*Alternaria* spp., *Penicillium* spp., *Trichocladium* sp., *Eurotium* sp, *Sterile fungus, Curvularia* sp.	14×10^3
April, 2009	*Trichocladium* spp., *Fusarium* spp. $6x10^3$	
May, 2009	*Aspergillus niger, Aspergillus* spp., *Emericella* sp., *Penecillium* spp.	11×10^3
Jun, 2009	*Penicillium* spp.	36×10^3
July, 2009	*Penicillium* spp. *Aspergillus* sp., *Trichophyton* sp.	7×10^3
August, 2009	*Aspergillus niger, Aspergillus* spp., *Penicillium* spp., *Eurotium* sp., *Rhizoctinia* sp., *Emericella* sp.	8×10^3
September	N	N

N: Data not obtained

contain 9 mL of sterilized distal water. The screw cup contents were mixed by scrolling and left to stabilize. One ml of suspension was withdrawn and added to another screw cup containing 9 mL sterilized D.W. this process repeated another time again to three dilution, was 10^{-1}, 10^{-2}, 10^{-3}, the suspension were left to stabilize, one ml from each dilution was cultured on potato dextrose agar (PDA) and the plates were incubated at 25±2ºC for 7 days as reported in (Al-Herthi, 2005). The resulting cultures were subculture onto similar media. With rare exceptions, blanks yielded on cultures. Standard light microscopy and phase contrast microscopy were used to examine fungal cultures and identify spores producing in culture. Phenotypic identification was based on the microscopic and microscopic morphology of the cultures and spores, respectively, using taxonomic keys (Scott, 2001).

Identification of bacteria: For identification of bacteria one ml from each dilution (as explained previously for fungi) added to Blood based nutrients are non selective media which are widely used for broad spectrum studies (Lacey and Venette, 1995), the cultures were incubated at 37ºC for 48 h. Identification is based primarily on morphology, gram stain, spore stain, motility, and biochemical tests were carried out (Morello *et al*., 2003). Moreover the API20E (Bio Merieux, Marcyl, Etoile, France) system was performed when necessary. The pure cultures were sub cultured on nutrient agar slants and preserved in the refrigerator at 4ºC until required.

RESULTS

Concentration of bacteria observed during dust storms are noted in Table 1, between November 2008 and July 2009 in Erbil city, Iraq.

When assessed by culturing the media concentration of total bacteria were ranged from 3×10^4 to 36×10^6 CFU/g, average of four replicates, these numbers were varied between months, the highest number 36×10^6CFU/g was recorded in January 2009, while for other months were 3×10^6 CFU/g dust or less.

Result identified presence of potential human, plants and animals pathogens, these isolates were composed of 10 bacterial genera. The dominant group of bacteria isolated from the dust was *Pseudomonas* 25.6%, including *P. corrugate*, *P. diminuta*, *P marginata* and *P. agaramicus*, followed by *Zanthomonas* 17.9% including *Z. orgzae*, *Staphylococcus* 12.8% including *S. xylosus*, *S. epidermidis*, *S. homonis* and *S. cohini*, *Bacillus* 10.2%, including *B. subtilis* and *B. licheniformis*, *Curtobacterium*, *Micrococcus* and *Streptococcus* 5.1%, *Ctrubacterium*, *Rhodococcus* and *Agrobacterium* 3.4%. Concentration of fungi varied a wide range from 6×10^3 to 1×10^4 CFU/g Table 2. Concentration were not affected by the seasons, there are substantial differences between the months. In particular, during Jun, February and November the concentration were markedly higher than other months. In fact concentration in Jun was substantially higher than any other period in the record.

Table 2 shows the frequency of occurrence of colony forming fungi, based on identification of different colonies. The dominant fungi was *Aspergillus* which comprised 23.4% of fungi colonies, followed by *Penicillium* 12.8%, *Emerciella* sp. 10.62%, *Tetracoccosporium*, *Eurotium* sp. 8.51%, *Rhizopus* sp., *Sterile fungus*, *Trichocladium* sp. 6.4%, *Alternaria* sp., *Phoma* sp., *Yeast*, *Curvularia* sp., *Ascochyta* sp., *Taeniolella* sp., *Trichophyton*, *Fusarium* sp., 2.12% of total isolated fungi.

DISCUSSION

The concentration of total bacteria and fungi analyzed by culture were 3×10^4 to 36×10^6 and 6×10^3 to 36×10^3 CFU/g for bacteria and fungi respectively Table 1 and 2, same results has been previously detected in the dust samples (Whitman *et al*., 1998) reported concentration ranging from 10^7 to 10^9 per g, and 0 to 10^7 (Kwaasi *et al*., 1998; Maier *et al*., 2004; Navarro-Gonzalez *et al*., 2003). The quantification of these results is based on colony forming unites i.e., including the possibility that a colony originates from an aggregate of several cells and spores, and that not all organisms are capable of producing a colony on artificial laboratory media. The preliminary results in this study proved that the concentration of cultivable bacteria and fungi in the dust are high, the results may change somewhat when the full dataset becomes available for each organisms.

In the present study a total of ten bacterial genera and 16 fungi were isolated from dust soil Table 1 and 2, and the dominant bacteria was *Pseudomonas*, followed by *zanthomonas*, *Staphylococcus*, *Microbacterium*, *Bacillus*, *Streptococcus*, *Micrococcus*, and the dominant fungi was *Aspergillus*, *Penicillium*, *Emerciella*, *Tetracoccosporium*, *Rhizopus*, *Alternaria*, *Phoma*, *Trichocladium*. Desert dust research in Kuwait and Iraq, Which is the same source of the dust as in studied region, identified 149 bacteria CFU, which included representatives from 10 genera, these genera included *Mycobacterium*, *Brucella*, *Coxiella burnetii*, *Clostridium perferingens*, and *Bacillus*, the link between airborne particulate inhalation and variety of respiratory diseases has long been established (Luski *et al*., 2011). Seven genera were isolated from the atmosphere over Erdemli Turkey, during Saharan dust event in March 2002 (Griffin *et al*., 2002).

Dust bacteria and fungi are ubiquitous organisms found in the dust and have been related to the development of three types of human disease hypersensitive responses (allergic reaction, infections and toxicosis) of the respiratory system (Edu *et al*., 2010). Presence of wide range of bacteria and fungi cause risk through inhalation of airborne microorganisms and their associated contaminants can cause a range of immunological and respiratory symptoms. *Pseudomonas* species was dominant group of bacteria observed in the dust and transferred to our region, these genera of bacteria and fungi may present in the soil, but inhalation of the cloud dust containing microorganisms obligatly make risk. Some of these species are pathogenic for human, plants and animals, *P. corrugate* are plant pathogenic bacteria (Smith *et al*., 1988), *Staphylococcus xylosus* is a commensal bacterium of the skin, and has the ability to produce enterotoxins D, C or E, and are opportunistic pathogens of animals and are multi drug resist. *Bacillus licheniformis* is commonly associated with food spoilage and poisoning, it cause bread spoilage, or more specifically, and Rope spores is what causes the spoilage, unfortunately these spores do not get killed during the baking process, and also cause septicemia (Salkinoja-Salonen *et al*., 1999), *Staphylococcus epidermigis* is opportunistic, endocarditis, urinary tract infection bacteria (Wisplinghoff *et al*., 2003). *Aspergillus* causing bread mold and seed decays, consists of approximately 185 species of which have been identified as causing opportunistic infections in man (Simmon-Nobbe *et al*., 2008), it is distributed ubiquitously in our natural environment and represents a dominant indoor pathogen, *Penicillium* causing blue mold root of fruits, *Fusarium* causing vascular wilts, root rots, and seed infections, *Alternaria* causing many leaf spots and blights, *Rhizopus* causing bread molds and soft root of fruits and vegetables (Agrios, 1997). In addition to the presence of these organisms, dust can indirectly impact human health by spurring toxic algal blooms in coastal environments (Holmes and Miller, 2004; Lenes *et al*., 2001; Walsh and Steidinger, 2001). Dust clouds may contain high concentration of organics composed of plant detritus and microorganisms (Griffin *et al*., 2002; Jaenicke, 2005). All of these potential dust cloud constituents may negatively influence human health with the greatest risk factors being frequency of exposure, concentration of and composition of particulates, and immunological status. Dust born microorganisms in particular can directly impact human health via pathogenesis, exposure of sensitive individual to cellular components (pollen and fungal allergens and lipopolysaccharides, etc.) (Griffin, 2007). There is no information on the dust constituents in this region, and the current study considered the first study of dust contents microorganisms in Erbil city.

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