Scientific Research and Essays Vol. 6(25), pp. 5435-5441, 30 October, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.1485
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

Aero-microbiological study on distribution pattern of bacteria and fungi during weekdays at two different locations in urban atmosphere of Gwalior, Central India

Pramod Kumar, Pawan Mahor, A. K. Goel*, D. V. Kamboj and Om Kumar

Biotechnology Division, Defence Research and Development Establishment, Gwalior-474002, India.

Accepted 20 September, 2011

The concentration and quality of microbes in urban atmosphere may affect human health and environment. In recent years, interest in air microbiology has been focused on air sampling strategies, indoor-outdoor distribution of microbes and climatic influences on microbial population. The weekdays distribution of aeromicrobial load at public places is not well established. Moreover, the research on airborne bacteria is under-acknowledged in India. In this study, air microbial load was estimated at two locations, a public garden (PG) and traffic circle (TC) at Gwalior, Central India during weekdays. The average weekday bacterial concentration at TC was found significantly higher (7120.48 cfu/m^3) than at PG (4804.76 cfu/m^3). The distribution of bacteria during weekdays (Monday to Saturday) was interesting as significantly higher bacterial load was found at TC than at PG. However, on weekend that is on Sunday, significantly higher count was observed at PG than at TC. The average weekly fungal count over TC (776.19 cfu/m^3) was also found higher than at PG (605.71 cfu/m^3). During week days, fungi also exhibited almost the similar distribution pattern as of bacteria at both sites. The correlation analysis of microbial load with meteorological factors (temperature, humidity and wind speed) revealed statistically non-significant effect. The study indicated that weekday variation in microbial load is mainly affected by daily activities as compared to meteorological factors in urban environment.

Key words: Airborne, bacteria, fungi, weekday variation, meteorological factors.

INTRODUCTION

Microorganisms are ubiquitous in the atmosphere but their proportion varies according to the environmental conditions and locations. Generally, a higher microbial concentration is found in urban areas than surrounding rural areas (Bovallius et al., 1978; Lighthart, 1997; Shaffer and Lighthart, 1997). Many activities like traffic, constructions and people gathering in urban areas contribute largely to outdoor microbial load (Fang et al., 2005, 2007). The concentration of airborne microorganisms shows topological, geographical, diurnal and seasonal variations. Airborne microbial quantity and quality can vary with time of the day, year and location (Abdel et al., 2009; Bovallius et al., 1978; Fierer et al., 2008).

Thus, their presence in air is a cumulative function of the geographical locations, anthropogenic activities and environmental factors (Burch and Levetin, 2002; Fang et al., 2007; Tang, 2009). Environmental conditions such as relative humidity (RH), temperature and wind velocity exert significant effect on the type of population and amount of microorganisms in the air (Harrison et al., 2005; Jones and Harrison, 2004; Mouli et al., 2005). Generally, microbes enter into atmosphere from natural (vegetation and soil) and anthropogenic sources but their survival and distribution depend on the cell structure of microbes and meteorological conditions (Abdel et al., 2009; Jones and Harrison, 2004; Lighthart, 1997). In addition to the more predictable sources like vegetation and soil, fecal material has also been found as an unexpected source of bacteria in the atmosphere (Bowers et al., 2011). Finally, the microbial concentration

*Corresponding author. E-mail: akgoel73@yahoo.co.uk. Tel: 91-751-2233742. Fax: 91-751-2341148.

in the atmosphere relies on abundance of source and factors controlling their release and dispersal from the surface boundary layer (Burge and Rogers, 2000).

Many airborne microorganisms are either pathogenic or can cause sensitivities due to prolonged exposure (Griffin, 2007). Airborne microbes attach to dust particles, condense and enter human body directly via inhalation or indirectly via ingestion of contaminated foods and water (Gorbushina and Palinska, 1999) resulting in the development of disease (Heldman, 1974). Airborne bacteria can also affect visibility, climate and the quality of life (Beggs, 2003; Griffin, 2007; Martiny et al., 2006). It is important to know the distribution pattern of live bioaerosols at different sites in the urban environment. Therefore, to evaluate the potential for microbial air pollution and other associated risks, current viable microbial levels must be monitored. This study was aimed at understanding the distribution pattern of microbes in the air.

MATERIALS AND METHODS

Air sampling site descriptions

Air samples were collected from two different locations in Gwalior, a historical city in Central India (longitude 78° 13′ E, latitude 26° 13′ N). The first location was a traffic circle (TC) near railway station where traffic remains fairly steady throughout the day. Traffic consisted primarily of auto rickshaws, cars, two wheelers and foot traffic. The second sampling location was a public garden (PG) located in the city which remained mostly free of automobile traffic. The sampling time was selected from 0900 to 1100 h, a time when people go to work. The garden site was selected as this remained unaffected of traffic and other anthropogenic activities during working days and during the weekend, there was moderate activity of people who visited garden with families for entertainment.

Sampling procedure

Samples for microbial analysis were collected using MAS 100 air sampler (Merck, Germany) from a height of 1.5 m from the surface to simulate human breathing zone. Samples were collected once in a day in duplicate for bacteria as well as fungi for regular seven days in a week (mean values were used for statistical analyses). Sampling was done at alternative weeks for three months during May to June, 2010. A 50 L of air was sucked on standard 90 mm Petri plates at each sampling. Petri plates were prefilled with 30 ml of microbial content test agar (Difco Laboratories, Detroit, Misch) containing (100 µg/ml cycloheximide (Sigma Chemicals Co, USA) for total bacteria and rose bangal agar (Difco Laboratories, Detroit, Misch) for total fungi. The plates were incubated at room temperature for 48 h for bacterial count and for 5 to 7 days for count and identification of fungi. Colonies were counted as per the "positive hole" method described in the manufacturers operating manual. The airborne bacterial and fungal concentrations were calculated and expressed as colony forming units per cubic meter of air (cfu/m^3).

Meteorological data

Temperature, relative humidity (RH) and wind speed were recorded at each location with handheld envirometer (Fisher Scientific, Control Company, TX, USA). During the study, temperature ranged between 28.6 and 41.5°C, RH ranged between 20 and 60% and wind speed varied between 0 and 10.7 m/s.

Taxonomic identification

A representative number of bacterial colonies from each sampling were selected on the basis of morphology, size, color and texture. The colonies were purified to a single clone on tryptic soy agar (TSA) plate using three way streaks. Isolates were subjected to Gram staining and preserved on 0.3% brain heart infusion (BHI) agar and 50% glycerol in tryptic soy broth (freezed at -86°C). The isolates were identified using a BD Crystal Autoreader for the identification of bacteria (Becton Dickinson & Co, MD, USA). Fungi were identified based on their culture characteristics, sporulation pattern and microscopic features.

Statistical analysis

Working days and weekend counts of bacteria and fungi were compared using paired *t*-test. Relationship between microbial counts and meteorological factors was examined by Spearman correlation analysis. A probability of less or equal to $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Distribution of airborne bacteria

A total 168 air samples (84 samples from each of the two locations) were collected for bacterial analyses. The count of culturable bacteria at TC was observed in the range of 1560 to 17060 cfu/m^3 with an average count of 7120.48 cfu/m^3 (Figure 1). The average bacterial count during working days (Monday to Saturday) varied between 6456 to 8410 cfu/m^3 and weekend count was 3973.33 cfu/m^3. The weekend bacterial concentration was significantly lower than during the working days ($p<0.05$). The bacterial load at PG site varied between 940 to 12260 cfu/m^3 with an average count of 4804.76 cfu/m^3. The bacterial counts during working days averaged between 6456 to 8410 cfu/m^3 and the count at weekend was 7580 cfu/m^3. The weekend bacterial concentration was significantly higher than counts on the working days ($P<0.05$). The average weekdays bacterial count at TC was significantly higher than at PG ($P<0.001$). The weekdays distribution of microorganisms was interesting as during working days higher bacterial load was observed on TC compared to PG ($P<0.0001$), whereas on weekend count was significantly higher at PG than TC ($P<0.05$). Lower microbial concentration at PG site may be due to less traffic and very little human activities during working days. At weekend, count may be high due to visit of families in garden. Human activities including movement, rafting, desquamated skin scales, sneezing and coughing are main contributors of elevated viable bacterial concentration in air (Roberts et al., 2006; Tham and Zuraimi, 2005). Therefore, lower concentration

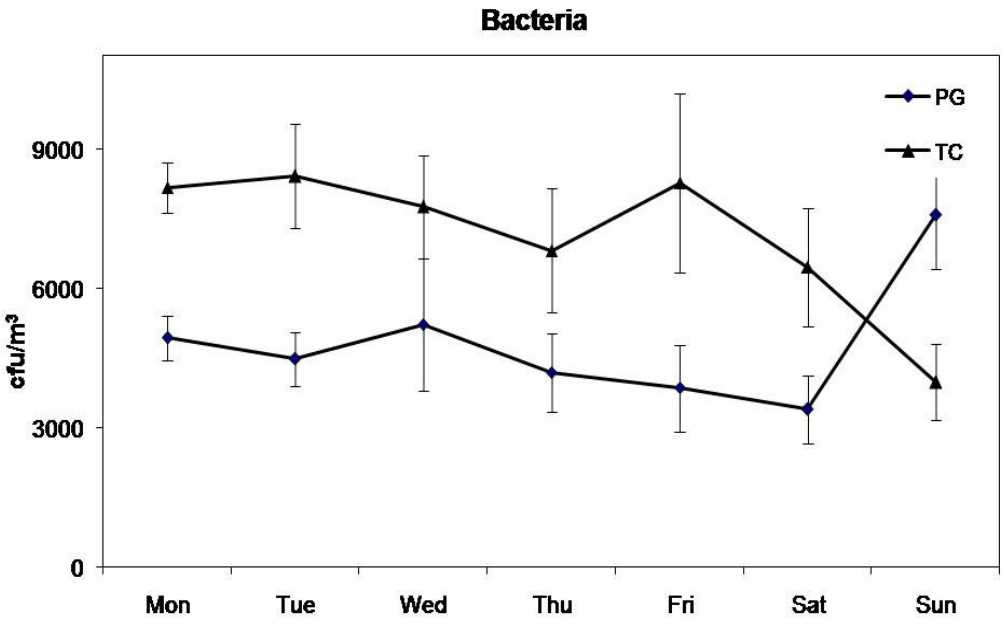

Figure 1. Weekly (Monday to Sunday) distribution of viable airborne bacteria at two different locations. The line graph represents average count in cfu/m^3 of six weeks and bar represents standard error of mean.

of bacteria was observed during working days and higher count on Sunday at the garden. The great proportion of Gram positive bacteria were observed from both the sites in this study. A total of 83 and 91% Gram positive bacteria were found at PG and TC sites, respectively. It was previously demonstrated that Gram positive bacteria have greater resistance and survival ability in atmosphere than Gram negative bacteria under strong sunlight (Shaffer and Lighthart, 1997).

A total of 29 species from 13 genera in 3 phyla (actinobacteria, fermicutes and proteobacteria) were identified from both locations (Table 1). The maximum bacterial diversity was associated with TC as 19 different species were identified whereas 16 species were identified from PG. *Acinetobacter, Bacillus, Micrococcus*, *Microbacterium, Pseudomonas* and *Staphylococcus* species were recovered from both locations. Most of these are either spore former or pigment producer or bacteria with high guanine (G) and cytosine (C) content because high G/C content DNA is more resistant to UV damage relative to low G/C content (Singer and Ames, 1970). Air is more suitable for spores than vegetative cells due to atmospheric stress like dryness, exposure to day light, UV damage, temperature, chemicals, humidity levels and oxygen (superoxide/peroxide/hydroxyl) radicals. Sporulation enables the bacterium to survive in the harsh environment. Cell wall pigment of bacteria also provides resistance to solar/UV radiations (Setlow, 2001). *Bacillus, Micrococcus*, *Microbacterium, Pseudomonas* and *Staphylococcus* were previously reported as the dominant bacteria in outdoor environments from different parts of the world (Fang et al., 2007; Gorny and Dutkiewicz, 2002; Gorny et al., 1999; Lighthart, 1997), but there proportions differed from place to place (Lighthart, 1997). This is mainly due to the characteristics of local environment and human activities. Our results also indicated that human movement or traffic significantly affect the airborne microbial proportion.

Distribution of airborne fungi

A total of 168 air samples (84 samples from each location) were also collected for fungal analyses. Airborne fungi also exhibited almost the similar distribution pattern round the week as shown by bacteria. The fungal load at TC ranged from 220 to 1560 cfu/m^3 with an average of 776.19 cfu/m^3 (Figure 2). The working days count averaged between 760 to 1000 cfu/m^3 and weekend count was 466.67 cfu/m^3. The weekend fungal concentration was significantly lower from working days ($P<0.05$). The load at PG site varied between 220 to 1020 cfu/m^3 with an average count of 605.71 cfu/m^3. The working days counts averaged between 503.33 to 653.33 cfu/m^3 and weekend count was 806.67 cfu/m^3. The weekend fungal concentration was significantly elevated from working days ($P<0.05$). The average weekdays fungal count over TC was found significantly higher than PG ($P<0.001$). Thus, during working days higher fungal load was observed at TC than at PG ($P<0.0001$), whereas on weekend, higher count was found at PG than at TC ($P<0.05$). Microbes in urban air are constantly

Table 1. Airborne dominating bacterial species identified at two locations at Gwalior.

Bacteria	TC	PG
Acinetobacter baumannii	-	+
Acinetobacter sps	+	+
Aeromonas sps	-	+
Arthrobacter sps	+	-
Bacillus amyloliquefaciens	+	-
Bacillus cereus	+	+
Bacillus mycoides	+	-
Bacillus pumilus	+	-
Bacillus thuringiensis	+	+
Enterobacter aerogenes	-	+
Pseudomonas aureofaciens	+	-
Pseudomonas putida	+	+
Pseudomonas stutzeri	-	+
Pseudomonas syringae	-	+
Pseudomons aurantiaca	-	+
Bacillus lichenoformis	+	+
Paenibacillus assamensis	+	-
Listeria innocua	-	+
Enterococcus avium	+	-
Staphylococcus gallinarum	-	+
Staphylococcus cohnii	+	-
Staphylococcus saprophyticus	+	+
Xanthomonas sp.	-	+
Micrococcus lylae	+	-
Microbacterium barkeri	+	+
Microbacterium aurum	+	-
Staphylococcus aureus	+	+
Staphylococcus epidermidis	+	+
Micrococcus luteus	+	+

stirred up by daily automobile traffic and other anthropogenic activities (Fang et al., 2005, 2007; Mouli et al., 2005). This was confirmed by higher concentration of both bacteria and fungi at TC which may be due to more traffic and crowd than PG. The microbial (bacteria and fungi) load at TC represented a specific pattern as higher count was observed from Monday to Saturday and significantly lower count on Sunday. The maximum count was found on working days. In a recent study on diurnal distribution of bacteria and fungi, a significantly higher fungal load was observed during working day with reference to weekend (Abdel et al., 2009). They also observed the elevated bacterial concentration on Monday but the difference was statistically insignificant. This can be due to the small sample size as they selected only Monday and Friday as sampling day in a week.

In the present study, we have identified 21 fungal species belonging to 13 genera from both the locations (Table 2). Maximum number of fungal species have been isolated from TC (15 species) belonging to 11 genera. From PG site, a total of 13 fungal species associated with 9 genera were isolated. *Alternaria, Aspergillus, Cladosporium, Curvularia, Drechslera* and *Fusarium* were common genera recovered from both sites. *Alternaria, Aspergillus, Cladosporium* and *Curvularia* were dominated over TC, whereas *Alternaria, Cladosporium* and *Fusarium* were dominated genera in the atmosphere of PG. Outdoor exposures to airborne microbes are associated with allergic respiratory symptoms, infection and asthma related death (Dales et al., 2004; Peternel et al., 2004). Many of the fungal species identified in the atmosphere like *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Penicillium citrinum* and *Fusarium* are common known to outdoor environment. However, these may cause allergic reactions if present in higher number (Horner et al., 1995). In a study from India, more than half of viable airborne fungi were found allergenic in skin prick test (Adhikari et al., 2004). The daily fungal spore concentration is associated with increase in number of emergency visit and hospital admission due to

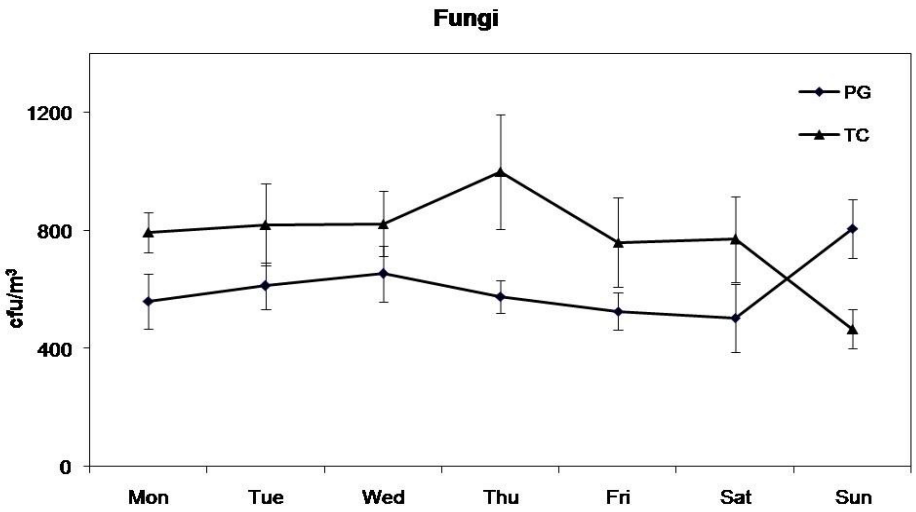

Figure 2. Weekly (Monday to Sunday) distribution of viable airborne fungi at two different locations. The line graph represents average count in cfu/m^3 of six weeks and bar represents standard error of mean.

Table 2. Percent distribution of airborne fungal species at two locations.

Fungi	TC	PG
Alternaria alternate	20.25	26.03
Aspergillus flavus	22.78	0
Aspergillus niger	1.27	5.48
Chaetomium indicum	2.53	0
Cladosporium cladosporioides	5.06	16.44
Cladosporium oxysporum	3.8	10.96
Cladosporium spaerospermum	6.33	2.74
Curvularia lunata	10.13	0
Curvularia pallescence	5.06	1.37
Drechslera australiensis	0	2.74
Drechslera rostrata	0	4.11
Drechslera tetramera	1.27	0
Fusarium moniliforme	0	2.74
Fusarium pallidoroseum	1.27	8.22
Fusarium solani	0	12.33
Neosartoria fischeri	0	1.37
Paecilomyces variotii	0	1.37
Penicillium citrinum	2.53	0
Phoma glomerata	1.27	0
Rhizopus	7.59	0
Trichothecium roseum	5.06	0

exacerbation in asthma attacks (Atkinson et al., 2006).

Effect of meteorological factors

A non-significant correlation of airborne bacteria and fungi was found with temperature, relative humidity (RH) and wind speed at both sites (Table 3). Spearman 'r' ranged from -0.085 to 0.198 for temperature, -0.069 to -0.128 for humidity and -0.179 to 0.211 for wind speed. At both sites, bacteria were negatively correlated with wind speed whereas, fungi were positively correlated. Their

Table 3. Correlation coefficients (spearman 'r') showing the effect of meteorological factors on bacterial and fungal concentrations.

		Temperature	RH	Wind speed
PG	Bacteria	-0.064	-0.026	-0.012
	Fungi	0.198	-0.0128	0.069
TC	Bacteria	-0.085	-0.069	-0.179
	Fungi	-0.013	-0.17	0.211

presence or entry in the atmosphere relies on availability of source, their release and dispersal from the surface (Burge and Rogers, 2000). During aerial dispersal, the survival and amount of microorganisms in air depends on meteorological conditions such as relative humidity, temperature, UV radiation and wind velocity (Cox, 1966; Jones and Harrison, 2004; Larson, 1973). The degree to which these factors influence the survival of microorganisms in aerosols depends strongly on the type of microorganism and the time it has to spend in the atmosphere. The quantity of microorganisms in air depends somewhat on meteorological conditions such as RH, temperature, UV radiation and wind speed (Cox, 1966; Jones and Harrison, 2004; Larson, 1973). The relative water content of the air is supposed to be important for survival of airborne microorganisms. A sudden change in RH from a favorable low or high RH to the more lethal intermediate RH range adversely affects the microbial survival in air (Hatch et al., 1970). Wind speed releases bacteria in air when it has to exceed a threshold speed to remove material from a surface whereas, at higher speeds bacterial concentrations may become diluted (Jones and Harrison, 2004). However, in this study, non-significant correlation of temperature, humidity and wind speed was observed on microbial concentration during week days. This may be due to the seasonal aerobiological study and short sampling time in this report. Previously, also in a similar aero-bacteriological study of vegetable market at Jabalpur, Madhya Pradesh, temperature and humidity were not found as the major factors for generation and aerosolization of airborne microorganisms in the environment (Pathak and Verma, 2009).

Other factors like dwellers, transporters, services, domestic animals, water spray and putrefactions were found the major contributors in microbial concentration (Pathak and Verma, 2009). The study concluded that weekly distribution of microbes over garden and traffic circle varied significantly between working days and weekend, and is affected by weekday traffic and anthropogenic activities on the sites.

ACKNOWLEDGEMENTS

The authors thank Director, DRDE, Gwalior for providing necessary facilities and financial support for the work. PK thanks Council of Scientific and Industrial Research (CSIR), New Delhi for providing Senior Research Fellowship.

REFERENCES

Abdel HAA, Khoder MI, Yuosra S, Osman AM, Ghanem S (2009). Diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt. Sci. Total Environ., 407: 6217-6222.

Adhikari A, Sen MM, Gupta-Bhattacharya S, Chanda S (2004). Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations. Sci. Total Environ., 326: 123-141.

Atkinson RW, Strachan DP, Anderson HR, Hajat S, Emberlin J (2006). Temporal associations between daily counts of fungal spores and asthma exacerbations. Occup. Environ. Med., 63: 580-590.

Beggs CB (2003). The airborne transmission of infection in hospital buildings: fact or fiction? Indoor Built Environ., 12: 9-18.

Bovallius A, Bucht B, Roffey R, Anas P (1978). Three-year investigation of the natural airborne bacterial flora at four localities in sweden. Appl. Environ. Microbiol., 35: 847-852.

Bowers RM, Sullivan AP, Costello EK, Collett JL, Jr, Knight R, Fierer N (2011). Sources of bacteria in outdoor air across cities in the midwestern United States. Appl. Environ. Microbiol., 77: 6350-6356.

Burch M, Levetin E (2002). Effects of meteorological conditions on spore plumes. Int. J. Biometeorol., 46: 107-117.

Burge HA, Rogers CA (2000). Outdoor allergens. Environ. Health Perspect., 108(Suppl 4): 653-659.

Cox CS (1966). Bacterial survival in suspension in polyethylene glycol solutions. J. Gen. Microbiol., 45: 275-281.

Dales RE, Cakmak S, Judek S, Dann T, Coates F, Brook JR, Burnett RT (2004). Influence of outdoor aeroallergens on hospitalization for asthma in Canada. J. Allergy Clin. Immunol., 113: 303-306.

Fang Z, Ouyang Z, Hu L, Wang X, Zheng H, Lin X (2005). Culturable airborne fungi in outdoor environments in Beijing, China. Sci. Total Environ., 350: 47-58.

Fang Z, Ouyang Z, Zheng H, Wang X, Hu L (2007). Culturable airborne bacteria in outdoor environments in Beijing,China. Microb. Ecol., 54: 487-496.

Fierer N, Liu Z, Rodriguez-Hernandez M, Knight R, Henn M, Hernandez MT (2008). Short-term temporal variability in airborne bacterial and fungal populations. Appl. Environ. Microbiol., 74: 200-207.

Gorbushina AA, Palinska KA (1999). Biodeteriorative processes on glass: Experimental proof of the role of fungi and cynobacteria. Aerobiologia, 15: 183-193.

Gorny RL, Dutkiewicz J (2002). Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. Ann. Agric. Environ. Med., 9: 17-23.

Gorny RL, Dutkiewicz J, Krysinska-Traczyk E (1999). Size distribution of bacterial and fungal bioaerosols in indoor air. Ann. Agric. Environ. Med., 6: 105-113.

Griffin DW (2007). Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clin. Microbiol. Rev., 20: 459-477.

Harrison RM, Jones AM, Biggins PD, Pomeroy N, Cox CS, Kidd SP, Hobman JL, Brown NL, Beswick A (2005). Climate factors influencing bacterial count in background air samples. Int. J. Biometeorol., 49: 167-178.

Hatch MT, Wright DN, Bailey GD (1970). Response of airborne *Mycoplasma pneumoniae* to abrupt changes in relative humidity. Appl. Microbiol., 19: 232-238.

Heldman DR (1974). Factors influencing airborne contamination of foods: a review. J. Food Sci. 39: 962-969.

Horner WE, Reese G, Lehrer SB (1995). Identification of the allergen Psi c 2 from the basidiomycete Psilocybe cubensis as a fungal cyclophilin. Int. Arch. Allergy Immunol., 107: 298-300.

Jones AM, Harrison RM (2004). The effects of meteorological factors on atmospheric bioaerosol concentrations--a review. Sci. Total Environ., 326: 151-180.

Larson EW. (1973). Environmental variables and microbial survival. In: Hers JFP, Winkler KC, editors. Airborne transmission and airborne infection: Oosthoek Publishing Co., Utrecht, The Netherlands. pp. 81-96.

Lighthart B (1997). The ecology of bacteria in the alfresco atmosphere. FEMS Microbiol. Ecol., 23: 263-271.

Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Ovreas L, Reysenbach AL, Smith VH, Staley JT (2006). Microbial biogeography: putting microorganisms on the map. Nat. Rev. Microbiol., 4: 102-112.

Mouli PC, Mohan SV, Reddy SJ (2005). Assesment of microbial (bacteria) concentrations of ambient air at semi-arid urban region: Influence of meteorological factors. App. Ecol. Environ. Res., 3: 139-149.

Pathak AK, Verma KS (2009). Aero-bacteriological study of vegetable market at Jabalpur. Iran. J. Environ. Health. Sci. Eng., 6: 187-194.

Peternel R, Culig J, Hrga I (2004). Atmospheric concentrations of Cladosporium spp. and Alternaria spp. spores in Zagreb (Croatia) and effects of some meteorological factors. Ann. Agric. Environ. Med., 11: 303-307.

Roberts K, Hathway A, Fletcher LA, Beggs CB, Elliot MW, Sleigh PA (2006). Bioaerosol production on a respiratory ward. Indoor Built Environ., 15: 135-140.

Setlow P (2001). Resistance of spores of Bacillus species to ultraviolet light. Environ. Mol. Mutagen. 38: 97-104.

Shaffer BT, Lighthart B (1997). Survey of Culturable Airborne Bacteria at Four Diverse Locations in Oregon: Urban, Rural, Forest, and Coastal. Microb. Ecol., 34: 167-177.

Singer CE, Ames BN (1970). Sunlight ultraviolet and bacterial DNA base ratios. Science, 170: 822-825.

Tang JW (2009). The effect of environmental parameters on the survival of airborne infectious agents. J. R. Soc. Interface., 6(Suppl 6): S737-746.

Tham KW, Zuraimi MS (2005). Size relationship between airborne viable bacteria and particles in a controlled indoor environment study. Indoor Air, 15(Suppl 9): 48-57.