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Study of Abundance and Characterization of Culturable Bioaerosol at Delhi, India

Bablu Kumar, Gyan Prakash Gupta, Sudha Singh and U.C. Kulshrestha*

School of Environmental Sciences, Jawaharlal Nehru University, New Delhi 110067 INDIA.

Abstract

Air particulates associated with biological origins are termed as bioaerosols. Bioaerosols consist of different species of bacteria and fungi including viruses, pollen, spores etc. Airborne bacteria and fungi can be toxigenic, allergenic and infectious. Infection can be caused by complete microorganisms as well as by its fragments or byproducts. The higher presence of bioaerosol can cause asthma and rhinitis, hypersensitivity pneumonitis, sick-building syndrome and organic dust toxic syndrome. This study reports the abundance of culturable airborne bacteria during monsoon and winter seasons at a site in Jawaharlal Nehru University campus, south Delhi. The results showed that the average concentrations of bacteria were higher during rainy season in comparison to winter season. The abundance is relatively higher than reported from temperate regions, which may be due to favourable climatic conditions of tropics. It was found that the percentage of Gram +ve bacteria dominated over Gram –ve bacteria during both the seasons. Among Gram +ve bacteria, cocci shape dominated over rod shape. Strong positive correlation of temperature with CFU indicated that the abundance of bacteria is largely controlled by ambient temperature.

Keyword: Bioaerosols, culturable bacteria, temperature, CFU, Gram +ve and Gram –ve.

1. Introduction

Air particulates having biological origins are termed as bioaerosols. Bioaerosols include bacteria, fungi, viruses, pollen and spores as well as their byproducts. They can be toxigenic, allergenic and infectious. Infection can be caused by complete microorganisms as well as by its fragments or byproducts (WHO, 1990). The presence of harmful bioaerosols can cause asthma and rhinitis, hypersensitivity pneumonitis, sick-building syndrome and organic dust toxic syndrome. The most obvious sources of bioaerosols are degraded plants and animal parts, water bodies, soil etc. Globally, the presence of bioaerosols has been reported from plants and soils vegetables, water bodies, sewage sludge, animal feeding, fermentation process, and agricultural activities, swine breeding farms, feedstuff-manufacturing factories, human (Pastuszka et al., 2000; Kulshrestha et al., 2012; Adhikari et al., 2005). The concentrations of bioaerosols have been reported from many locations worldwide. Bioaerosols studies have been reported at office building, hospital ,cave, calf house, public places, lake, recreation facilities, island, food stuff, school, flood sites and home etc. Some studies report the effect of meteorological parameters viz. temperature, humidity, wind velocity and wind direction on bioaerosols (Giorgio et al., 1995; Wang et al., 2010).

Though bioaerosols is an important area of research, it has not been very well attempted in India. Very few studies limiting to fungi mainly, have been attempted on bioaerosols in India. Delhi is densely populated rapidly growing city which is surrounded by various types of industries. Improper sanitation and waste disposal practices in the city allow generation of large amount of microbes in air. Considering the importance of bioaerosols, this short-time study has been carried out which is first of its kind report from Delhi. The study was aimed to find out the concentration of culturable bacteria in air along with their Gram characterization. An attempt has also been made to correlate the concentration of bacteria with meteorological parameters.

2. Materials & Methods

2.1. Sampling site

Samples were collected in Jawaharlal Nehru University (JNU) campus at the building of School of Environmental Science. JNU campus lies in extreme South of Delhi, having mini forest area in its surroundings. The campus is located away from any industrial activities. The nearest two busy roads run north-south, 1 km east and 1 km west to the site respectively. The traffic density of these roads is of the order of 10^6 vehicles per day. A slum area is located in the north-west of JNU Campus. In south and north of JNU campus, two densely populated areas are located having human population of ~ 0.2 million. In JNU campus, no major air pollution sources exist except vehicles like cars and bikes used by the students, faculty, visitors and the kitchens of various cafeteria existing in different hostels and buildings. Approximate 10000 inhabitants are residing inside JNU campus. It is likely that suspended particulate matter contamination may occur from the construction work going on within JNU campus.

2.2. Collection and characterization of culturable airborne bacteria

Samples were collected in widely used rich medium Luria Broth (LB). This meduim is popular among bacteriologists because it permits fast growth and good growth yields for many species (Sezonov et al., 2007). For this study purpose, the LB was procured from Himedia. The collection medium for air sampling was prepared by dissolving 2% LB in water. It was autoclaved for 15 minutes at 15 lb/in^2 pressure and 120°C temperature. Air sampling was carried out for three days on randomly basis during monsoon and winter seasons at the top most floor of the building (~18m height from the ground). Samples were collected using a handy air sampler (Envirotech 821). Around 1.5 litre volume of air was passed through an impinger containing 15 ml of 2% LB solution. This solution also contained cycloheximide (0.5 mg/ml) to prevent the growth of Fungi. All essential materials related to sampling were sterilized by autoclaving to restrict the growth of microbes before sampling. Immediate after sampling, 100 µl volume was taken out from the impinger which was spreaded on a LB agar plate. The plates were prepared by mixing LB(2%), agar (2%) and cycloheximide (final concentration 0.5 mg/ml) in sterilized condition. These plates were incubated at 37^0C for 18 hours. A negative control was also taken in which no air was passed through LB Broth solution. This control was treated in the same manner as the samples. After incubation, the plates were taken out and the colonies were counted. Bacterial abundance as CFU/m^3 was calculated by using the standard formula used worldwide.

After incubation, distinct individual colonies were picked up which were different in size, shape, colour, transparency, surface and elevation and further subcultured on LB Agar plate to ensure that it was not contaminated with other colony. Gram staining was done by standard Gram staining protocol using Crystal violet, Iodine and Safranin for all subcultured colonies to determine the percentage of Gram +ve and Gram -ve bacteria in air.

3. Results & Discussion

3.1. Concentration of airborne Bacteria

Table1. shows the concentrations of bacteria (CFU/m^3) during monsoon and winter seasons along with average concentrations. The concentration of bacteria varied from 0.43 - 3.35 x 10^7 CFU/m^3 which is relatively higher than the range reported from temperate regions (Table 1). The average concentrations of bacteria during monsoon and winter seasons were 2.37×10^7 and 0.57×10^7 CFU/m^3 respectively. On an average, we observed relatively higher values of bacteria (CFU/m^3) as compared to temperate regions (Table 1). It could be due to suitable climatic conditions of tropical region as well as the local activities which can give rise to airborne bacteria. Humans on campus may be significant contributor of bacteria as reported by Pastuszka et al., 2000; Kulshrestha et al., 2012; Kumar et al., 2011. Apart from these, warm weather of tropics, open waste disposal, cafeteria and vegetation etc may also contribute bacteria in air. Another additional factor of contribution could be the soil dust coming out from

the digging of land for the construction of buildings in the surroundings of the site. The presence of suspended soil dust in air supports microbes. The average of CFU/m^3 was 4.16 times higher during monsoon season than winter season, which could be due to higher degradation of plants and other living materials (Lighthart and Shaffer, 1994) surrounding the building. The abundance of bacteria in this study has been noticed comparable with those reported from other locations worldwide. The detailed comparison of the abundance of bacterial aerosol is given in Table 1. In this study, the concentration of bioaerosols is in the same range as reported in the residences after flood reclamation at Colorado, USA (Fabian et al., 2005), green waste composting at south east England, UK (Taha et al., 2006) while it is very high as compared to the values reported at Marseilles, France (Giorgio et al., 1995) , Laval, Quebeck, Canada (Payment et al., 1983), Dhunhuang, China (Wang et al., 2010) etc. As mentioned earlier, the higher concentration at this site could be due to favourable climatic conditions (tropical region), higher population of human etc. The variability of airborne bacteria is reported globally due to difference in climatic conditions, culturable media, geographical location and sampling time.

Table 1: Comparison of bacterial CFU/m^3 at different sites of the world.

Site	**Environment**	**CFU/m^3**	**Reference**
Dhunhuang (China)	Open cave	$1.1x10^3$	Wang et al (2010)
Colorado (USA)	After flood reclamation	$\sim10^7$	Fabian et al (2004)
Marseilles (France)	City	791	Giorgio et al (1995)
South East England (UK)	Green waste compost facilities	$3.7\ x10^7$	Taha et al (2006)
Laval. Quebeck (Canada)	Apartment building	17.2	Payment et al (1983)
South Delhi (India)	Urban area (Monsoon)	$23.7x10^6$	Present study
South Delhi (India)	Urban area (Winter)	$5.7x10^6$	Present study

3.2. Characterization of Bacteria

It was observed that on various dates of sampling, the size, shape, colour, margin, consistency, optical characters and surface of the colonies varied to some extent. Based on these features, we selected colonies for further analysis. These clusters were subcultured on separate plates containing LB agar. This ensured that the subcultured colonies were not contaminated by other types of colonies. Gram staining was performed for the characterization of these separately subcultured colonies during both monsoon as well as winter seasons. On an average, the percentage of Gram +ve bacteria was found higher than Gram –ve bacteria during both the seasons (Table -2).

However, daily variation was very large which may be due to variable environmental conditions especially meteorological factors such as temperature and wind velocity. Higher Gram +ve bacteria than Gram –ve bacteria are reported globally by various workers (Giorgio et al., 1995). Among Gram +ve bacteria, mainly cocci dominates over rod shape bacteria (Table -2) which is similar to the observations reported worldwide in different environments such as buildings (Payment et al., 1983), underground concourse (Seino et al., 2005), residential rooms (Pastuszka et al., 2000), office buildings (Tsai and Macher, 2005). The detailed comparison of Gram staining result with its location and environment in India and other countries of the world is given in Table 2. The higher number of Gram + ve cocci could be due to the presence of humans. Human skin inhabits many species of Gram +ve cocci bacteria (Commission of the European Communities, 1993). In general, cases of bacterial infections are seen higher during monsoon period as compared to winter season at Delhi. The possible reason could be higher abundance of pathogenic bacteria in air during monsoon season.

Table 2: Comparison of percentage of Gram staining at different sites of the world.

Type of Bacteria	**Present study**	**Porquerolles island (France)** (Giorgio et al., 1995)	**Multiple Office buildings, (USA)** (Tsai and Macher, 2005)	**Underground concourse, Tokyo (Japan)** (Seino et al., 2005)	**Apartment building, Laval, Quebeck (Canada)** (Payment et al.,1983)
Gram +ve cocci	54	35	31	76	73.5
Gram +ve rod	26	45	49	8	14.2
Gram–ve cocci	10	0	3	6	0.3
Gram –ve rod	10	20	17	10	12

3.3. Correlation of bacterial counts with meteorological parameter

Table 3 shows pearson's correlation coefficient of the bacterial counts and temperature, relative humidity and wind speed. Correlation of bacterial CFU with meteorological parameters viz. Temperature (T), relative humidity(RH) and wind speed (WS), revealed that bacterial CFU had strong correlation with temperature (r = 0.94) . This indicated that temperature is an important factor among all meteorological parameters which controls bacterial population in air. Similar observations have been reported by other workers from different parts of the globe. On the other hand, unlike observations reported by (Wang et al., 2010), bacterial abundance was poorly correlated with relative humidity. This is possibly due to the fact that relative humidity remained almost constant during the sampling dates. A poor correlation observed between the abundance of bacteria and wind speed could also be due to very less variability in wind speed during the sampling dates.

Table 3: Correlation coefficients of bacterial CFU with Meteorological parameters.

	T	**RH**	**WS**	**CFU**
T	1.00			
RH	-0.42	1.00		
WS	-0.67	0.50	1.00	
CFU	0.94	-0.09	-0.52	1.00

4. Conclusion

The average concentration of airborne bacteria at JNU site in Delhi varied from 0.43 - 3.35 x 10^7 CFU/m^3 which is relatively higher than the range reported from temperate regions. The study showed higher concentration of airborne bacteria during monsoon season than in winter season. On an average, the percentage of Gram +ve bacteria was found higher than Gram –ve bacteria during both the seasons. Among Gram +ve bacteria, mainly cocci dominates over rod shape bacteria. The strong correlation between temperature and abundance of bacteria indicated that temperature is one of the most important meteorological factors controlling bacterial abundance. These preliminary results suggested to carry out further comprehensive studies on airborne bacteria at more number of sites and longer duration.

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