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Distribution Pattern of Airborne Bacteria and Fungi at Market Area

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Abstract: Markets are characterized by high human activity site, responsible for generation of higher quantity of bioaerosols. Exposure to these bioaerosols often associated with a wide range of adverse health effects including contagious infectious diseases, acute toxic effects, allergies and cancer. In the present study, the current atmospheric load of bioaerosols and the influence of meteorological factors on these bioaerosols at Sardar market, Jodhpur (India) were investigated. Gram negative bacilli were found to be in higher count among all bacterial group and Aspergillus sp. prevailed among fungi. Both bacteria and fungi showed significant seasonal variations.

Key words: Market · Bioaerosols · Gram Negative Bacilli · Aspergillus · Allergies

INTRODUCTION

Presence of microorganisms in the air is ubiquitous but their proportion varies according to the environmental conditions and locations. Markets are characterized as a human activity enriched site and also a highly trafficked site. People in these areas are actively engaged in various activities, responsible for generation of higher quantity of bioaerosols. Bioaerosols are airborne particles that are living (bacteria, viruses and fungi) or originated from living organisms [1]. Spore-forming bacteria and fungi are able to survive in bioaerosols and stay viable for a long time in the air [2] but the situation becomes worse when they are able to multiply in these aerosols.

It is generally known that bioaerosols present in the air can affect human health, causing mainly respiratory and related diseases transmitted via respiratory route, allergic and toxic reactions [3]. In addition, long-term contact of people with bioaerosols can be a source of serious illnesses; can influence a person’s mental power and learning ability. Some previous study has also reported high level of potential hazardous bioaerosols in the similar environments [4]. Exposure to these bioaerosols can impose adverse effect on local inhabitants as well as tourists. For the above reasons, it is important to monitor the sanitary state of the air in places of increased risk of bio aerosol contamination, where people spend time every day. The present study therefore was an attempt to evaluate the quantity and quality of potentially hazardous bioaerosols, represented in the air of Sardar market, Jodhpur, Rajasthan (India) to the best of our knowledge where no such study has been attempted till now.

MATERIALS AND METHODS

Jodhpur is the second largest city in Rajasthan at an altitude of 250-300 m (26.29 °N latitude; 73.03 °E longitude). The city is known as the "Sun City" for the bright, sunny weather it enjoys all year. Jodhpur is a popular tourist destination, featuring many palaces, forts and temples, set in the stark landscape of the Thar Desert. It lies near the geographic centre of Rajasthan state, which makes it a convenient base for travel in a region much frequented by tourists. The climate of the city is generally dry, hot and semi-arid with three distinct seasons: winter (November to February), summer (March - June) and monsoon (July to October). The city experiences extreme climatic conditions which are categorized by very hot and dry summer and cold and chilly winters. In summer, the maximum temperature is around 44°C and the minimum temperature is around 37°C. In winters, the maximum temperature is around 27.5 °C and the minimum temperature is around 10°C. The average annual rainfall is approximately 32 cm.

Sampling Site: Sardar Market lies right in the middle of the city centre and one of oldest markets of Jodhpur that has kept alive the old haat bazaar culture. This market is

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very congested and crowded, having around 6,000 to 7,000 tiny shops squeezed into both sides of the narrow lanes in this market, offer a wide range of items one can think of. Hundreds of people and tourist flock here every day. In addition, large amounts of fresh vegetables, grocery and other items are transported to the market from different regions.

Sampling Procedure: Air samples were taken from market area, monthly from July 2010 to June 2011, by settle plate method. Nutrient agar (NA) (HiMedia Laboratories Limited, Mumbai, India) was used for the sampling and cultivation of bacteria. For isolation of fungi, Potato dextrose agar (PDA) (HiMedia) supplemented with 10 mg/L chloramphenicol was used. Plates of NA and PDA were exposed to air for 30 min and were setup at a height representative of the normal human breathing zone, that is, 1.5 m above floor level [5]. Nutrient agar plates were incubated at 37°C for 48 h to allow the growth of aerobic bacteria, while PDA plates were incubated for up to 5 days at 25°C to allow the growth of fungal colonies. The average of colony forming units (CFU) of both bacteria and fungi was calculated and converted to organisms per cubic meter of air (CFU/m3). However, results obtained by settle plate method are less accurate than those from impaction methods with the use of an air sampler, this method is still quite popular in India and some other countries [6,7]. This method is cheap, simple and allows the drawing of correct conclusions on types of microorganisms present in the air.

Bacterial colonies were initially characterized by morphology and microscopic appearance and identified further by biochemical tests according to Bergey's Manual of Systematic Bacteriology [8]. A wet mount preparation of each fungal colony was prepared by using Lacto phenol-cotton-blue solution and examined microscopically. Identification of fungi was based mainly on colonial appearance, microscopic examination of the spore and hyphal characteristics of the stained preparations [9].

Meteorological Data: During the whole study, parameter such as temperature and humidity was also measured monthly. During the whole study, temperature ranged between 24.5-41.6°C whereas, humidity ranged between 36-84% at sampling site.

Statistical Analysis: In the present study, Pearson's correlation coefficient procedure was used to estimate the impact and degree of effectiveness of meteorological factors (temperature and humidity) on airborne bacterial and fungal concentration [10]. The statistical significant difference in the concentration of airborne bacteria and fungi among the samples and among the months were determined by one-way ANOVA test.

RESULTS

The mean monthly concentrations of total airborne bacteria at Sardar market are represented by Fig. 1A. The bacterial concentrations ranged between 173.33 to 287.5 cfu/m^3 with peak value in September and lowest in February. Gram negative bacilli which comprised *Enterobacter aerogenes, Escherichia coli, Pseudomonas sp.* and *Serratia marcescens* were observed as dominant among all bacteria and constituted 41.40% of total bacterial count (Fig.1B). The second most commonly isolated bacteria belonged to Gram positive cocci (36.17%) and identified as *Staphylococcus aureus*, *Micrococcus luteus* and *Micrococcus kristinae* (Fig.1B). Endospore forming Gram positive bacilli (Bacillus sp.) represented the third bacterial group and constituted 22.43% of

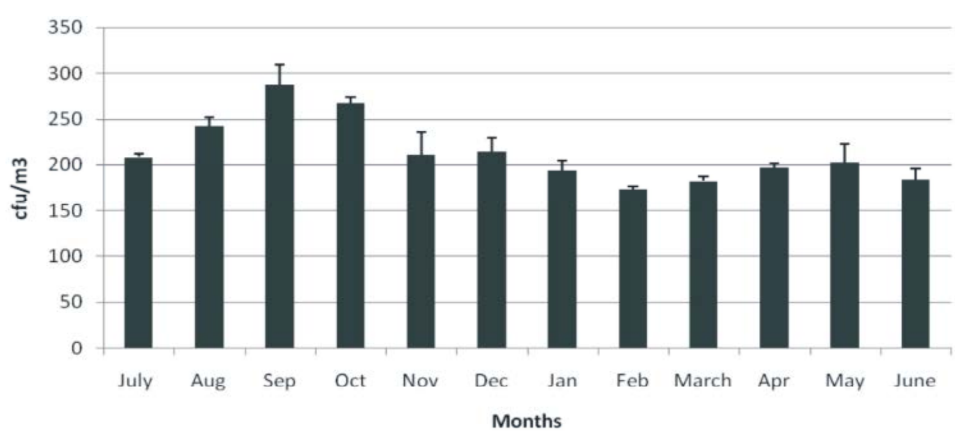

Fig 1A: Monthly fluctuation in concentration of total airborne bacteria in the Sardar Market (mean ± S.D.)

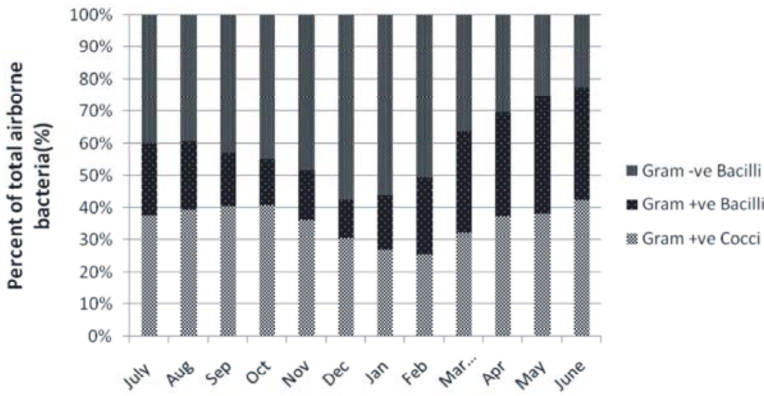

Fig 1 B: Distribution of various groups of airborne bacteria in each month

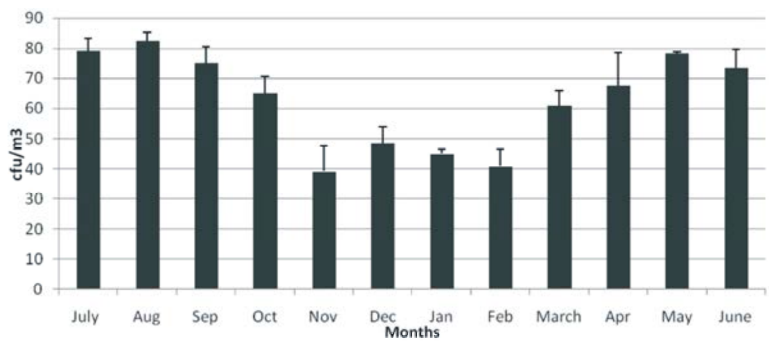

Fig 2 A: Monthly fluctuation in concentration of total airborne fungi in the Sardar Market (mean ± S.D.)

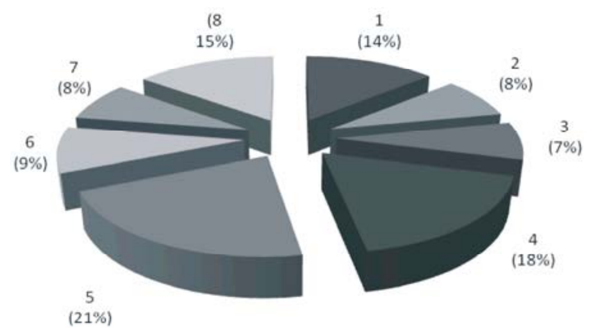

Fig 2 B: Distribution of various fungal species in monsoon 1-A.*niger*, 2-A.*flavus*, 3-A.*fumigatus*,4-A.*alternata*,5-C.*cladospoiodes*,6-F.*oxysporum*,7-F.*solani*, 8-*Helminthosporium* sp.

total bacterial count. It included *Bacillus subtilis*, *Bacillus megaterium, Bacillus lentus* and *Bacillus pumilus* (Fig.1B).

The mean monthly concentrations of total airborne fungi at Sardar market are represented by Fig. 2A. The fungal concentrations ranged between 39.16 to 82.5 cfu/m^3 with peak value in August and lowest in November. Among the fungi the *Aspergillus* sp. prevailed and formed 48.45% of total fungal count. It comprised *A.niger, A.fumigatus and A.flavus*. Other fungal isolates, constituted 51.55% of total fungal count were identified as Penicillium sp., Rhizopus sp., *Alternaria alternata,* Helminthosporium sp.*, Cladosporium cladosporioides, Fusarium solani* and *Fusarium oxysporum.*

Seasonal variation in different bacterial groups and different fungal species was also recorded in which Gram positive cocci and Gram positive bacilli were found to be dominant in summer. During winter, Gram negative bacilli were dominant whereas in monsoon Gram negative bacilli along with Gram positive cocci were dominant. In fungi highest count of *Aspergillus sp.* were observed in summer (Fig.2D) but in monsoon and winter highest count of *Cladosporium cladosporioides* and *Alternaria alternata* were observed (Fig.2B & Fig.2C).

Temperature was correlated positively weak with total airborne bacteria (r=0.064488) and positively strong with Gram positive bacteria (r=0.802386) but negatively strong with Gram negative bacteria (r=-0.6907). Humidity was correlated positive with total airborne bacteria (r=0.43976) and positively strong with Gram negative bacteria (r=0.602126) but negatively weak with Gram positive bacteria (r=-0.06753) (Table: 1).

Table 1: Correlation coefficients ('r') showing the effect of meteorological factors on bacterial concentrations at market

	Total airborne bacteria	Gram positive bacteria	Gram negative bacteria
Temperature	0.064488	0.802386	-0.6907
Humidity	0.43976	-0.06753	0.602126

Table 2: Correlation coefficients ('r') showing the effect of meteorological factors on fungal concentrations at market

	Total airborne fungal count	Total *Aspergillus* Count	Other fungal sp. count
Temperature	0.797232	0.80264	-0.30254
Humidity	0.002035	-0.67694	0.780209

Table 3: ANOVA for Bacteria

Source of Variation	SS	df	MS	F	P-value	F crit
Between months	58767	11	5342.454545	34.046	6.01174E-12	2.22
Within months	3766	24	156.9166667			
Total	62533	35				

Table 4: ANOVA for Fungi

Source of Variation	SS	df	MS	F	P-value	F crit
Between months	11901	11	1081.909091	39.342	1.20944E-12	2.22
Within months	660	24	27.5			
Total	12561	35				

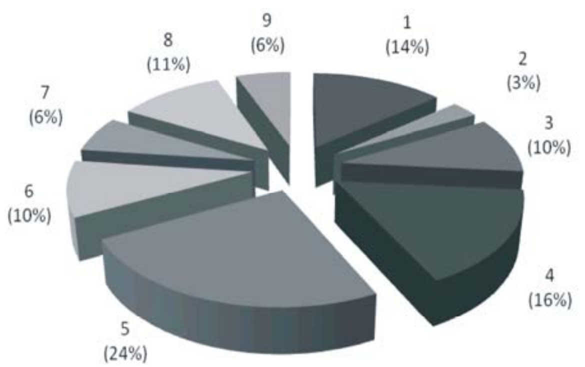

Fig 2 C: Distribution of various fungal species in winter 1-A.*niger*,2-A.*flavus*,3-A.*fumigatus*, 4-A.*alternata*,5-C.*cladospoiodes*,6-F.*oxysporum*,7-F.*solani*,8-*Helminthosporium* sp., 9-*Penicillium* sp.

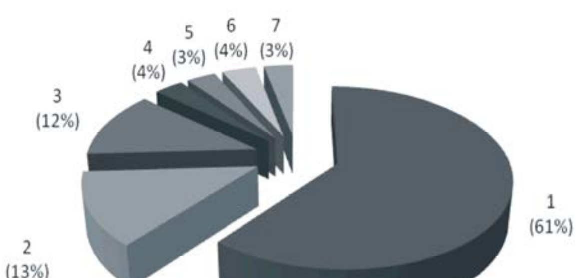

Fig 2 D: Distribution of various fungal species in summer 1-A. *niger*, 2-A. *flavus*, 3-A. *fumigatus*, 4- F. *oxysporum*, 5-F.*solani*, 6-*Penicillium* sp., 7-*Rhizhopus* sp.

Temperature was correlated positively strong with total airborne fungal count (r=0.797232) and with total *Aspergillus* count (r=0.80264) but negative with other fungal species count (r=-0.30254) whereas, humidity was correlated positively weak with total fungal count (r=0.002035) and positively strong with other fungal species (r=0.780209), but negatively strong with total *Aspergillus* count (r=-0.67694) (Table: 2).

From the analysis of variance (ANOVA) (Table: 3&4) under degree of freedom of V1=11, V2=24, the F value of 34.046 and the critical value (Fcv) of 2.22 for bacteria and the F value of 39.342 and the critical value (Fcv) of 2.22 for fungi were obtained. Thus, we could reject Ho in favour of Ha. This means that the difference in the concentration of airborne bacteria and fungi among the samples and among the months was substantially significant.

DISCUSSION

In the present study, a total of 11 bacterial and 10 fungal species were isolated and identified. Previously Rao *et al*. [11] isolated 8 bacterial and 15 fungal species from a vegetable and cereal market of Vijaywada. Similarly Pathak and Verma reported 11 bacterial species in their study of a vegetable market [4] and Reddy and shrinivas isolated 20 fungal species from a vegetable market [12].

Most of the airborne microbes in outdoor environment originate from natural sources such as soil, water, animal and humans, but activities like traffic, people gathering, industrial operations and agricultural processes in urban areas also contribute largely to outdoor microbial load [13]. In market areas poor sanitary measures also contribute to buildup and spread of various microorganisms [4]. In our study, high population density, heavy traffic and unhygienic conditions at the site may be the predominant source of microorganisms. A significant correlation between population density and concentration of microorganisms in outdoors has previously been reported by Kumar *et al* [14] found significantly higher count of both bacteria and fungi at TC (Traffic Circle) than PG (Public Garden) during weekdays. They stated that this may be due to high traffic and more human activity. Similarly other studies oriented towards the density of human, traffic and the density of microbial flora in outdoor environment also reported by some researchers [15-17].

The survival and quantity of microorganisms in air, depends on meteorological conditions such as relative humidity, temperature, UV radiation and wind velocity [18, 19]. 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. In the present study, highest total airborne bacterial concentration was observed in the month of September (monsoon).These findings are similar to the results of Shrivastava [20] and Donderski *et al.* [21] who also reported higher bacterial concentration in monsoon, probably due to higher relative humidity and rainfall at that period.

In the present study, seasonal variation in different bacterial groups was also recorded in which Gram positive cocci and Gram positive bacilli were found to be dominant in summer. Whereas, during winter Gram negative bacilli were dominant. Our results are in agreement with Verma and Pathak [22] and Pathak and Verma [4], who reported similar results during their study. Relative abundance of Gram-positive cocci and Gram positive bacilli bacteria in summer might be explained on the bases of their structural cell wall composition, which results in more global resistance to hostile conditions of the aerial environment, such as desiccation or sun radiation. Whereas, during the winter season low temperature and moderate humidity favors the survival of most of the airborne Gram negative bacteria especially members of the Enterobacteriaceae.

In the present study, the *Aspergillus* sp. prevailed among the fungi followed by Cladosporium and Alternaria sp. These findings were in accordance with the finding of Reddy and Srinivas [12], in which the most common fungal isolates from a vegetable market were *Aspergillus* sp. followed by Cladosporium and Alternaria sp.

In this study, fungi also showed a significant seasonal variation, characterized by a distinct peak in August i.e monsoon period. These observations are in agreement with Hazarika *et al*. [23] and Shukla and Shukla [24] who also reported higher fungal count in monsoon period which may be due to wet condition, high humidity and presence of plenty of organic food. In our study, highest count of *Aspergillus sp.* during summer are in agreement with the fact that spores of this species are generally well adapted to survival in the absence of available water and nutrient in the atmosphere [25]. Whereas, highest count of Cladosporium and Alternaria sp. In winter (dry season) may be due to their nature of conidia as they both produce dry conidia in chains and greater dispersal of dry powdery spores in air [26].

The present study revealed that both nonpathogenic and pathogenic form was prevalent in the market area. Although, most of the microorganisms present were mainly noninfectious but, inhalation of non-infectious microorganisms and their constituents can also cause inflammation of the respiratory system while antigens and allergens may activate the immune system and cause allergic and immunotoxic effects [27-29]. In our results high concentration of Gram negative bacteria is serious as these bacteria alone are responsible for mucous membrane irritation, extrinsic allergic alveolitis, organic dust toxic syndrome, bronchitis, asthma and infection [30].

In addition, presence of filamentous fungi, their spores and by-products of microbial metabolism, as particulate liquid or volatile organic compounds can be components of aeroallergens [31]. They can cause many health problems, including allergic and toxic reactions.

CONCLUSION

In the present study a total of 11 bacterial and 10 fungal species were isolated. Gram negative bacilli were found to in higher count among all bacterial group and *Aspergillus* sp. prevailed among fungi. Both bacteria and fungi showed significant seasonal variations. Results of this study showed considerable bioaerosol contamination in market area so there is a need to develop a control program and good hygiene practice. Also necessary to develop the standards of air quality related to bioaerosol contamination in such crowded settings.

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