

ASSESSMENT OF MICROBIAL (BACTERIA) CONCENTRATIONS OF AMBIENT AIR AT SEMI-ARID URBAN REGION: INFLUENCE OF METEOROLOGICAL FACTORS

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Abstract. In the present study, outdoor airborne microflora (bacteria) at different locations, viz; institutional, health care, commercial, traffic, industrial and agricultural areas of Tirupati — a semi-arid urban region, southern peninsular India was investigated during winter season, 2004. Concentrations of airborne viable bacteria averaged between 19 ± 5 CFU/m³ (IE) and 3 ± 5 CFU/m³ (SVU) and observed the following trend among the locations: IE > TG > CBS > TUDA > RUYA > SVU. Airborne Gram-positive bacteria were most abundant, with more than 60 to 90% of the measured population at each location. Developed regression models have been explained about 50% (or greater) variation in bacteria concentration at each location (except RUYA), due to the effect of meteorological factors – temperature, RH, and wind speed. Among these factors, wind speed had the most pronounced influence on bacterial concentration, with the regression coefficient (β) varied between 0.225 and 2.092, followed by the temperature. The overall air quality index (A_{BQI}) with respect to bacterial composition of aerosol is found to be 22.33 which signifies that the quality of air is good. The results reveal that the airborne bacteria are contributed from terrestrial (soil) sources greatly followed by the little contribution from point sources.

Keywords. Bioaerosol, airborne bacteria, meteorological factors, multiple linear regression, quality index, India.

Introduction

Earth's atmosphere is known to team with airborne microorganisms, though the high light intensities, extreme temperature variations, low concentrations of organic matter, and a scarcity of water, make the atmosphere as unsuitable environment habitat for microbial growth [1]. Biological material may contribute about 20%, 22% and 10% to the total airborne particulate by volume in remote continental, populated continental and remote maritime environments respectively [2]. Most of them originate from natural sources such as soil, lakes, animals and humans [3]. Moreover, agricultural practices, healthcare units and industrial operations such as sewage treatment, animal rendering, fermentation processes, and food processing plants also emit viable microorganisms into the air [4].

Exposure to bioaerosol pollution is now an almost inescapable feature of urban living throughout the world, which associated with a wide range of adverse health effects including contagious infectious diseases, acute toxic effects, allergies and cancer [4,5]. Inhalation is of the predominant route of exposure resulting in adverse health effects. Other types of exposure, namely ingestion and skin contact may also be present besides inhalation [6]. Although the relationship is still poorly defined, increased mortality and

morbidity believed to be caused by urban air microbial (bioaerosol) pollution are of great concern. Among the microorganisms present in the atmosphere, bacteria are often the highest in number, despite their high death rate due to environmental factors producing stress of various kinds, of the major being dehydration stress [7]. Though, most of the bacteria, or bacterial agents are not very potent allergens with the exception of spore forming actinomycetes; bacterial cell wall components, such as endotoxin (most prevalent in gram negative bacteria) and peptidoglycens (most prevalent in gram positive bacteria) are crucial agents with important pro-inflammatory properties that may induce respiratory symptoms [8].

It is important to note that geography and climate play an important role in determining the outdoor air microbial concentrations because the transport of bioaerosol is primarily governed by hydrodynamic and kinetic factors, while their fate is dependent on their specific chemical makeup and the meteorological factors to which they are exposed. The most significant environmental factors influencing the viability of microorganisms are temperature, relative humidity (RH), and wind velocity [9]. Also, the additional influences are exerted through oxygen, air ions, solar irradiance, and open-air factors. Hence, the monitoring of outdoor airborne microorganisms is necessary to evaluate the risk on human health and to study its evolution, and the interest in bioaerosol characterization has increased over the last few decades [10]. Most of these studies were carried through airborne fungi [11]. But up to now there are the limitations of the data available from monitoring for the bacteria found in the atmosphere [12].

The present study aim to investigate the current atmospheric load of airborne bacteria at different locations in Tirupati, a world famous pilgrim centre, where no survey of airborne bacteria has been attempted till now. Moreover, to estimate the influence of meteorological factors on bioaerosol along with bioaerosol pollution through modelling and quality indexing approach respectively.

Materials and Methods

Study area

Tirupati, a holy pilgrimage town for devotees of Lord Sri Venkateswara is situated in Chittoor district of southern Andhra Pradesh state in India at an altitude of 182.9 m (13.05 0 N latitude; 79.05 0E longitude). Tirupati — a semi-arid region prevailing the continental type of climate with three distinct seasons: winter, summer and monsoon, represents an urban area surrounded by major industrial and agricultural activities [13]. The meteorological data (weekly average) for the study period are given in Table 1. Sampling points were selected for the collection of airborne bacteria, based on the specific activity of the area in different parts of Tirupati. Sri Venkateswara University (SVU) campus, which represents the institutional area encompassed with educational institutions and good plantation. Tirupati urban development authority (TUDA) premises, represents the higher commercial prone with constant automobile traffic, vegetable market and residential density beside the site. Government hospital (RUYA) located towards north of the town with dense of forest represents the health care zone. Sri Venkateswara dairy firm (SVDF), is surrounded with cultivating fields located towards the west of the town represents the agricultural area.

Table 1 Weekly average level of meteorological factors during the study period (January to February, 2004) at Tirupati.

Week No.	Temperature (°C)		Relative humidity (%)		Wind vel. (km/hr)
	Min.	Max.	I	II	
1	17.90	28.66	79.00	49.29	8.10
2	15.30	28.43	70.86	36.43	8.60
3	14.01	30.00	78.86	35.71	6.28
4	19.98	30.89	77.57	49.57	6.03
5	19.50	31.46	78.71	41.57	7.01
6	18.61	31.53	73.00	42.71	7.44
7	15.96	31.16	64.43	33.71	6.88
8	15.53	33.37	51.57	23.71	7.07
9	16.60	34.47	56.00	20.00	6.48

While central bus station (CBS) located at center of the town, passing national high way and railway track beside the site, represents the severe traffic prone, whereas industrial estate (IE) located towards the east of the town is encompassed with different types of major and small scale industries along with food processing units and sewage treatment plants.

Sampling and Analysis

Airborne bacteria were collected by impaction onto an agar medium, using a portable Mini-Patrisol Air Sampler PM10 (Model 2100, Rupprich & Patashnik, USA) operating at about 10 l/m³ for 2 to 8 minutes, with a frequency of about once in a week during winter season (i.e. from January to February, 2004) placing at about 1.5 m above the surface, to simulate the human breathing zone. Before or after each sampling, the sampler surface was disinfected with a 70% ethonal solution. Sampling has been carried out for all the six sampling points on each sampling day during daylight hours, usually between 09.00 and 17.00 hrs. A modified version of the National air monitoring schedule (MNMS), excluding weekends, was used to assure that the sampling events were uniformly distributed among days during the workweek.

Sampled Nutrient agar plates were incubated at 30 °C and the aerobic bacteria were enumerated after 48 hours [14]. Cycloheximide, final concentration of 0.5 mg/ml, which has been previously shown not to affect bacterial counts [15], was added to the media to inhibit growth of fungi. Further on each plate, approximately 50% of the entire colonies were isolated for partial identification by light microscopy based on Gram reaction and bacterial morphology.

Modelling approach

Modelling technique in environmental research is gaining popularity because of the possibility that exists for the representation of complex problems in one model, which can be used to analyse and forecast the real problems [16,17). In the present study, multiple stepwise linear regression procedure was used to estimate the impact of meteorological factors (temperature, RH and wind velocity) on airborne bacteria concentration and the form of this linear regression model is:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \varepsilon \quad \text{Eq. 1.}$$

where Y is bacteria concentration, X1 is temperature, X2 is relative humidity, and X3 is wind velocity. β_0 is a constant representing the general level of bacteria irrespective of the effect of meteorological factors. β_1 is regression coefficient indicating the marginal effect of temperature, β_2 is regression coefficient indicating the marginal effect of RH and β_3 is regression coefficient indicating the marginal effect of wind velocity.

Results and Discussion

Variation in ambient air levels of bacteria

Table 2 presents the average levels of total culturable bacteria along with statistical results for each location. Concentration of airborne bacteria varied in the range of 1 to 32 CFU/m³ (Fig. 1) for different locations in Tirupati during the study period (January to February, 2004); the obtained bacterial counts were consistent with previous data where airborne bacteria varied between 10 to 100 CFU/m³ [18]. Among the locations studied, IE showed the highest level (19 CFU/m³) where the food processing units and sewage treatment plants existing might have contributed greatly. Also, the bare soil surrounding the location may have contributed significantly, which is further evident from the higher percentage of Gram positive bacteria [19]. Subsequently SVDF (16 CFU/m³) and CBS (15 CFU/m³) showed the greater levels of airborne bacteria. High background level of bacteria at SVDF might be due to constant input from the vegetation along with soil [20]. Abundance of bacteria at CBS may be due to many factors such as vehicle traffic, turbulent airflow, and amount of suspended dust and density of people carrying germs. It is further evident from the strong correlation with atmospheric pollutants (NO, CO, Hydrocarbons), which may have a protective effect on microorganisms, depending on microbial species [21]. While TUDA is with moderate concentration, both SVU and RUYA showed the lowest levels of bacteria. It represents the better cleanliness along with the maintenance of sterile conditions at respective sites, whereas the moderate concentration at TUDA is due to the rotting of wastage vegetables and the density of people carrying germs and also the urban air is constantly stirred up by a steady stream of automobile traffic. But the level of bacteria at each location are well below the threshold value (TLV), 50 CFU/m³ (WHO).

Table 2 Average ambient air levels of bacteria (CFU/m³) along with statistical results at each location of Tirupati.

Location	Mean \pm SD	Variance	Kurtosis	Skewness	Minimum	Maximum
SVU	3 \pm 1	1.50	-0.2857	8.37E-17	01	05
RUYA	4 \pm 2	2.50	-0.5143	0.2711	02	07
TUDA	10 \pm 2	5.19	-0.5948	0.3827	07	14
CBS	15 \pm 1	5.36	-0.7002	-0.26	13	17
IE	19 \pm 5	24.50	0.4301	0.8172	13	29
SVDF	16 \pm 3	11.11	-0.0517	0.2663	11	22

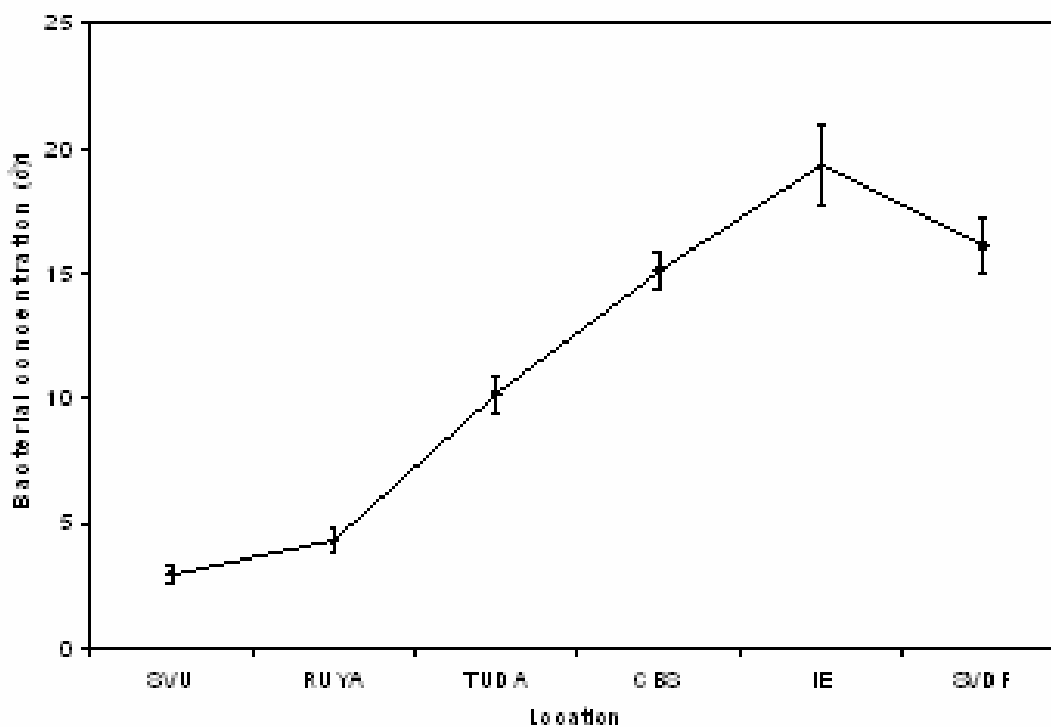


Figure 1 Mean level of airborne bacteria showing the standard errors at each location of Tirupati

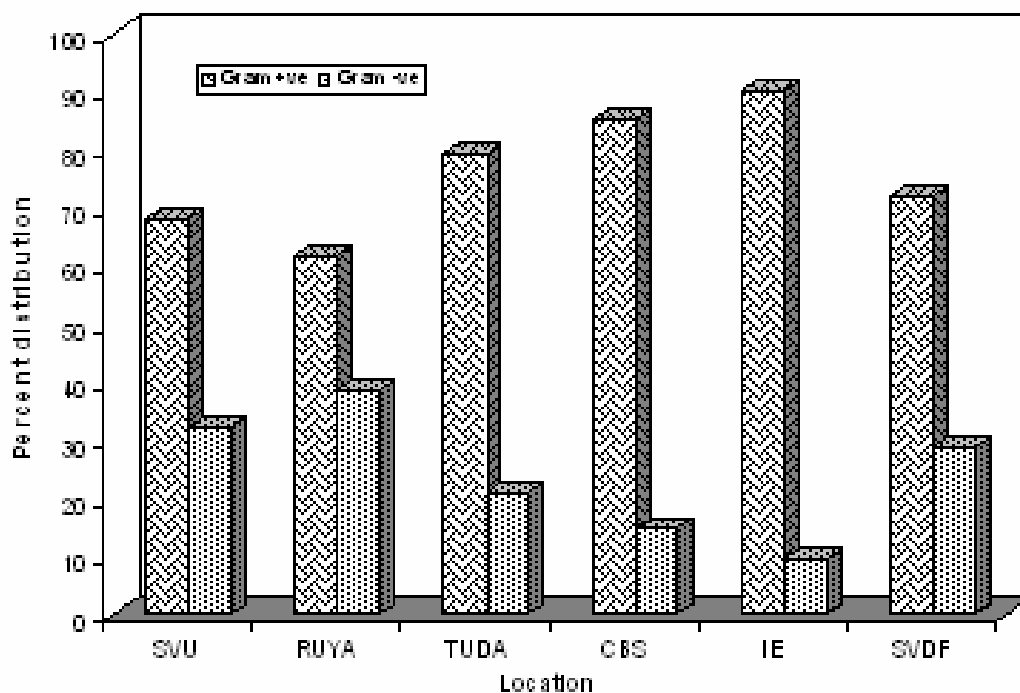


Figure 2 Frequency distribution of Gram positive and Gram negative bacteria at each location of Tirupati

Kurtosis calculations on the data set have given a negative value at each location except industrial estate (Table 2). This characterises a flatness of the distribution of the bacteria compared with the normal distribution. Whereas the positive kurtosis at industrial estate indicates a relatively peaked distribution, which signifies that there are sporadic high emissions of bacteria from certain sources causing a peaked distribution. Skewness calculations in a data set characterize the degree of asymmetry of a distribution around the mean. Positive and negative skewness indicate the distribution with an asymmetric tail extending toward values that are more positive and negative respectively. As for asymmetry in the data set is concerned this study has shown a wide variability (Table 2), which is mainly the result of the meteorological effects, and further of the widely changeable source emission pattern. Standard deviation calculations on the data set show a lower dispersion of the values of the bacteria while the variance measure the extent to which the actual observations vary from the central value during the study period.

The different forms of airborne bacteria, as well as their Gram reaction showed un similar pattern of percentage distribution at each and every location of Tirupati (Fig. 2). The concentration of airborne Gram positive bacteria were significantly larger than airborne Gram negative bacteria (which was less than 10%) and is in agreement with other reports [22]. It was previously demonstrated that Gram positive bacteria have greater resistance and survival ability outdoors than Gram negative bacteria under strong sunlight [23]; that characterize the troposphere of Tirupati; can only be found in the spore form. From the results (Fig. 2) it is evident that Gram negative bacteria has been occurred in high concentration at RUYA (38.46%) and SVU (32%) followed by

SVDF (28.58%) where the richest plantation is available, when compared with other locations. Increase in Gram negative bacteria in urban areas has been reported in previous studies [18] suggesting that sampling environment had a qualitative influence on airborne contamination and the additional sources of contamination such as hospital incinerator which has previously been reported to produce bacteria in the airborne state may have increased the Gram negative bacteria in respective locations. Apart from this, plants may release the Gram negative bacteria heavily. Abdel Hameed and Khoudr [20] reported that the suspended particulate in agricultural fields may contain about 103 CFU/gm of Gram negative bacteria and contribute about 2/3 to the total airborne bacteria. At each site, the greater contribution of gram positive bacteria has been observed, which indicates that the bacteria are highly contributed from the soil at Tirupati.

Influence of Meteorological Factors

The impact of meteorological factors such as temperature, RH and wind velocity on airborne bacteria concentration has been examined through multiple linear regression modelling approach, which provides the effect of each meteorological factor on bacteria concentration. Apart from the point sources, the environmental factors can affect the level of bioaerosol in atmosphere. The data for the study period has been used to fit the regression models for each location separately through stepwise regression procedure with SPSS software version 11.5, and the resulted models along with R² and F-value are presented in Table 3.

Table 3 Regression models developed for each location at Tirupati

Location	Regrssion Model	R²- value	F- value
SVU	-4.920 + 0.241 (temp) - 0.051 (RH) + 0.746 (wind vel.)	0.572	2.232
RUYA	9.910 - 0.312 (temp) + 0.0095 (RH) + 0.225 (wind vel.)	0.275	0.831
TUDA	-1.450 + 0.181 (temp) + 0.0909 (RH) + 0.346 (wind vel.)	0.466	1.453
CBS	3.291 + 0.0419 (temp) - 0.0008 (RH) + 1.659 (wind vel.)	0.798	6.579
IE	11.205 - 0.745 (temp) + 0.204 (RH) + 2.092 (wind vel.)	0.769	5.556
SVDF	-10.269 + 0.740 (temp) - 0.0684 (RH) + 1.865 (wind vel.)	0.504	1.695

Regression model showed that the meteorological factors have significant influence on airborne bacteria concentration. The models obtained can explain about 27.5, 46.6, 50.4, 57.2, 76.9 and 79.8 percent of variation in bacteria concentration in terms of wind velocity, temperature and RH at different locations; RUYA, TUDA, SVDF, SVU, IE and CBS respectively. The regression model developed for both CBS and IE are well fitted and were statistically significant ($p = 0.048$ and 0.000 respectively) where as other models do not show the significant value ($p = <0.05$) but the model at each site explained the significant variation in bacteria, which signifies that the bacteria at each site might have contributed greatly from similar source, probably terrestrial sources along with few more point sources with respect to the site.

Wind velocity showed a very good positive correlation at each location (Table 3) signifying that the bacteria concentration will increase with increasing wind velocity. Wind velocity has showed the highest regression coefficient ($\beta=2.092$) at IE and lowest at RUYA ($\beta=0.225$), means the increase in 1 km/hr leads to a marginal increase of 2.092 CFU/m³ at IE and 0.225 CFU/m³ at RUYA respectively. The influence of wind velocity as a dilution and survival factor of airborne bacteria has been largely demonstrated in dispersion models and environmental reports [24], and the results are in good agreement with previous reports [25].

Temperature is also a significant variation factor for airborne bacteria, which governs the rate of change of water vapor and the rate of change of heat between the surface and environment. It also affects the viability of airborne bacteria through the evaporation of their cellular water. In the present study temperature showed the positive correlation at TG ($\beta=0.740$), SVU ($\beta=0.241$), TUDA ($\beta=0.181$) and CBS ($\beta=0.0419$) and also the negative correlation at RUYA ($\beta=0.312$) and IE ($\beta=0.745$) respectively. Moreover, most environmental studies have reported the significant effect of temperature on bacterial counts depending on species and sampling environments [26]. The highest positive correlation ($\beta=0.740$) was observed at SVDF where the activity is agricultural and most of the bacteria might have contributed from vegetation. It shows that the release of bacteria will be increased with increasing temperature by reducing the binding force [27]. The highest negative correlation ($\beta=0.745$) was observed at IE where the bacteria might have contributed from terrestrial (bare soil) along with point sources, due to damaging effects of UV radiation [28].

Relative humidity (RH) showed a relatively low significant effect on airborne bacteria. It showed a positive correlation at RUYA ($\beta=0.0095$), TUDA ($\beta=0.0909$) and IE ($\beta=0.204$) and also the negative correlation at SVDF ($\beta=0.0684$), SVU ($\beta=0.051$) and CBS ($\beta=0.0008$) respectively. Among all the locations a significant correlation was observed only at IE ($\beta=0.204$) i.e. the bacteria concentration increases with increasing RH which is in good agreement with other reports [29]. Though the negative correlation at SVDF and SVU is not significant which also coincides with other previous reports [30], and there is no obvious relationship between RH and airborne bacteria at CBS ($\beta=0.0008$). The results reveal that the concentration of bacteria may increase either with the increase or decrease in RH, because the higher percent of RH favors the viability where as the lower percent of RH favors the spore release in greater number.

Assessment of Bioaerosol Pollution

Air quality index (AQI) shows how clean or polluted air is, and what associated health affects might be a concern for human beings (EPA). Air quality indices (AQI's) obtained for each site along with percentage of deviation with mean value were presented in Table 4.

Table 4 Air quality index (ABQI) with respect to ambient air levels of bacteria at Tirupati.

Location	A _B QI	% of Deviation with mean value
SVU	06.00	-73.13
RUYA	08.00	-64.17
TUDA	20.00	-10.43
CBS	30.00	34.35
IE	38.00	70.17
SVDF	32.00	43.41
Overall (Mean)	22.33	

The indices enable us to assess the quality of air at each location and can also be used to compare with each other. The higher the ABQI value the greater the level of bioaerosol pollution and greater the health concern. An ABQI value of 100 generally corresponds to the National Air Quality Standard for the pollutant, which is the level, set to protect public health. ABQI values below 100 are generally thought of as satisfactory. When ABQI values are above 100, air quality is considered to be unhealthy at first for certain sensitive groups of people, thus for every one as ABQI values get higher. The ABQI for each location individually and the overall ABQI for Tirupati are found to be well below the permissible value (Table 4), which indicates that the overall air quality of Tirupati with respect to ambient air levels of bacteria is very good. Among the different locations studied, IE showed the good air quality (ABQI > 33) and all other locations showed very good quality of air (ABQI < 33) which reveal that the region is free from bioaerosol pollution due to the contamination airborne bacteria. The trend of air quality for different locations in Tirupati is as follows: SVU > RUYA > TUDA > CBS > SVDF > IE.

Conclusion

A study on ambient air levels of bacteria and the influence of meteorological factors on airborne bacteria concentration have been carried out at different locations of Tirupati, a world famous pilgrim centre. Highest bacterial levels were observed at IE (19 CFU/m³) followed by SVDF (16 CFU/m³) and CBS (15 CFU/m³) and the lowest at SVU (3 CFU/m³). Airborne Gram-positive bacteria contributed highly, with more than 60 to 90% of measured population at each location, which signifies that the bacteria might have released from terrestrial (soil) sources greatly. Among the meteorological factors – temperature, RH and wind velocity, the wind velocity had showed the pronounced effect on bacterial concentrations with positive correlation ($\beta=0.225$ to 2.092). The resulted regression models have explained about 27.5 to 79.8% of variation of the airborne bacterial concentration at different locations. The overall BAQI was found to be 22.33, which reveals that the quality of air at Tirupati is good in terms of bioaerosol (airborne bacteria) pollution.

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