Evaluating the colonization and distribution of fungal and bacterial bio-aerosol in Rajkot, western India using multi-proxy approach



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Received: 2 March 2019 / Accepted: 24 March 2019 / Published online: 3 April 2019 C Springer Nature B.V. 2019

Abstract

Bio-aerosol is an emerging pollutant of the technological age. Air pollution related episodes that are a region-specific phenomenon in our atmosphere, with bio-aerosols being the main area of the problem. The present research was focused on assessing the particulate, and culturable concentration of bacteria at five different spatially located sites in the Rajkot city and surroundings, in the western part of India. The highest $(108.33 \times 10^9 \text{ CFU m}^{-3})$ and lowest $(318 \times 10^3 \text{ CFU m}^{-3})$ bacterial concentrations were found in dump site and residential area, respectively. With reference to particulate concentration, higher (101.79 ± 8.09) concentrations were reported in the industrial area than other sampling locations. All sampling sites under the present study displayed greater variability of bacteria than that of particle concentration. The growth potential of various bacterial isolates from perspective bioaerosol was measured spectroscopically by measuring OD at 600 nm in rich medium. The isolate 1A displayed significantly higher growth compared to all other isolates after 24 h. Outcomes of the current work suggested that bacterial concentration was observed in the respirable fraction (<2.5 μ m) and so had the potential to penetrate the deeper part of the lungs. In addition, meteorological parameters (i.e., wind speed, temperature, and relative humidity) were measured to understand whether they had any effect on biotic matter. The temperature and relative humidity are the most important meteorological parameters responsible for the enhanced viability of bacteria. Land use and land cover feature were also studied to understand the spatial characteristics of bio-aerosol in the study area. This viewpoint summarizes available information on bio-aerosols and its impact on human health, devising strategies to understand characteristics of bio-aerosols and emphasizing the vital gaps in available knowledge such as to develop a relationship between biological agents and solid/liquid or a mixture of both to the assessment of dispersion behavior and toxicological nature during exposure.

Keywords Bio-aerosol · Urban air · Bacteria · PM2.5 · LULC · India

Abbreviations

VOC	volatile organic compounds
LULC	land use and land cover
NDVI	normalized difference vegetation index
SRTM	Shuttle radar topographic mission
DEM	digital elevation model
LBA	Luria-Bertani Agar
Т	temperature

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WS	wind speed
RH	relative humidity
PM _{2.5}	particle concentrationn

Introduction

Bio-aerosols pose a major environmental concern due to their threat to public health and potential influence on the surrounding environment (Madureira et al. 2018; Humbal et al. 2018). Along with the presence of life-threatening hazardous chemicals such as heavy metals, volatile organic compounds (VOCs), and toxic gases, air pollutants are known to be composed of bio-aerosols that constitute live microbes such as viruses, bacteria, fungi, and their toxic products (Kim et al. 2017). The dispersal of such biotic factors through the air causes innumerable mild to acute adverse effects such as localized and systemic organ failures, cancer, and immuno-

modulatory responses in humans and husbandry animals (Humbal et al. 2018). The resilience of these microbes over a broad range of physiochemical conditions makes them potentially harmful to humankind. Some of the well-known airborne viruses include Influenza virus, Chicken Pox, Small Pox, and HINI Influenza virus responsible for the deterioration of respiratory and multiple organ functions in humans (Núñez et al. 2016). Pathogenic bacteria such as Bordetella pertussis, Bacillus anthracis, Corynebacterium diphtheriae, and Neisseria meningitidis are known to cause many infections leading to respiratory failures and neurological damage to humans (Global Burden Diseases 2013). Harmful fungi such as Aspergillus spp., Fusarium spp., Scedosporium spp., and Mucorales spp. are a most common bio-aerosol component and are known to cause acute toxicity, hypersensitivity (mainly asthma), invasive mycoses, and respiratory abnormalities (Jung et al. 2009). Endotoxins and β -glucans are among the routinely found toxic microbial products which cause a severe allergic or anergic effect on the individual's immune system which may lead to autoimmune diseases and cancer (Kim et al. 2017). Effects due to bio-aerosols have been observed in recent short-term and long-term studies, which are associated with daily concentration variations in air pollution and health, and cohorts of personal exposure over different periods (Wu et al. 2015). Exposure to bio-aerosols has been seen to show health effects even at miniscule levels (Jhonson and Choi 2012). With the advancement in technology and industrialization, bio-aerosol has become an emerging pollutant of our age and rapidly grasping the reputation of major air contaminant (Bragoszewska et al. 2018; Humbal et al. 2018). The occurrence of bio-aerosols has substantially enhanced over the last decade. Cases of detrimental effects on respiratory, circulatory system, cardiovascular, immune, and nervous systems are increasingly reported which led to enhanced morbidity and mortality among the population (Johnson and Choi 2012). Nowadays, a sustained severe haze even affects people's routines in the urban and rural area, by measures like solid and hospital waste dumping zones, slaughterhouses, poultry farm, suspension of production at industrial plants, suspension of constructive activities, and so on (Humbal et al. 2018).

In most of the developed countries, effective legislation is implemented to manage and eliminate air pollution-related issues. Innumerable problems arising from bio-aerosols engage many air pollution experts throughout the globe to understand and control the events of bio-aerosol dispersal and its health hazards. Johnson and Choi (2012) suggest continuous efforts in observations and health effects of bio-aerosols to assess individual exposure on human health. Moreover, many studies reported that the mortality rate is increasingly high in the last few years due to the effects of the incremental concentration of bio-aerosols (Mclean et al. 2004). Causality is the next question; the primary and secondary particles associated with air pollution, virus, and fungi were thought to be a very critical factor (Humbal et al. 2018). Many studies (Anderson et al. 1976) indicated that a large portion of the aerosol is created by fungal spores in the surrounding environment. Kumar and Attri (2016) reported the longer period of survival ability with extreme environmental condition of fungal spores. Agriculture and open land/soil are the potential sources of fungi in ambient air. The quantity (number) and quality (composition) nature of fungi depend on different type of plants, various locations, and several types of anthropogenic and natural activities (Hawkes et al. 2010). Spores (2–20 μ m) are a suitable medium of the movement of fungi to the new host through the air, water, and soil. However, Egan et al. (2014) pointed out the air, as a suitable and pervasive medium for dispersion of spores and related biotic matter.

On the other hand, the meteorological parameters especially temperature (T), relative humidity (RH), and wind speed play an essential role in the dispersion of particle/biotic matter from one place to another (Hwang et al. 2016). Also, previous studies indicate the bio-aerosol concentration at different weather conditions; however, dispersion behavior of biotic matter and bio-aerosol concentration and its relationship with the meteorological condition at a various location in urban areas have been only preliminary recognized. Moreover, a few studies (Gautam et al. 2016; Buttner and Stetzenbach 1993) have mentioned the concentration levels in the different area and its related sources. Therefore, it is needed to understand the influence of meteorological parameters on the bioaerosol concentration and its dispersion behavior in different locations of the urban area.

Spatial elimination of bio-aerosols will need substantial new directions or resources from sweeping societal changes and the local and international community. Countries require to switch to modification of production techniques, transportation network enhancements, non-polluting energy sources, and the introduction of new intervention techniques to economic growth. These effective strategies will quite be difficult. Influential vested interests to be required to overcome strong opposition. Fortunately, to control air pollution, all needed requirement such as technical, institutional/organizations, and tools related to the policy already at hand. They are very much available and can be arranged to obtain short- and long-term achievements.

Here, we prepared land use and land cover (LULC) maps by using remotely sensed data to understand the spatial characteristics of the bio-aerosol in the study area. The remotesensing data capture high-resolution information with more detail and yield high-level classification accuracy of the given area (Hilker et al. 2009). We have used Sentinel-2 remotesensing data for our analysis. The Sentinel-2 mission, launched on 23 June 2015, by European Commission's Copernicus Programme, has a higher spatial and spectral resolution (10 m 4 bands, 20 m 6 bands, 60 m 3 bands; 13 spectral bands) (Malenovsky et al. 2012). Several studies using remote-sensing data have assessed the land use and land cover in specific regions (Giriraj et al. 2009; Reddy et al. 2015). However, no systematic high-resolution LULC study has been published in the Rajkot area in western India. Although a few low-resolution estimates have attempted LULC classes based on Landsat images, here, we focus on the analysis of LULC and normalized difference vegetation index (NDVI) using a high-resolution satellite mission to understand the land cover pattern. The LULC is the key driver of environmental changes (Goldewijk and Ramankutty 2004).

The present study focuses on understanding the spatial characteristics of levels of bio-aerosol concentration in the Rajkot City and surroundings (Fig. 1)-is one of the smart cities of Gujrat in India. To achieve the aim of the present study, microbiological analysis, the concentration level of culturable bacterial aerosol in different sites of Rajkot City (i.e., main urban site, industrial site, dumping site, slaughterhouse, and poultry form) is done. Presented works are focusing on the culturable biotic matter (i.e., bacteria, fungi, virus, etc.), as a biotic matter is susceptible and having good interaction with fine particles, generated due to anthropogenic activities in the urban area. In addition, meteorological influence of the behavior of bio-aerosol is also reported. Therefore, the relationship between aerosol and associated biological agents responsible for behaviors like dispersal, total potential health hazards, and toxicology level during exposure to bio-aerosol on health needs to be identified. The LULC features are also recognized to support the findings. The outcomes of this study contribute awareness about the typical behavior of bio-aerosol and its possible impact on human health especially in the urban outdoor air quality of developing countries, where different types of source (i.e., industries, slaughterhouse, poultry form, etc.). In this view, the reported data also specify the urgent need to control air pollution, thereby reducing respiration diseases as the result of exposure to bio-aerosol.

Experimental methodology

Study area description

Rajkot is a typical urban area, located in Gujrat state in western part of India, lies between 22°10'N and 22°25'N and 70°40'E and 70°55'E, and covers an area of 350 km² (Fig. 1a, b). The elevation range varies from 100 to 150 m above sea level (masl) in the study area. The major part of the study area occupied by agriculture land. We designed our sampling strategy to document spatial variation in LULC pattern of the study area. We have collected samples from five different locations in the study area (Fig. 1a). The selected sampling locations were characterized by LULC activities (i.e., slaughterhouse, poultry form, industrial activities, center point, and academic area) to cover total area and its possible measure sources of the city. Total ten representative samples have been taken in each selected locations. To measure bio-aerosol/ PM_{2.5}, we utilized five fine particle samplers running (flow rate of 16.67 lpm) (Gupta et al. 2019). All samplers were installed in and around the source locations, and the samples were collected at the height of 1.5 m (general human head height) from the ground level. The sampling was performed with a total of 50 measurements from five locations for 24 h during spring (March to June) and winter (October to December) of 2018. The meteorological parameters (i.e., T and RH) were also recorded simultaneously during the bioaerosol sampling. On the other hand, fine particle samplers (5

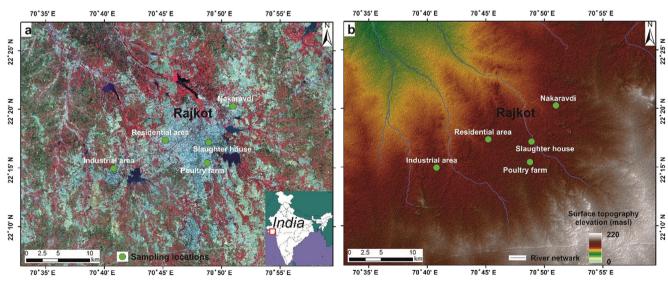


Fig. 1 Overview maps of the study area in Rajkot in Gujrat state in western India. **a** Sentinel 2 imagery (bands 3, 4, and 8). **b** Regional topographic view from Shuttle Radar Topographic Mission (SRTM) data,

3 arcsec resolution, in the study area. The green color circle shows sampling locations. The continuous blue line indicates the drainage network in the study area

nos.) were used to assess fine particle concentration at selected locations in the study area.

Sampling locations and analysis methods

The samples (fine particles; > 2.5 μ m = aero-dynamic diameter range) were collected using fine particle sampler at sampling locations. The used samplers comprised a small pump with a constant flow rate of 16.7 lpm during air sample measurement. For analysis point of view, samples were divided in two divisions (i.e., microbiological analysis and chemical analysis). Samples were put in Petri dishes with media (Luria Bertani Agar, Miller) at different T (i.e., 25 °C and 37 °C for fungi and bacteria, respectively) to the assessment of proper growth. The microbial count was scored using a polish standard (Bragoszewska et al. 2018). The measurements of biotic matter in the air are quite difficult; however, the applied methods are very suitable to assess the concentration of airborne biotic matter/living micro-organisms through counting the number of colonies in Petri dishes. Li et al. (2015) indicated that only 10% of the total micro-organism could be included using culture-based study due to the nonculturable nature of most of the microbes; however, it supplemented required information to evaluate the actual human exposure and standards/policy development to know the comprehensive levels of different types of living micro-organism in the ambient urban air. PN-EN12322 (PKN 2005) and ISO 11133 (BSI 2014) standards were used to guality control during analysis. In addition, SPSS (version 24) used to perform the statistical analysis of collected data sets. Descriptive analysis was presented the concentration values (mean and standard deviation). A non-parametric method was applied on collected data as data were not normally distributed. Spatial variation at sampling locations was identified by using the Mann-Whitney test on collected data sets. To evaluate the statistical dependence between meteorological parameters (i.e., T & RH) and biotic matter (i.e., fungi and bacteria), Spearman's rank correlation was applied at different locations. Significant differences were reported during the consideration of probability lower than 0.05 (p < 0.05).

Remote-sensing data

In order to understand the spatial characteristics of bio-aerosol in the study area, we processed sentinel 2 remotely sensed imagery acquired on 22nd December 2018, to derive LULC classes for the study area. The sentinel 2 mission provides free images with global coverage. Sentinel 2 is a high-resolution mission with a spatial resolution of 10 m to 60 m, which carries optical instruments with 13 bands ranging from 443 to 2190 nm (Erinjery et al. 2018). The sentinel 2 data were downloaded from the earth explorer data hub. For the present study, we processed four bands of 10-m resolution for the classification: blue (band 2, 490 nm), green (band 3, 560 nm), red (band 4, 665 nm), and near-infrared (band 8842 nm) (Fig. 1a). In addition to this, LULC classes classified based on the spectral signature of the image. We created multiple signatures on the satellite image to categorize different LULC classes. Further, the evaluation process of the collected signature was done using the histogram, contingency matrix. The thematic raster layer was produced by using maximum likelihood classifier. In addition to these bands, we used SRTM-derived digital elevation model (DEM) and sentinel 2derived NDVI layer for overall accuracy during the classification. The NDVI layer was created using near-infrared (band 8) and red (band 4) for classification. Further, we used a maximum likelihood classification approach for classification (Laurin et al. 2013). For classifier training and validation of the results, we acquired in situ measurements during the field campaign in 2018. We collected reference data from random locations (n = 62) for five (n = 5) category (such as agriculture land, built-up area, vegetation, water body, and barren land) in the study area (Fig. 2). The reference data represent the land cover types within the study area, which can be defined in the context of a classification approach. The overall accuracy, user's accuracy, and kappa statistics were derived from the error metrics to find the reliability and accuracy of the maps produced.

Result and discussion

Land use and land cover map

The overall accuracy obtained was the maximum likelihood classification with data set sentinel 2 bands 2, 3, 4, 8 and NDVI. Figure 2 depicts the spatial distribution of land use and land cover pattern for the study area. This is the first time (to our knowledge) that high-resolution LULC map was prepared for the Rajkot in the western part of India. The confusion matrix used to see accuracy in the thematic map and their comparison between accuracy (Table 1).

The confusion matrix helps to identify the confusion between classes, as well as potential sources of error (Foody 2002). Further, the user accuracy and producer accuracy for land use classes were calculated and attributed in Table 1. Furthermore, we also derived quantitative metrics from confusion metrics, to understand the overall accuracy and confidence intervals during the measurements. In addition to this, we achieved 82% overall accuracy for our assessment, which is below 85% level which can be satisfactory for management, planning, and environmental purposes (Anderson et al. 1976). However, several studies indicate 78–88% (Xian et al. 2009) and 65–82% (Homer et al. 2002) overall

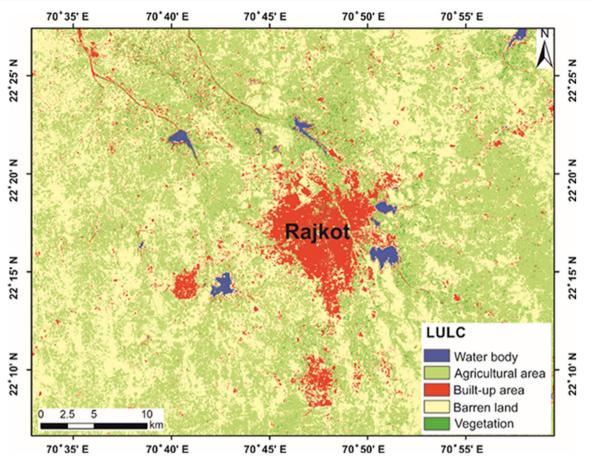


Fig. 2 Overview maps of the LULC for the study area in Rajkot in Gujrat state in western India. Sentinel 2 imagery (bands 3, 4, and 8) is used to prepare LULC map

accuracy for their assessment. Therefore, while aspiring to uphold the accepted standards, the overall accuracy obtained during the present study is quite satisfactory, as it resembles that of analogous studies.

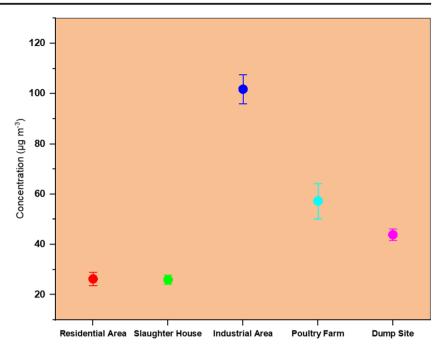
Particle concentrations at different selected locations

Figure 3 shows the variation of different concentration levels of fine particles in selected locations. The particle

Maximum likelihood classification		Observed classes					Total	User accuracy
		Waterbody	Agriculture area	Built-up area	Barren land	Vegetation		
Predicted classes	Water body	11	0	0	0	0	11	100
	Agriculture area	0	14	0	0	9	23	61
	Built-up area	0	0	12	0	0	12	100
	Barren land	2	0	0	10	0	12	83
	Vegetation	0	0	0	0	4	4	100
Total		13	14	12	10	13	62	
Producer accuracy		85	100	100	100	31		
Overall accuracy				82%				
Kappa				78%				
Expected				0.2032				
Product matrix				781				
Cumulative sum of	f the product matrix			3844				

Table 1 Confusion matrix and accuracy measures for land use and land cover classification

Fig. 3 PM_{2.5} concentration at study chosen sites in the urban area of Rajkot District, India



concentrations were found $(26.26 \pm 3.76, 25.93 \pm 2.55, 101.79 \pm 8.09, 57.26 \pm 9.86, and 43.89 \pm 3.20)$ in the residential area, industrial area, slaughterhouse, poultry farm, and dump site, respectively (Fig. 3). The variation was clearly indicating that the concentration of industrial area was reported 4, 4, 2, and 1.33 times higher than residential area, slaughterhouse, poultry farm, and dump site, which is due to heavy construction, transportation, waste disposal discharge, etc. that generally take place (Bauleo et al. 2019). The lower concentrations were found in the residential area, slaughterhouse, poultry farm, and dump site because extreme conditions, such as high T and urban activities, might contribute to decreased levels of biotic matter (Bragoszewska et al. 2018).

Genitsaris et al. (2017) have reported that activities are very significant criteria to effects of the level of concentration instead of seasonal variation, as they found higher concentration in winter comparing to spring; however, Bragoszewska et al. (2018) present the opposite trend. The contrast outcomes might be results of similar positive T levels which promotes the growth of biotic matter in different seasons. Therefore, the present study focused on different types of activities in the urban area and its level comparison. Moreover, especially in Asian countries and other countries of the world, there is no exiting micro-biological air pollution standards developed as per available literature, it can be observed that the reported particle concentration in few study areas (i.e., residential area, urban area, poultry farm, and slaughterhouse) below and above the Indian standards and WHO standards, respectively. That is why field study is required to take action to understand the level and its possible impact of bio-aerosol on human health from different activities in the urban area.

Bacterial aerosol concentrations at different selected locations

Figure 4 shows the concentrations of culturable bacterial of aerosol in different study locations. The monitored concentration of bacterial aerosol in different locations is 318×10^3 CFU m⁻³, 150×10^3 CFU m⁻³, 283.3×10^4 CFU m⁻³, 781.6×10^4 CFU m⁻³, and 108.3×10^9 CFU m⁻³ for residential area, poultry farm, slaughterhouse, industrial area, and dump site, respectively.

Goyer et al. (2001) indicated that microbiological contamination is possible if the total air-borne biotic matter concentration is 1000 CFU m⁻³. As per the previously reported results (Goyer et al. 2001), the present results indicate the significant amount of bacterial exposure in the urban area which shows the severe impact on human health by direct or indirect exposure.

The reported values in dump site and industrial area are higher than other selected locations (i.e., residential area, poultry farm, and slaughterhouse), which are also presented in previous studies (Buttner and Stetzenbach 1993). The higher biotic concentrations are found in industrial and dump site because extreme conditions, like a massive amount of discharge/waste material deposited, high vegetation areas might contribute to increase the bacterial concentration (Buttner and Stetzenbach 1993). In the absence of policy and standards, there is a chance to more exposure to bioaerosol on human and environment as well.

In the updated information on bio-aerosol by Robert Sauve Occupational Health and Safety Research Institute, the biotic concentration is 1000 CFU m⁻³; then, the influence in the

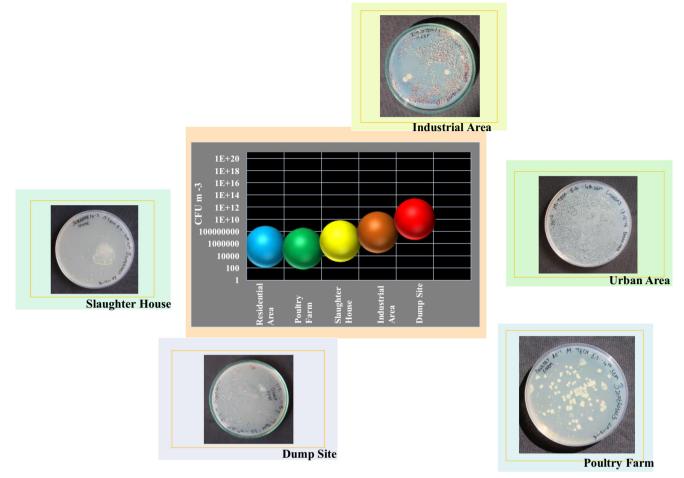


Fig. 4 Concentration of culturable bacterial aerosol in the study locations

microbial is possible; then, the reported higher concentration of biotic matter in the present study is indicating that further investigation of the situation needs to be taken by strong action (Goyer et al. 2001).

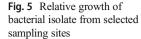
Growth curve

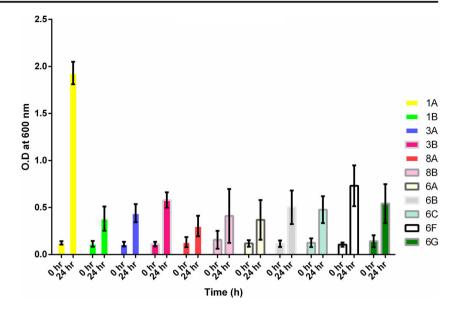
Bacterial microbes were isolated from the filter by enriching them on Luria-Bertani agar (LBA) medium incubated at 37 °C. A total of 11 different bacterial colonies were observed and were separately enriched on the LBA at 37 °C (Fig. 5). Upon attaining the pure culture of each bacterial isolate, each culture was monitored for relative growth at 24 h on Luria-Bertani broth incubated at 37 °C at 150 rpm. The growth was measured as a function of optical density measured at 600 nm. Relative growth studies suggest that isolate 1A displayed maximum growth within 24 h, while isolates 1B, 3A, 3B, 8A, 8B, 6A, 6B, 6C, and 6G displayed 3-fold less growth compared to 1B after 24 h. Strain 6F displayed 2-fold less growth compared to 1A and displayed marginally higher growth compared to other isolates. Strain 3B displayed the least growth of all after 24 h.

The present experiment suggests the study and robust growth potential of isolate 1A which might give him an advantage for outnumbering other organisms for the establishment. Such a strong growth may also contribute in potential infectivity of bioaerosol linked with strain 1A. Other isolates may contribute to lower to moderate infectivity.

Influence of meteorological parameters

The meteorological influence on incremental particle concentration and biotic matter are quite complex and vary with different locations or geographical environment (Li et al. 2011). The measurement of meteorological parameters (i.e., temperature (T), wind speed (WS), relative humidity (RH), and fine particle concentration ($PM_{2.5}$)) was done to understand the influence of environment on the biotic matter (i.e., fungi and bacteria). Meteorological parameters were reported at the same time of monitoring at different selected locations, which are presented in Table 2.





T and RH variation

The significant growth was reported for culturable bacteria especially in the spring/winter than summer (Li et al. 2011). Similarly, the plant growth which is observed very high during spring and winter than summer season is probably the support to increase the different types of media where microorganisms can grow appropriately (Zhong et al. 2016). The reported T ranged in the present study from 22 to 31 °C in all selected locations of the study area. The results of statistical analysis (i.e., correlations) indicated that the bio-aerosol concentration significantly negatively correlated with the concentration of bio-aerosol-a higher concentration of bio-aerosol would report as the T decreases (Table 3). The correlation coefficient (Spearman correlation) was -0.042 (p < 0.05), -0.026 (p < 0.05), -0.033 (p < 0.05), -0.023 (p < 0.05), and -0.122 (p < 0.05) for residential area, slaughterhouse, industrial area, poultry farm, and dump site, respectively. The reported T data range 27-31 °C, which are quite high that indicate correlation with the growth of bio-aerosol negatively-as the T decreased than the concentration of bio-aerosol increases (Zhong et al. 2016).

The low RH values support bio-aerosol viability, as low RH will affect the metabolism activities of biotic matter/ microorganisms. The monitored RH values were relatively changed moderately little between different selected locations; the average was 63.32%, 63.32%, 60.01%, 63.32%, and 61.93% (Table 2). The observed results are supported by previous studies (Li et al. 2015), where a positive correlation between the concentration of bacteria and RH is reported. In support of the results, Jones and Harrison (2004) observed that increased humidity due to moisture present in the air promotes the growth of bacteria.

WS variation

Wind speed plays an important role to disperse particle from one place to another place (Gautam et al. 2016). Similarly, in the case of bio-aerosol, it is proved by an earlier study (Montero et al. 2016) where they mentioned the levels of bio-aerosol concentration varying according to different wind speed. For example, a significant correlation was observed between wind speed and coarse bio-aerosols especially during the summer season (Montero et al. 2016). The advection of bio-aerosol generally reported high at higher WS and then finds low bio-aerosol concentration (Jones and Harrison 2004). In the present study, a negative correlation was observed between WS and bacteria (Table 2). Moreover, to get

Table 2 Meteorological parameters and particle concentration during different sampling locations sampling locations	Parameters	WS (m s ⁻¹) Mean \pm SD	T (°C)	RH (%)	PM _{2.5} (µg m ⁻³)
sumpting rocations	Residential area	3.39 ± 0.74	29.31 ± 1.50	63.32 ± 9.69	26.26 ± 3.75
	Slaughter house	3.39 ± 0.74	29.31 ± 1.50	63.32 ± 9.69	25.92 ± 2.54
	Industrial area	3.42 ± 0.74	31.11 ± 3.01	60.01 ± 9.34	101.79 ± 8.09
	Poultry farm	3.39 ± 0.74	29.31 ± 1.50	63.32 ± 9.69	57.26 ± 9.88
	Dump site	3.43 ± 0.74	30.11 ± 3.45	61.93 ± 8.40	43.89 ± 3.20

 Table 3
 Correlation analysis between bio-aerosol concentration and meteorological parameters

	Bacteria	WS (m s^{-1})	T (°C)	RH (%)	$PM_{2.5}~(\mu g~m^{-3})$
Residential	area				
Bacteria	1				
WS	-0.030	1			
Т	-0.042	0.051	1		
RH	0.120	0.058	-0.383	1	
PM _{2.5}	0.003	- 0.058	0.070	0.047	1
Slaughter l	nouse				
Bacteria	1				
WS	-0.010	1			
Т	-0.026	0.033	1		
RH	0.220	0.049	-0.40	1	
PM _{2.5}	0.040	- 0.034	0.031	0.026	1
Industrial a	irea				
Bacteria	1				
WS	- 0.011	1			
Т	- 0.033	0.049	1		
RH	0.092	0.060	-0.411	1	
PM _{2.5}	0.002	- 0.020	0.053	0.010	1
Poultry far	m				
Bacteria	1				
WS	- 0.020	1			
Т	- 0.023	0.055	1		
RH	0.140	0.044	-0.266	1	
PM _{2.5}	0.004	- 0.052	0.022	0.030	1
Dump site					
Bacteria	1				
WS	- 0.040	1			
Т	-0.122	0.001	1		
RH	0.110	0.041	-0.221	1	
PM _{2.5}	0.014	- 0.034	0.010	0.011	1

precise values for the relationship between bacteria and WS, the number of data sets needs to be analyzed. To understand the nature of the dispersion of bio-aerosol, detail ranges of wind velocity are highly required.

PM_{2.5} variation

The biological and morphological characteristics have been significantly changed due to combination of biotic matter (i.e., bacteria, virus, molds, and yeasts) and PM. In this view, it is very important especially in developing countries where high PM emissions generally reported due to manmade activities (i.e., construction, industrial, transportation, etc.) (Humbal et al. 2018). In current study, good relationship (p > 0.05) has been reported between PM_{2.5} and biotic matter especially in industrial and dump site, where increase in air-

borne biotic matters were observed as $PM_{2.5}$ concentration levels increased (Table 3). Hence, good relationships were observed between $PM_{2.5}$ and bacteria in all selected locations. The reason behind is the associated components with particle supports to grow for biotic matter (Humbal et al. 2018).

Identification of different types of fungi and their possible impact on human health

Table 4 shows the fungi types and their possible health impact at different sampling sites. At few sites (i.e., industrial area, slaughter house, and poultry farm), the variation in fungi types was observed higher than residential area and dump site (Table 4). Normally, the presence of fungi was higher in industrial area, slaughter house, and poultry farm than residential area (except for the little higher in dump site). The variation in types of fungi at sampling locations might be due to presence of geographical parameters, slaughter activities, disposal of waste material, waste discharge, microclimate, indoor levels, and association of number of people. The identification of observed types of fungi showed that significant variation found between sampling locations, where Mucor, Alternaria, and Candida were found in industrial, slaughter house, and poultry farm, respectively. Khan and Karuppayil (2012) suggested that the presence of fungi in industrial area and slaughter house could cause severe health issues in worker at working place. Moreover, Aspergillus was very common in all sampling sites. Several researchers (Baxi et al. 2016; Husman 1996) indicated the hazardous nature of Candida fungi near to industrial area, poultry farm, and slaughter house, where the significant number of *Candida* is responsible of several types of disease (i.e., sinus infections, urinary infections, fatigue, and skin infections). Similarly, many studies reported the harmful impact of Mucor and Alternaria in terms of significant diseases (i.e., allergic infections and asthma) in industrial area and slaughter house, respectively (Baxi et al. 2016). In addition, Aspergillus was reported very common type of fungi in each sampling sites which may cause different types of diseases (i.e., asthma, sinus infections, post-operational infections, and respiratory diseases), as mentioned in previous studies (Inal et al. 2007; Husman 1996). Therefore, the present work mentioned that the few locations (i.e., industrial area, slaughterhouse, and poultry farm) in urban areas can be cause of high exposure to biotic matter on human health than residential area, especially in developing countries. Hence, the comparative monitoring of fungi between different locations is quite important in the urban area of developing countries.

Sr. no.	Sampling Site	Fungus Name	Health Effect	Reference (year)
		Mucor	Allergic diseasesAllergic rhinitisAllergic conjunctivitis	Husman et al. (1996)
1. Industrial	Industrial Area	Aspergillus	 Respiratory infections Aspergillosis Postoporational infection Skin infection Sinuses infection 	Baxi et al. (2016) Inal et al. (2007)
2.	Residential Area	Aspergillus	 Respiratory infections Aspergillosis Asthma Skin infection Sinuses infection 	Baxi et al. (2016) Inal et al. (2007) Khan et al. (2012)
3. Slaughter House		Aspergillus	 Respiratory infections Postoporational infection Allergic diseases Skin infection 	Husman et al. (1996) Baxi et al. (2016)
	Slaughter House	Alternaria	 Allergic diseases Allergic rhinitis Allergic conjunctivitis Skin infection 	Husman et al. (1996) Inal et al. (2007)
		Candida	Urinary tract infectionsBlood infectionDigestive issues	Baxi et al. (2016)
		Penicillium	 Lung infection Hpersensitivity Pneumonitis Allergic diseases Allergic Respiratory diseases 	Halonen et al. (1997)
4. Poultry Farm		Aspergillus	Respiratory infectionsAspergillosisSkin infectionSinuses infection	Husman et al. (1996) Baxi et al. (2016) Khan et al. (2012)
	Poultry Farm	Candida	 Skin infection Urinary tract infections Digestive issues Sinus infections 	Baxi et al. (2016) Khan et al. (2012)
5.	Dump Site	Aspergillus	Respiratory infectionsSinuses infectionAspergillosis	Husman et al. (1996) Baxi et al. (2016) Khan et al. (2012)
	Dump Site	Fusarium	CancerSevere burnEye infectionsSkin infections	Amaissle et al. (1989)

 Table 4
 Identified fungus types at study locations and their possible health effects

Conclusion

The present study provides a comprehensive multi-proxy record such as meteorological, remote sensing, and microbiological based ere to investigate the levels of biotic matter in the residential area, slaughterhouse, industrial area, poultry farm, and dump site in the Rajkot City and surroundings. The outcomes of the study indicated that the number and concentration of biotic matter were highly variable, especially in the dump site and industrial area. This could be the first study to report the bacterial concentration in India to cover maximum possible source locations and indicates higher respiratory exposure to biotic matter in the urban area. Waste material deposition and farming activities can be associated with higher bio-aerosol concentration in the urban area. Dumping sites and industrial area (generation of waste materials) might make a significant contribution to increasing the levels in the urban area. The relative growth studies suggest that isolate 1A has the fastest growth potential and may be responsible for higher infectivity as compared to other isolates. The higher growth potential also allows the bacteria to overpass the effectiveness of immune response thereby increasing the chance of spreading through nasal or oral pathways. Some bacterial species are also known to cause skin infections where such a rapid growth may be optimal characteristic. The outcomes of the present study investigated the lacuna of standard approaches and policies to compare concentration in different locations in the urban area. In addition, information about the biotic matter at different locations of the urban area is not only provided dispersion behavior and possible deposition in the deeper part of lungs but can also suggest the possible source of bio-aerosol concentration. Such elevated levels of microbes are mainly responsible for respiratory diseases, skin diseases, and immunomodulatory responses. In addition, the study mentioned about few locations like industrial area and slaughterhouse are the specific areas of high exposure to biotic matter on living things. Comparative monitoring of biotic matter is quite important in developing countries. The present study demonstrates the enumeration of only culturable microbes; however, some harmful non-culturable microbes may also pose a significant threat to human health which otherwise may be detected only by advance molecular biology methods like metagenomics.

Acknowledgments Marwadi University, Rajkot, Gujarat, India provided us the required funding and support during fieldwork and analysis.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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